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

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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 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

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During salt restriction, the ability of renal endogenous dopamine to inhibit sodium transport is abolished. 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 (AT1) receptors, and the intrarenal dopaminergic system, via D1-like and D2-like receptors, exert counter regulatory effects on sodium balance. Dopamine can also negatively regulate renin production, while angiotensin II (AT2) can increase dopamine turnover. By contrast, D1-like and AT2 receptors cooperate to inhibit renal proximal sodium transport, causing natriuresis. In the current study, we tested the hypothesis that the previously reported attenuated natriuretic effect of D1-like receptor agonists in normotensive humans 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 D1-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 D1-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) was excluded from data analysis (Figure 1, Table 1).

All subjects attained sodium balance with urinary sodium excretion of 52.8±7.4 mmol/24 h on Day 5 of the LS diet (50 mmol/24 h) and 296±5.10 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 on LS and HS diets were similar and unchanged with fenoldopam, 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. Fenoldopam 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. Fenoldopam and enalapril given together decreased diastolic and mean BPs on both salt diets (Table 2).
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 (Table 4B) unlike UNaV, 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 4A).40 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

Table 1. Baseline characteristics of seven salt-resistant subjects who completed both phases of the trial (HS and LS diets)

<table>
<thead>
<tr>
<th>Variable</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male:Female</td>
<td>5(71%):2(29%)</td>
</tr>
<tr>
<td>Caucasian</td>
<td>4</td>
</tr>
<tr>
<td>African-American</td>
<td>2</td>
</tr>
<tr>
<td>Asian</td>
<td>1</td>
</tr>
<tr>
<td>Age (yrs)</td>
<td>32.25 (8.86)</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>25.83 (3.20)</td>
</tr>
<tr>
<td>Systolic BP (mmHg)</td>
<td>110.31 (3.84)</td>
</tr>
<tr>
<td>Diastolic BP (mmHg)</td>
<td>73.54 (3.33)</td>
</tr>
<tr>
<td>Mean arterial BP (mmHg)</td>
<td>84.14 (8.33)</td>
</tr>
<tr>
<td>BUN (mg/dL)</td>
<td>10.85 (2.28)</td>
</tr>
<tr>
<td>Serum creatinine (mg/dL)</td>
<td>0.97 (0.19)</td>
</tr>
<tr>
<td>Serum sodium (mmol/L)</td>
<td>137.31 (1.59)</td>
</tr>
<tr>
<td>Serum potassium (mmol/L)</td>
<td>4.08 (0.24)</td>
</tr>
<tr>
<td>Serum cholesterol (mg/dL)</td>
<td>170.50 (30.03)</td>
</tr>
<tr>
<td>HDL risk ratio</td>
<td>4.07 (1.48)</td>
</tr>
<tr>
<td>LDL risk ratio</td>
<td>2.81 (1.11)</td>
</tr>
</tbody>
</table>

Data are presented as number (percentage) or mean (SD). Chest x-ray and EKG in all subjects were normal. Urinary protein/creatinine ratios in all subjects were <0.2. EKG, electrocardiogram; BMI, body mass index.

Figure 2. (A) High salt (HS), relative to low salt (LS) diet, increases urinary dopamine. (B) Example of timed measurement of UNaV showing that fenoldopam (Fen) increases sodium excretion (UNaV) after 30 minutes in salt-resistant subjects on LS diet who were pretreated with enalapril (Enal). Data are shown as mean±SEM (n=7/group). (A) Urinary dopamine was higher on Day 5 of HS relative to LS. (B) Timed graph of UNaV (mEq/min) measured at half-hourly intervals before (control), during (fenoldopam), and after discontinuing (postcontrol) fenoldopam infusion. Salt-resistant subjects given enalapril (2.5 mg×2 doses) represent the Control.
diet (absolute and %, Table 4B, Figure 5B) as with \( U_{Na}V \). 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).

**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>
<td>62 ± 1</td>
<td>61 ± 1</td>
<td>61 ± 5</td>
<td>62 ± 3</td>
<td>61 ± 2</td>
<td></td>
</tr>
<tr>
<td>t test, LS versus HS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td></td>
</tr>
<tr>
<td>Systolic BP (mmHg)</td>
<td>LS</td>
<td>114 ± 2</td>
<td>113 ± 3</td>
<td>111 ± 5</td>
<td>112 ± 3</td>
<td>109 ± 3</td>
<td>111 ± 5</td>
<td>NS</td>
</tr>
<tr>
<td></td>
<td>HS</td>
<td>117 ± 4</td>
<td>115 ± 3</td>
<td>120 ± 2</td>
<td>114 ± 3</td>
<td>112 ± 2</td>
<td>120 ± 2</td>
<td></td>
</tr>
<tr>
<td>t test, LS versus HS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td></td>
</tr>
<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</td>
</tr>
<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></td>
</tr>
<tr>
<td>t test, LS versus HS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td></td>
</tr>
<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</td>
</tr>
<tr>
<td></td>
<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></td>
</tr>
<tr>
<td>t test, LS versus HS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</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.

**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>
</tr>
</thead>
<tbody>
<tr>
<td>PAH clearance (ml/min per 1.7 m(^2))</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></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>
<td></td>
</tr>
<tr>
<td>t test, LS versus HS</td>
<td>NS</td>
<td>NS</td>
<td>P=0.003*</td>
<td>NS</td>
<td>NS</td>
<td>P=0.002*</td>
<td></td>
<td></td>
</tr>
<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>
<td></td>
</tr>
<tr>
<td>t test, LS versus HS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</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.
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>
<tbody>
<tr>
<td>Creatinine clearance (ml/min per 1.7 m²)</td>
<td>LS</td>
<td>104±14</td>
<td>101±16</td>
<td>96±12</td>
<td>120±13</td>
<td>115±13</td>
<td>115±13</td>
<td><strong>P=0.006</strong></td>
</tr>
<tr>
<td></td>
<td>HS</td>
<td>128±15</td>
<td>137±15</td>
<td>116±7</td>
<td>143±10</td>
<td>152±8</td>
<td>145±6</td>
<td>NS</td>
</tr>
<tr>
<td>Filtered sodium load (mmol/min)</td>
<td>LS</td>
<td>15.4±2.0</td>
<td>14.8±2.2</td>
<td>13.9±2.1</td>
<td>17.5±1.8</td>
<td>16.8±1.8</td>
<td>16.8±2.4</td>
<td>NS</td>
</tr>
<tr>
<td></td>
<td>HS</td>
<td>17.1±2.4</td>
<td>1.2±2.6</td>
<td>16.4±1.5</td>
<td>20.6±2.3</td>
<td>20.2±1.0</td>
<td>19.7±0.9</td>
<td>NS</td>
</tr>
<tr>
<td>Lithium clearance (ml/min per 1.7 m²)</td>
<td>LS</td>
<td>11.7±1.0</td>
<td>13.2±1.3***</td>
<td>10.6±0.8</td>
<td>11.8±1.1</td>
<td>14.7±1.9**</td>
<td>7.9±0.5</td>
<td>**P&lt;0.01,***P&lt;0.001 versus LS, LS Enal, Post Fen, and Post Fen + Enal</td>
</tr>
<tr>
<td></td>
<td>HS</td>
<td>12.3±1.1</td>
<td>15.5±1.7***</td>
<td>13.6±2.6</td>
<td>13.1±0.9</td>
<td>15.2±1.5***</td>
<td>12.1±1.6</td>
<td>NS</td>
</tr>
<tr>
<td>Urine flow (ml/min)</td>
<td>LS</td>
<td>11.7±1.0</td>
<td>13.2±1.3***</td>
<td>10.6±0.8</td>
<td>11.8±1.1</td>
<td>14.7±1.9**</td>
<td>7.9±0.5</td>
<td>**P&lt;0.01,***P&lt;0.001 versus LS, LS Enal, Post Fen, and Post Fen + Enal</td>
</tr>
<tr>
<td></td>
<td>HS</td>
<td>12.3±1.1</td>
<td>15.5±1.7***</td>
<td>13.6±2.6</td>
<td>13.1±0.9</td>
<td>15.2±1.5***</td>
<td>12.1±1.6</td>
<td>NS</td>
</tr>
<tr>
<td>t test, LS versus HS</td>
<td>U_FenV (mmol/min)</td>
<td>LS</td>
<td>0.19±0.02</td>
<td>0.24±0.02†</td>
<td>0.18±0.01</td>
<td>0.16±0.02</td>
<td>0.28±0.05†</td>
<td>0.18±0.01</td>
</tr>
<tr>
<td></td>
<td>HS</td>
<td>0.37±0.05</td>
<td>0.62±0.07†</td>
<td>0.30±0.01</td>
<td>0.33±0.03</td>
<td>0.43±0.08</td>
<td>0.29±0.04</td>
<td>NS</td>
</tr>
<tr>
<td>Fractional excretion of lithium (FELi, %)</td>
<td>LS</td>
<td>1.4±0.3</td>
<td>2.2±0.7†</td>
<td>1.5±0.4</td>
<td>0.8±0.1</td>
<td>1.6±0.2†</td>
<td>1.2±0.2</td>
<td>NS</td>
</tr>
<tr>
<td></td>
<td>HS</td>
<td>2.1±0.6</td>
<td>3.6±1.0†</td>
<td>1.8±0.2</td>
<td>1.7±0.3</td>
<td>2.2±0.4</td>
<td>1.8±0.2</td>
<td>NS</td>
</tr>
<tr>
<td>t test, LS versus HS</td>
<td>Lithium clearance (ml/min per 1.7 m²)</td>
<td>LS</td>
<td>26.1±2.8†</td>
<td>26.4±2.2</td>
<td>20.6±1.8*</td>
<td>21.5±1.3</td>
<td>28.8±1.8***</td>
<td>23.6±2.5</td>
</tr>
<tr>
<td></td>
<td>HS</td>
<td>27.5±1.9</td>
<td>33.3±5.5†</td>
<td>18.1±18.5</td>
<td>27.1±1.5</td>
<td>29.9±2.4</td>
<td>32.2±2.7</td>
<td>NS</td>
</tr>
<tr>
<td>Absolute proximal reabsorption, APR (ml/min)</td>
<td>LS</td>
<td>77.8±14.5</td>
<td>75.5±16.6</td>
<td>75.4±16.6</td>
<td>98.7±13.7*</td>
<td>73.3±13.22</td>
<td>90.7±17.5</td>
<td>NS</td>
</tr>
<tr>
<td></td>
<td>HS</td>
<td>110.1±16.8</td>
<td>93.2±9.7***</td>
<td>87.8±14.4</td>
<td>96.0±19.2</td>
<td>112.6±6.4</td>
<td>115.8±5.6</td>
<td>NS</td>
</tr>
</tbody>
</table>

*P<0.05 versus LS Enal, †P<0.05 versus HSC, RM ANOVA, Fisher’s LSD
†††P<0.001 versus LS Enal, ‡‡‡P<0.001 versus LS Enal, Post Fen, and Post Fen + Enal
△P<0.05 versus LS Enal, Post Fen, and Post Fen + Enal
◆P<0.05 versus LS Enal, RM ANOVA, Fisher’s LSD
◆◆◆P<0.001 versus LS Enal, RM ANOVA, Fisher’s LSD

**P<0.01,***P<0.001 versus LS, LS Enal, Post Fen, and Post Fen + Enal
Table 4. Continued

<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>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>
</tr>
<tr>
<td>t test, LS versus HS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>P=0.036*</td>
<td>NS</td>
<td>NS</td>
</tr>
<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>$^+$P&lt;0.05 versus LS Enal</td>
</tr>
<tr>
<td>t test, LS versus HS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
</tr>
<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>$^+$P&lt;0.05 vs LS Enal, Paired t test</td>
</tr>
<tr>
<td>t test, LS versus HS</td>
<td>NS</td>
<td>NS (P=0.053)</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>P=0.024*</td>
<td>P=0.02*</td>
<td>$^+$P&lt;0.05 versus LSC, LS Fen, LS Fen + Enal, RM ANOVA, Fisher’s LSD</td>
</tr>
<tr>
<td>Absolute distal reabsorption of sodium, ADR$_{Na}$ (mmol/min)</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>$^+$P&lt;0.05 versus HSC, Post HS Fen + Enal, RM ANOVA, Fisher’s LSD</td>
</tr>
<tr>
<td>t test, LS versus HS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS (P=0.06)</td>
<td>P=0.03*</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>$^+$P=0.01 versus LS C and LS Enal</td>
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<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>$P=0.001^*$</td>
<td>$^+$P&lt;0.05 versus HSC, paired t test</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.\(^4\) 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\(^4\) 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 4B, C 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

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, \(U_{\text{Na}}V\) (mEq/min) with control and Fen on LS and HS diets. Effect of Fen (Δ Fen-C) on LS versus HS and percentage effect of Fen (% Δ Fen-C) on LS and HS. (B) Left to right, fractional excretion of sodium, \(\text{FENa}\), % with Fen and control on LS and HS diets. Effect of Fen (Δ Fen-C) on LS versus HS and percentage effect (% Δ 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 (Δ Fen-C) on LS versus HS and percentage effect of Fen (% Δ 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 (FEK) potassium clearances were higher on LS than HS diets (not shown). FEK 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. Selective gene deletion of aromatic amino acid decarboxylase (essential for renal dopamine synthesis) in the renal proximal tubule in mice produces salt-sensitive hypertension, 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 and acts on D1-like and D2-like dopamine receptors, causing natriuresis. The natriuretic effect of dopamine is impaired in salt-depleted states.

We submit evidence, for the first time in normotensive salt-resistant humans, that the RAS and dopaminergic system interact in regulating renal sodium transport on LS and HS diets. We confirm previous reports of natriuresis with D1-like receptor stimulation in normotensive humans on normal and HS diets, but not on LS diets.
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\(^{22,50,51}\) 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)\(^{40}\) 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\(^{57}\) during salt- and volume-depleted states\(^{58}\) because of lithium reabsorption beyond the proximal tubule. Increased renal nerve activity during salt depletion\(^{59}\) 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.\(^{60}\)
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 receptors 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\textsuperscript{64} 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\textsuperscript{65,66}. The use of exogenous lithium and a water load\textsuperscript{41} 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,\textsuperscript{21,22,24,31,32,34} similar to the effects of low doses of dopamine in humans.\textsuperscript{24} 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,\textsuperscript{67} 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 D\textsubscript{1}-like receptors) and the RAS (via the AT\textsubscript{1} receptors) in the proximal tubule that may influence sodium transport on LS diets, hitherto only described in \textit{in vitro} and \textit{in vivo} animal studies. These findings support the notion that dietary salt restriction stimulates the RAS,\textsuperscript{68} providing a mechanism by which dietary salt restriction fails to enhance the natriuretic effect of agents acting beyond the loop of Henle.\textsuperscript{69}

### 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\textsuperscript{<120/80} mmHg,\textsuperscript{48} 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 measurements. 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)\textsuperscript{40} 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.\textsuperscript{70}

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,\textsuperscript{22} 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.

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**Figure 8.** Comparison of percentage effect of fenoldopam (Fen) alone versus fenoldopam + enalapril (Fen + Enal) on sodium transport in the distal nephron on LS and HS diet. Data are expressed as percentage effects compared with pre-infusion values, mean±SEM. *P<0.05, one-way ANOVA, Holm–Sidak. The percentage decrease in absolute distal reabsorption of sodium in response to fenoldopam (Fen) alone on LS and HS was different from the percentage change with the combination of Fen + Enal on LS.
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 (FENa), lithium (FEli), and potassium (FEK), fractional (FPR) and absolute proximal reabsorption of sodium (APRNa), distal sodium delivery, and fractional (FrDR) and absolute distal reabsorption of sodium (ADRNa) were measured²²/calculated²⁴ 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-control periods in all subject groups are expressed as mean±SEM (Tables 2–4). The percentage changes in the above parameters compared with the pre-infusion values were calculated using two-group comparison t test for parametric data and Mann–Whitney Rank-sum test for nonparametric data. The fold-change from the pre-infusion (control) with fenoldopam and enalapril versus fenoldopam alone on a LS diet were measured at half-hour intervals and compared using two-way ANOVA, Bonferroni post-hoc. Differences between and within study days were analyzed by a blinded observer using repeated measures one-way ANOVA with Holm–Sidak post-hoc, and paired t test or Wilcoxon Rank-sum test when applicable. P<0.05 was considered significant. Details of the clinical protocol are in the online supplement.

ACKNOWLEDGMENTS
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Excerpts from this work were presented as a poster presentation at the American Society of Hypertension Annual Meeting (May 19–22, 2007) in Chicago, IL, and as an oral presentation at the 3rd World Congress on Bioavailability and Bioequivalence Pharmaceutical R&D Summit (March 26–28, 2012) in Hyderabad, India.

DISCLOSURES
None.

REFERENCES


This article contains supplemental material online at http://jasn.asnjournals.org/lookup/suppl/doi:10.1681/ASN.2014100958/-/DCSupplemental.
# Supplement 1: Screening Questionnaire

<table>
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<th>Name</th>
<th>SS No</th>
<th>Contact Address/Phone</th>
<th>Subject Number</th>
<th>Date of Birth</th>
<th>Age</th>
<th>Sex</th>
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</thead>
</table>

**BP (recorded)**  
**Height (M)**  
**Weight (Kg)**  
**BMI (kg/M²)**  
**None**  
**Race**  
**Ethnicity**  

<table>
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<th>Date of Screening Interview</th>
<th>YES</th>
<th>NO</th>
<th>NA</th>
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<th>NO</th>
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</thead>
</table>

- Heart disease
- Psychiatric disease
- Psychotropic medications
- Sulfite allergy
- Enalapril allergy
- Chronic use of NSAIDs
- Hereditary angioneurotic edema
- Severe Drug Allergy
- Asthma
- Seizure disorder
- Glaucoma
- Thyroid disease
- Diabetes
- Smoking
- Alcohol
- Vegan
- Women: Pregnant
- Women: Lactating

- Food
- Wheat
- Soya
- Nuts
- Milk
- Eggs

- YES
- NO
Supplement 2: Screening Criteria for Recruitment

1. 12 Lead EKG: No Left Ventricular Hypertrophy (LVH)

2. Chest X-Ray: No cardiomegaly

3. Hematological Profile
   Hemoglobin 13.5 –17.5 g/dL (Males) and 12.0 –16.0 g/dL (Females)
   White blood cell count 4-11 X 10^3/μL

4. Biochemical Profile
   Sodium 135-145 mmol/L,
   Potassium 3.5 –5.1 mmol/L
   Chloride 98-106 mmol/L,
   Bicarbonate 22-29 mmol/L,
   Glucose 70 – 115 mg/dL,
   BUN 7-18 mg/dL,
   Creatinine 0.6 –1.2 mg/dL,
   Calcium 8.2-10.2 mg/dL
   Mg 1.3 – 2.1 mmol/L,
   Total Cholesterol < 200 mg./dL, with low Risk Ratio,
   Urinary Protein/Creatinine ratio <0.2,
   Urine analysis: without active sediment.

5. Serum Beta HCG (Females only): Negative
Salt and Natriuretic Mechanisms Natarajan et al SUPPLEMENT

Supplement 3. Consent to Participate in Research

Normotensive Humans

Project Director: Aruna Natarajan, MD

Principal Investigator: Aruna R. Natarajan, MD   Phone: 202-668-1335
Co-Investigator: Pedro A. Jose, MD, PhD   Cell Phone: 703-405-9661

Sponsor: National Center for Research Resources, National Institutes of Health, DHSS

The Georgetown University Institutional Review Board has given approval for this research project. For information on your rights as a research subject, call the Institutional Review Board office: 202-687-1506.

Introduction

You are invited to consider participating in this research study. We will be evaluating the mechanism of salt excretion by the kidney in response to Fenoldopam, a drug that dilates blood vessels. Specifically, we will study the effect of Enalapril, a drug used in hypertension, on Fenoldopam's ability to increase salt excretion in the urine. You will also receive Lithium, Inulin and Para-amino-hippuric acid (PAH), all of which are standard drugs used to test renal function in humans.

THIS IS NOT A TREATMENT PROTOCOL.

This form will describe the purpose and nature of the study, its possible risks and benefits, other options available to you, and your rights as a participant in the study. Please take whatever time you need to discuss the study with your physicians, hospital personnel and your family and friends. The decision to participate or not is yours. If you decide to participate, please sign and date the last line of this form.

The research is being sponsored by the National Center for Research Resources, National Institutes of Health. The NIH is called the sponsor, and Georgetown University is being paid by the NIH to conduct this study with Aruna Natarajan, MD as the primary investigator.

Background and purpose of the study

This study aims to study the factors which affect salt excretion by the kidney, by studying 2 drugs, Fenoldopam, and Enalapril and their interaction with one another in normotensive and hypertensive people (subjects) when they are on low salt and moderate salt diets.

Fenoldopam dilates (widens) blood vessels, increases the flow of blood to the kidneys and increases the elimination of water and salt (sodium) from the body. It acts by enhancing the effect of dopamine, which is produced in the kidney, and increases salt excretion. This drug
has been studied extensively in animals, and has been well tolerated in healthy men and women with normal blood pressure and high blood pressure. It has been shown to lower blood pressure in some individuals with high blood pressure. Fenoldopam has also been used safely as a treatment for heart failure and retention of excessive body fluid in selected individuals on an experimental basis.

Another substance produced in the body is Angiotensin II, which decreases salt excretion in the urine and thus helps to retain salt and water in the body. We would like to know whether Fenoldopam increases salt excretion in normotensive and hypertensive individuals, and whether this effect is modified by Angiotensin II. We will administer a drug widely used in treating hypertension, Enalapril, which decreases the body's production of Angiotensin II, and study its effect on Fenoldopam-induced salt excretion.

The amount of salt intake in the diet may affect the actions of the drugs we use. Also, some individuals have salt-sensitive hypertension that is their blood pressures are higher with increased salt intake. So, we will study the effects of these drugs in conditions of low and moderate salt diet. We will study normotensive and hypertensive men and women, and see if salt excretion in response to Fenoldopam in these two groups varies depending on salt diet and intake of Enalapril.

To assess effects of any drug, we need to compare these effects with the normal functions of the kidney in excreting certain standard drugs. So you will also be given lithium, PAH (para-amino-hippuric acid) and inulin, which are all standard drugs used to assess renal function. We will also test your urine for some substances that help you excrete salt in the urine, called sodium transporters.

Past research has indicated that essential hypertension has a heritability as high as 30% -50%. We hope to understand the genetic factors that may influence the occurrence of this widespread disease, which is known to be aggravated by increased salt intake. Could it be related to inefficient excretion of salt by the kidney?

To learn more about the potential genetic factors which may influence how an individual responds to Fenoldopam, and Enalapril, we would like to test your blood for some of the recently identified abnormalities in the genetic makeup of some genes that influence salt excretion. For example, abnormalities in the makeup of a specific gene, GRK4, appear to be present in the random population, but also appear to have a higher incidence in people who have high blood pressure. Similarly, interactions with other genes controlling the renin-angiotensin system, which is responsible for production of Angiotensin II (described earlier in this form), may contribute to the development of high blood pressure.

We will also look for known abnormalities in the makeup of genes, which may affect the actions of Angiotensin II.

This information will be kept completely confidential and anonymous for research and educational purposes only.

You were selected for this study because you are in good health, between 18 and 55 years of age, have a normal blood pressure, and have volunteered to participate.
Women of childbearing potential must agree to avoid pregnancy. Their physician may counsel them about avoiding pregnancy.

**Total number of subjects**

About 48 people (20 normotensive and 28 hypertensive people) will take part in this study. Participants in the study are referred to as "subjects." All subjects will be participating at this site, GCRC of Georgetown University.

**General plan of this study**

This study involves placing subjects with normal and high blood pressure on diets with low and moderate amount of salt, and testing how well their kidneys excrete salt in the urine when treated with drugs that increase salt excretion by stimulating dopamine in the kidney, or inhibiting Angiotensin II in the kidney. Every subject will be tested with low salt diet and moderate salt diet.

**Investigational Procedures**

Procedure for testing Enalapril's and Fenoldopam's effect on salt excretion in urine in low salt and salt loaded states

If you choose to participate, you will be hospitalized for a total of 6 days (3 days in a row, followed by 3 further days in a row after an interval of one month). Five days before hospitalization, a medical history, physical exam, blood test and urine sample will be done. You will be placed on a special diet containing a low/ moderate amount of salt. This will be determined in a random manner. Your meals will be given to you daily, as packets for the day, which you can pick up from the GCRC, Georgetown University Hospital. It is critical that you follow the diet strictly, avoiding coffee, colas, and all snacks, which are sources of extra salt. You will also be asked to collect several 24 hour urine samples at home, and to report to the hospital daily, to pick up your meals for the day to have your weight, heart rate, blood pressure checked, and to submit your urine sample for analysis. The salt in the urine will help us monitor that you are following the prescribed diet. Once you are found to be in metabolic 'balance', which is determined by your intake-output balance, you will receive a dose of Enalapril/placebo by mouth on the evening of the 5th day, and admitted to the hospital as an in-patient. The dose of Enalapril you will receive is **2.5 mg/dose in 2 doses, 12-14 hours apart.** (The dose used to treat high blood pressure is from 2.5-40 mg twice daily)

From midnight to 5pm. on the next day (Day 6), you must stay in bed and eat no food. At 6am. on the 6th day, or the evening prior to Day 6, 3 small IV (intravenous) catheters will be placed in the veins of your arms. You will drink a standard amount of water adjusted for your weight. Inulin and PAH, standard agents to assess kidney function, will be given through the IV lines. These are harmless medications and are used mainly to see how well the kidney can excrete them. 1 dose of lithium will be given by mouth. Lithium is given to determine its excretion by the part of the kidney we are interested in studying, which is the proximal tubule, and the part where salt is maximally reabsorbed into the blood. We will be giving 600 mg of lithium
carbonate (Eskalith, Smith Kline Beecham) immediate release, which is 1/3 of the starting
dose used to treat psychiatric conditions. You will be asked to urinate every half-hour and
continue to drink water regularly. Blood samples will be drawn every half-hour between 10.30
am. and 4.30 pm. Between 11.30 am. and 2.30 pm, you will receive a third drug,
Fenoldopam, as an IV infusion at 0.05 g/Kg/min for a duration of 3 hours. This dose is half of
the minimum dose used to reduce blood pressure in patients with high blood pressure. In
therapeutic trials in the past, this dose results in the same drug levels in the body in subjects
with normal and high blood pressures. Blood pressure and pulse will be checked regularly
using an automated device, and a cuff around your arm. At 4.30 pm, you may get out of bed,
eat and walk around, but you must remain in the hospital. Again, you will receive an oral dose
of placebo/Enalapril (the opposite of the one you got the day before). You will spend the night
in the hospital. At 07.30 am. on the next day (Day 7) you will receive a second dose of
placebo/Enalapril by mouth. From 10.30 am. to 4.30 pm. on the 7th day, the infusions and tests
described earlier are repeated. If you received enalapril on the 5th and morning of the 6th days,
you will receive placebo (inactive medication) on the evening of the 6th day and the 7th day
and vice versa.

At 4 pm. on the 7th day, you may go home and eat a normal diet. After one month at home, the
entire process as for Days 5-7 is repeated, except you will be on low salt diet, if you were on
moderate salt diet the first time, and vice versa. Again, all meals will be supplied by the
GCRC, and must be picked up by you daily, when you will also submit urine for testing, and
have a brief physical examination. Urine analysis for sodium will help us follow sodium
balance and make sure you are following the diet strictly.

You will be asked to make an outpatient visit one week after each hospitalization for a final
physical exam, blood test and urine test.

**How your treatment will be determined in this study**

You will undergo low and moderate salt diet regimens, 5 days of each diet. These will be
scheduled four weeks apart, and the order in which you are assigned these diets will be
‘randomized’. This means that like flipping a coin, there is an equal chance of starting out with
a low salt diet, as starting out with a moderate salt diet. We will make the randomization
procedure such that half the subjects will start with low salt, and the other half will start with
moderate salt. The allocation will be made by a computerized system. All subjects will have
both diets, four weeks apart. Similarly, there will be randomization of whether you get
Enalapril or placebo (an inactive medication) first, but you will always get Fenoldopam. These
randomizations will all be double blinded, that is, neither you nor the administering
nurse/physician will know which treatment you have received. In all cases, you will be
monitored closely, just as if you had received the drug. This process is necessary to reduce or
minimize any ‘bias’ that could occur in interpretation of the data, and also in the symptoms you
may experience after taking the drug. Of course, you will know whether you are on low or
high salt diet, as you will guess from the taste! In the event of an emergency, only Dr. Aruna
Natarajan and Dr. Pedro Jose are authorized to break the code, and determine which diet/drug
you are being treated with, so that you receive appropriate treatment.
Length of the study for each subject

We expect that you will be in the study for 5 days as outpatient on the diet, 3 days and 2 nights as inpatient for the drug trial. 4 weeks later, you will spend another 5 days on the diet as outpatient, and 3 days and 2 nights as an inpatient on the drug trial. So each subject spends 10 outpatient days (on diet) and 6 inpatient days (on drug trial).

Possible benefits of participating in the study

You might benefit from this study if we were to discover that you are sensitive to salt, and this may help you decide to make some lifestyle changes, such as reduce the amount of salt in your diet, which may prevent hypertension later on in life. However, we cannot guarantee that you will experience medical benefits from participating in this study. Others may benefit in the future from the information we obtain while you are in this study.

If you are taking any over-the-counter drugs, herbal supplements, etc. which you have purchased from the drug store, grocery store, etc., you should advise your physician.

Possible side effects and other risks of participating in the study

You may experience some side effects as a result of the study medicines and treatments you receive.

Very likely, less serious:

Sodium and potassium supplements taken on the day of renal function testing may cause some loose stools, and abdominal discomfort. Potassium tastes bad and could cause nausea. We are giving the supplements slowly, and giving the potassium in a little orange juice to mask the taste.

You will miss work during the days you are an in-patient in the study (total of 6 days). On the days you have to report to the GCRC to pick up your meals, have your physical exam and have your urine tested, you will need to spend about 1-2 hrs at the GCRC every morning. (total of 10 days).

The risk of blood drawing, and IV catheter placement, includes uneasiness associated with needles, excessive bleeding at the site, or the formation of a small clot in the vein at the site of puncture. These complications will be watched for and promptly treated.

During the entire study, a total of about 450 cc (1 unit or pint) of blood will be obtained for the laboratory tests required by the protocol. Other blood draws, such as for blood donation or for other tests, could result in an excessive amount of blood being donated during the study. Therefore, if you are asked to donate blood by anyone, you should inform them of this study and decline to donate blood for at least 1 month after end of the trial.
Less likely, but serious:

Fenoldopam may increase or decrease blood pressure during the infusion. For this reason, we will monitor blood pressure every 15 minutes while you are on the Fenoldopam infusion. However, these changes will be by less than 10 points. Sometimes heart rate can increase by about 10 points. We will be watching for these side effects. The drug lasts a very short time (6 minutes) in the body, so if we see significant changes in blood pressure or pulse, we will stop the drug immediately, and the effects will be reversed. Most of these changes are seen at a dose of 0.1: g/Kg/min, while we will be giving you a dose of 0.05: g/Kg/min. Fenoldopam has also been reported to cause flushing, palpitations or headache. However, about 950 patients with heart failure and high blood pressure have received oral Fenoldopam for 1-16 weeks without significant problems. The minimum dose that was used in these patients was twice the dose you will receive.

Fenoldopam contains sodium metabisulfite, a sulfite that may cause allergic symptoms that could be severe, such as asthma, or even life-threatening allergic reactions. Subjects who are allergic to sulfites will be excluded from the study, as will subjects with asthma, who have a higher tendency to be allergic to sulfites.

The pressure in your eyes could increase with Fenoldopam. For this reason, we will exclude patients with glaucoma. The effects on the eye are reversible and will reverse within a few minutes of stopping the drug.

Fenoldopam can also cause low potassium levels in your body. For this reason, you will get potassium capsule, while on the drug, and your blood will be checked for potassium levels. We will also follow a continuous EKG on you during the infusion, which can help us detect a change in potassium levels.

Non-specific symptoms such as chest pain, a low grade fever, limb cramps, nervousness and anxiety, reaction at the injection site and headache have been described at the doses we will use in less than 10% of the subjects tested, which should resolve on stopping the drug.

Drug Interactions There is limited information about the risks of administering Fenoldopam with enalapril, as we will be doing in this study. Fenoldopam has been administered without problems with drugs such as digitalis and trinitroglycerine. However, the action of Fenoldopam is short-lived, therefore, on stopping the drug, any drop in blood pressure should resolve within a few minutes with standard treatment, that is extra IV fluids.

Enalapril may also reduce blood pressure. Enalapril will be administered in the hospital, under supervision. Generally, Enalapril is given in doses ranging from 2.5 mg twice a day to 40 mg twice a day, to treat hypertension. We will be giving 2.5 mg per dose of Enalapril for two doses separated by 12-14 hrs, which is a low dose, to enhance salt excretion, but has a minimal effect on blood pressure. However, we will monitor blood pressure closely to make sure it is not low. If blood pressure decreases more than 20 points, or if you have symptoms of lightheadedness, we will discontinue the study and reverse fall in blood pressure with appropriate treatments, by giving intravenous fluids.

Other adverse effects of enalapril, which have been reported, include jaundice, rash, persistent cough and high levels of potassium. We will be measuring potassium levels. The other effects will resolve on stopping the drug.
Rare:
These are extremely unlikely. However, rarely, Enalapril can cause swelling of the face and windpipe. This is like an allergic response to the drug, may obstruct breathing, and be life threatening. Therefore, we would like to know immediately if you have any sensation of choking or swelling in your face, tongue or throat. In such a case, the drug will be discontinued, and you will be excluded from the rest of the trial. If you have had an allergic reaction to Enalapril in the past, or have a hereditary condition where you have experienced swelling of the face, windpipe or difficulty in breathing due to allergy/reaction to some external factor, you will not be able to participate in the study as Enalapril may be dangerous for you.

Drug Interactions with Enalapril

When given with diuretics or anti-inflammatory drugs, Enalapril may cause a fall in blood pressure. We will ask that you let us know if you are taking any over-the-counter anti-inflammatory drugs such as Motrin. Enalapril can cause higher blood levels of lithium, which are addressed in the next paragraph.

Lithium

You will receive a single dose of 600 mg Lithium Carbonate (Eskalith), (2 capsules by mouth) immediate release on each day of the drug trial, when you are an in-patient. The dose we are giving is less than the maintenance dose used to treat psychiatric disorders (which is 900 mg/day) and 1/3 the dose used to treat acute mania (which is 1800 mg/day). The reason we need to give you Lithium is to measure its excretion in the urine, which is the best indicator of how well a certain part of the kidney is functioning. This part of the kidney plays a critical role in controlling salt excretion, so we need to know how well it is working. High levels of lithium in the body are associated with diarrhea, vomiting, tremor, drowsiness and muscle weakness, and even a change in consciousness. We will be giving very low doses of lithium as a single dose: levels tested in subjects so far have been well below the toxic range.

Drug Interactions with Lithium

We will ask that you take no other medications when Lithium is given, as it can interact with several over-the-counter drugs, such as anti-inflammatory drugs, which would increase Lithium levels. Other drugs and situations may also cause an increase in Lithium levels, even if the dose is low, such as: Flagyl, enalapril, calcium channel blockers, some antidepressants which are serotonine inhibitors, and antiseizure drugs, and in patients who have thyroid disease. Lithium levels may also be higher when you are on a low salt diet, and so we will follow levels closely, about 8-12 hrs after administering the drug. We will thus exclude all subjects on any of these drugs, those with thyroid disease, and ask you to let us know if you are on any medications, including over-the-counter and herbal remedies.
Lithium has also been reported to cause increased thirst, dry mouth, and occasionally cold and painful fingers and toes, all of which resolve on stopping the drug. Very rarely, a syndrome with increased pressure in the brain, with impairment of vision has been reported with lithium toxicity. We do not anticipate this will occur in the very low dose we are prescribing, under careful supervision, and with close monitoring of serum lithium levels.

Other persons may learn that you have contributed a sample of blood for genetic analysis. This risk is small, since the DNA samples will be made anonymous after testing for the specific mutations.

A health care worker may be involved in a needle-stick injury with exposure to your blood. In such an event, as certain mandatory testing for communicable diseases such as hepatitis and HIV need to be done, we will obtain a separate consent from you, before your blood is tested.

We will take reasonable safeguards to minimize known and potential risks but unknown and/or unanticipated side effects might occur. Most side effects will go away when the study drugs are stopped. The effects on blood pressure are reversed by stopping the drugs mentioned above.

**Who can participate**

This study is designed for men and women between the ages of 18 years and 55 years. You must be in good general health, with no chronic diseases. Your suitability for this study will be determined by a detailed history, physical exam demonstrating normal blood pressure, ECG, Chest Xray and lab tests to determine your body chemistry and lipid levels, and your signing of the consent form. During the study, your urine will be checked while you are on the diet to make sure you are following it as instructed.

**Who cannot participate**

You cannot participate if you are pregnant, breastfeeding (for women), have a chronic disease such as asthma, arthritis, diabetes, or seizure disorder. You cannot participate if you have a hereditary predisposition to develop swelling of the face or airway as an allergic response, if you have had an allergic response to sulfa drugs, or a past history of allergic response to Enalapril. You cannot participate if you have a past history of heart failure. If you do not achieve salt balance after being on the diet for 7 day, you cannot participate in the study.

**Avoidance of Pregnancy**

The medicines and procedures used in this study may be unsafe for an unborn baby, an infant, sperms, and eggs. If you, as a subject of study, are a woman of child bearing potential, you must agree to avoid pregnancy during your participation in this study. If you, as a subject, are a man, you must agree to not conceive a child during your participation in this study. If you do become pregnant during the study or if you father a child during the study, you should immediately notify Dr. Aruna Natarajan at 202-668-1335 (Pager). In addition, if you are already pregnant or are breast feeding, you cannot participate in this study.
Other treatment options

You do have the option not to participate in this study.

Confidentiality of the data collected during the study

Every effort will be made to keep your medical records confidential as well as other personal information that we gather during this study. However, we cannot guarantee absolute confidentiality.

Whenever data from this study are published, your name will not be used. The material used for genetic testing will be coded such that the information cannot be traced back to you. Only Dr. Aruna Natarajan and Dr. Pedro Jose, the investigators will have access to the code.

Individuals from the Georgetown University IRB, Georgetown University Hospital and Medical Center, the Clinical Trials Office, and the U.S. Food and Drug Administration, may look at medical and research records related to this study, both to assure quality control and to analyze data. We will disclose personal information about you to others as required by law.

Data security

If information about your participation in this study is stored in a computer, we will take the following precautions to protect it from unauthorized disclosure, tampering, or damage:
- Password Protection
- Encryption when being transferred

Only authorized users will have access.

New findings

Throughout the study, we will tell you about new information we receive about treatments that may be appropriate for you, about the treatments under research in this study, and any information that may affect your interest in remaining in the study.

Costs to you for participating

Qualified study subjects will not have to pay for the study drug, diet, or investigations used for research purposes.

Payments to you for participating
Qualified study subjects will be paid for participating in this study. Payments will be made as follows: 500 $ per subject which will be paid for successful completion of both arms of the study, low and moderate salt diet. If you withdraw from the study before both arms of the study are completed, you will be paid on a prorated basis for the number of days spent in the study. In addition, all expenses incurred by you for parking while participating in the study will be reimbursed to you.

Materials obtained from you in this research may be used for commercial purposes. It is the policy of Georgetown University Medical Center, MedStar, Inc., and their affiliates not to provide financial compensation to you should this occur.

**Compensation in case of injury**

We will make every effort to prevent study-related injuries and illnesses. If you are injured or become ill while you are in the study and the illness or injury is due to your participation in this study, you will receive emergency medical care. The costs of this care will be charged to you or to your health insurer. No funds are available from Georgetown University, Georgetown University Hospital, their affiliates, the District of Columbia government or the federal government to compensate you for a study-related injury or illness.

**Your rights as a participant in the study**

Participation in this study is entirely voluntary. You have the right to leave the study at any time. Leaving the study will not result in any penalty or loss of benefits to which you are entitled. Should you decide to leave the study, the procedure is the following: You will be paid compensation on a prorated basis for the number of days you spent in the study. Should you decide not to participate or to withdraw, your medical care will not be affected nor will your relations with your physicians, other personnel and the hospital or university. Your care, however, may subsequently be managed by different researchers or physicians.

**Problems and questions**

Call Dr. Natarajan at 202-668-1335 (Pager) day or night if you have questions about the study, any problems, unexpected physical or psychological discomforts, any injuries, or think that something unusual or unexpected is happening. If Dr. Natarajan is not available, Dr. Pedro Jose, the co-investigator may be contacted at Cell Phone number 703-405-9661. The project will be covered 24 hrs/day, 7 days/week, and we will let you know the specific individual to contact, when you are enrolled. Call the Georgetown University Clinical Trials Office at 202-687-0381 with any questions or concerns about bills you have received from the hospital or your study physician that you feel may be related to your participation in this research study.

Call the Georgetown University IRB office at 202-687-1506 with any questions about your rights as a research subject.

**Research Subject Advocate**
A nurse with expertise in clinical research studies is available to talk with you. Her job is to help ensure that you are properly informed and protected as you participate in this research study. She is not directly associated with this research study. If you have any questions about what is being done in this study, why it is being done, or if you have any other questions or concerns either now or during the study, please page Judith Baigis, RN, PhD, Research Subject Advocate, General Clinical Research Center, Professor, School of Nursing and Health Studies, Georgetown University Medical Center, Pager No. 202-542-9813.

**Withdrawal by investigator, physician, or sponsor**

The investigators, physicians or sponsors may stop the study or take you out of the study at any time should they judge that it is in your best interest to do so, if you experience a study-related injury, if you need additional or different medication, or if you do not comply with the study plan. They may remove you from the study for various other administrative and medical reasons. They can do this without your consent.

**Investigator's statement**

I have fully explained this study to the subject. I have discussed the procedures and treatments, the possible risks and benefits, the standard and research aspects of the study, and have answered all of the questions that the subject and the subject's family members have asked.

Signature of investigator ___________________________ Date

**Subject's consent**

I have read the information provided in this Informed Consent Form (or it was read to me by __________________________________). All my questions were answered to my satisfaction. I voluntarily agree to participate in this study.

[Upon signing, you will receive a copy of this form, and the original will become part of your medical record.]

Signature of witness ___________________________ Date ___________

Your signature ___________________________ Date ___________

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Supplemental Figures

FIGURE 1. Enalapril (Enal) alone has no effect on distal sodium delivery or transport

Data are shown as Mean ± S.E.M. or Median (Range), with *P<0.05 vs. C and Post control (not shown) RM ANOVA, or Mann-Whitney Rank-Sum /paired t-test (LS vs HS) respectively

A. L to R, Distal delivery of sodium (mEq/min) with Control (C) and Enal on Low (LS) and High Salt (HS) diets, Effect of Enal (Δ Enal - C) on LS vs HS and Percentage effect of Enal (% Δ Enal -C) on LS and HS

B. L to R Absolute Distal Sodium Reabsorption (ADR$_{Na}$, mEq/min) with Enal and Control (C) on Low (LS) and High Salt (HS) diets, Effect of Enal (Δ Enal - C) on LS vs HS and Percentage effect (% Δ Enal -C) on ADR with Enal compared to C on LS and HS

C. L to R, Fractional Distal Reabsorption (FrDR,%) with Enal and Control (C) on Low (LS) and High Salt (HS) diets, Effect of Enal (Δ Enal - C) on LS vs HS and Percentage effect of Enal ( % Δ Enal-C) on LS and HS
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FIGURE 2. Enalapril (Enal) does not affect fenoldopam-mediated distal sodium transport on high or low salt diet

Data are shown as Mean ± S.E.M. or Median (Range), with *P<0.05 vs. Enal and Post control (not shown) RM ANOVA or Mann-Whitney Rank-Sum/paired t-test (LS vs HS) respectively

A. L to R, Distal Delivery of Sodium (mEq/min) with Enal and Fen+ Enal on Low (LS) and High Salt (HS) diets, Effect of Fen + Enal (Δ Fen + Enal – Enal) on LS vs HS and Percentage effect of Fen + Enal (% Δ Fen + Enal – Enal) on Distal Delivery on LS and HS

B. L to R, Absolute Distal Sodium Reabsorption (ADR$_{Na}$, mEq/min) with Enal and Fen + Enal on Low (LS) and High Salt (HS) diets, Effect of Fen + Enal (Δ Fen + Enal – Enal) on LS vs HS and Percentage effect of Fen + Enal (% Δ Fen + Enal – Enal) on LS and HS

C. L to R, Fractional Distal Reabsorption (FrDR, %) with Enal and Fen + Enal on Low (LS) and High Salt (HS) diets, Effect of Fen + Enal (Δ Fen + Enal – Enal) on LS vs HS and Percentage effect of Fen + Enal (% Δ Fen + Enal – Enal) on LS and HS
TABLE 1

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Table 1. Comparison of the percentage effects of fenoldopam and fenoldopam and enalapril on low or high salt diet  
Percentage effects of fenoldopam alone (Fen, %) and fenoldopam +enalapril (Fen + Enal, %) on the parameters shown.  
Data are expressed as Mean ± S.E.M. *P<0.05, **P<0.01,  
paired t-test, Fen (%) vs Fen +Enal (%) on Low salt and High Salt diet respectively.
Table 2. Comparison of the difference in APR\textsubscript{Na} with fenoldopam and fenoldopam and enalapril treatment of salt-resistant subjects on low salt diet, $P= 0.026$, Rank Sum analysis of medians.

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<th>APR\textsubscript{Na} (mEq/min)</th>
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