Effect of Sodium Balance and Calcium Channel Blocking Drugs on Blood Pressure Responses

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SUMMARY To study the role of calcium movements in mediating the effects of sodium chloride on the response of blood pressure to angiotensin II (ANG II), we infused ANG II before and after giving calcium channel blocking drugs (nifedipine and diltiazem) and calcium infusions to normal subjects during high and low sodium intakes. ANG II was also infused in nine patients with essential hypertension eating a low sodium diet. In preliminary studies, the effects of nifedipine, 20 mg p.o., on blood pressure and plasma renin activity were determined. Sensitivity to infused ANG II was calculated as the slope of the linear regression of the increase in diastolic blood pressure (DBP) expressed as a function of the ANG II infusion rate (mm Hg/ng ANG II/kg/min). During intake of a high sodium diet (Na, 200 mEq/day) both drugs significantly (p<0.05) reduced ANG II sensitivity, while on a low sodium diet (10 mEq Na), neither drug reduced ANG II sensitivity. There was a significant (p<0.001) inverse correlation between the initial ANG II–DBP sensitivity and the change in sensitivity induced by the calcium channel blocking drugs in normal subjects (r = —0.78) and in hypertensive patients (r = —0.70). Five hypertensive patients had greater than normal ANG II–DBP sensitivity that was significantly (p<0.05) reduced by nifedipine. Calcium infusion did not affect the ANG II–DBP sensitivity on either diet. The results suggest that in normal subjects increased DBP responses to ANG II, induced by an increase in sodium intake, are partially mediated by increased extracellular to intracellular calcium movements, since they are blocked by the structurally different calcium channel blocking drugs nifedipine and diltiazem. In hypertensive patients on a low sodium diet, increased DBP responses to ANG II infusion were blocked by nifedipine, indicating they are at least partly mediated by increased extracellular to intracellular calcium flux. (Hypertension 10: 239–248, 1987)

KEY WORDS • sodium balance • blood pressure • calcium channel blockers • angiotensin II • norepinephrine • essential hypertension

Although sodium balance influences the actions of angiotensin II (ANG II) on the adrenal and vascular systems in humans, the mechanisms by which it does so are uncertain. Increased salt intake increases blood pressure sensitivity to ANG II, while a low sodium intake has the opposite effect. In rats, a low sodium diet decreases and a high sodium diet increases the number of vascular ANG II receptors. Chronic but not acute lowering of plasma ANG II by converting enzyme inhibition in rats on a low sodium diet increases the number of ANG II vascular receptors. In humans, however, converting enzyme inhibition only partially inhibits the reduced blood pressure response to ANG II during a low sodium diet, and prolonged ANG II infusion in normal subjects on an ad libitum diet does not affect subsequent ANG II–blood pressure response, suggesting other factors are involved. Another factor that could influence the sensitivity of vascular tissue to ANG II is calcium movement, since calcium is important in the vascular response to ANG II. Therefore, we studied the effect of nifedipine and diltiazem, calcium channel blocking drugs with different molecular structures, on the ability of ANG II to increase blood pressure in normal subjects eating high and low sodium diets; 2) the effect of nifedipine on the ability of norepinephrine to increase blood pressure in normal subjects eating high and low sodium diets; and 3) the
effect of calcium infusions on the blood pressure responses to ANG II administration during high and low sodium diets. In addition, as the blood pressure response to angiotensin II infusion is increased in some patients with essential hypertension, especially in those with the low renin type, we administered nifedipine to patients with essential hypertension during a low sodium diet to determine its effects on the blood pressure response to stimulation by ANG II.

Subjects and Methods

Normal subjects, men and women, ranging in age from 19 to 45 years, were fed constant diets containing sodium, 200 mEq/day, and potassium, 80 mEq/day, or sodium 10 mEq/day, and potassium, 80 mEq/day, for 4 days from the kitchen of the Clinical Research Center. On the fourth day a 24-hour urine sample was collected for measurements of sodium, potassium, and creatinine concentrations. On the fifth day of the diet, the subjects were admitted to the Clinical Research Center at 0730 to 0800. After admission, subjects voided, were weighed, and then lay down for at least 1.5 hours to ensure a steady state plasma renin activity (PRA) and blood pressure. During recumbency arterial pressure was recorded automatically every 2 minutes by an arteriosonde (Roche Diagnostics, Nutley, NJ, USA). After the 1.5 hours of recumbency blood was drawn for measurement of PRA. After this point the procedures in each study varied. In Group 1 (n = 8; 200 mEq Na, 80 mEq K diet) and Group 2 (n = 6; 10 mEq Na, 80 mEq K diet), 20 mg of nifedipine was given by mouth and blood studies were obtained every half hour for 3 hours (see Table 1). In Group 3 (n = 5; 200 mEq Na, 80 mEq K diet), ANG II was infused at 3, 6, and 9 ng/kg/min for 20 minutes each. One hour after the ANG II infusion was stopped, blood samples for PRA were again drawn and 20 mg of nifedipine was given by mouth. One hour later, blood was again drawn and ANG II was infused as described. In Group 4 (n = 11; 10 mEq Na, 80 mEq K diet), the study was similar to that performed in Group 3. In Group 5 (n = 7; 10 mEq Na, 80 mEq K diet for 4 days), the study was the same as in Group 4 except that ANG II was infused at 6, 9, and 12 ng/kg/min instead of 3, 6, and 9 ng/kg/min. In Group 6 (n = 9; 200 mEq Na, 80 mEq K diet) and Group 7 (n = 8; 10 mEq Na, 80 mEq K diet), the study was similar to that in Group 4 except that 60 mg of diltiazem instead of nifedipine was given by mouth and ANG II infusion was repeated 2 hours later. This was done since diltiazem has a slower onset of action than nifedipine. In Group 8 (n = 8; 200 mEq Na, 80 mEq K diet) and Group 9 (n = 7; 10 mEq Na, 80 mEq K diet), ANG II was infused as in Group 3, and, 1 hour after the ANG II infusion was stopped, calcium gluconate (558 mg of elemental calcium) was infused in 5% dextrose in water for 3 hours. During the last hour of calcium infusion the ANG II infusion was repeated. Blood was drawn for ionized calcium determinations before and after each hour of calcium infusion. In Group 10 (n = 7; 200 mEq Na, 80 mEq K diet for 4 days) and Group 11 (n = 7; 10 mEq Na, 80 mEq K diet for 4 days), norepinephrine was infused at 1, 3, and 6 μg/70 kg/min, each for 20 minutes, and blood for PRA was obtained just before the infusion. One hour after the norepinephrine infusion was stopped, blood samples were again obtained and 20 mg of nifedipine was given by mouth. One hour later, blood was again drawn and norepinephrine was infused as described. No subject took part in more than two of these groups.

Nine patients with essential hypertension were also studied. All had normal renal function. All patients had discontinued antihypertension medication (β-blockers, methyldopa, diuretics, or captopril) for at least 7 days before the study began; the mean number of days was 10.1. In addition, all subjects were given a low sodium diet. PRA was determined after furosemide administration, and secondary causes of hypertension were excluded on a previous admission as described before. After admission to the Clinical Research Center these patients were fed a 10 mEq Na, 80 mEq K diet for 4 days. On the fifth day they were infused with ANG II before and 1 hour after receiving 20 mg of nifedipine. Blood pressure was measured and blood samples obtained in the same manner as described for normal subjects in Group 3. The diastolic blood pressure (DBP) during infusion of ANG II did not exceed 115 mm Hg.

Arterial pressures in each subject were the means of 10 determinations at 2-minute intervals during 10-minute averages of the control periods and at the end of each ANG II or norepinephrine infusion rate. ANG II (CIBA, Summit, NJ, USA) and norepinephrine were infused by a motor-driven syringe (Harvard infusion pump, Model 900; Millis, MA, USA), and calcium gluconate (558 mg of elemental calcium) was mixed in 250 ml of 5% dextrose solution and infused for 3 hours by an IVAC (Model 530; San Diego, CA, USA) infusion pump. An electrocardiogram was obtained every 45 to 60 minutes during infusion of the calcium gluconate; no changes were seen in any subject.

PRA, serum sodium concentration, and potassium concentration were measured as previously reported. PRA was measured by radioimmunoassay of angiotensin I (ANG I) generated during incubation for 2 hours at pH 7.4. Interassay replicate determinations of the activity of two plasma samples in a total of 34 consecutive routine assays gave a mean coefficient of variation of 20%. Within the same assays 41 duplicates had a mean difference of 7.9%.

Ionized calcium was measured by an instrument (ICAT) from Radiometer (Copenhagen, Denmark). At a level of 1.24 mM, the interassay variability was 2.3% and the intra-assay variability was 1.2%. Sensitivity to infusion of ANG II (including a zero infusion rate) was calculated as the slope of the linear regression of DBP (4 points) on ANG II infusion rate (mm Hg/ng ANG II/kg/min). All slopes had a regression coefficient (r > 0.80), and all were significant (p at least < 0.05). Statistical analysis was performed using Student’s tailed t test for paired samples and linear
regression. The mean calculated sensitivity of the DBP to ANG II in normal subjects during the 10 mEq Na+, 80 mEq K diet (same diet as the hypertensive patients) was 1.4 ± 0.8 (mean ± 2 SD) mm Hg/ng/kg/min. Based on this value, patients with essential hypertension were categorized into those with normal ANG II–DBP sensitivity (4 patients) and those with increased ANG II–DBP sensitivity (5 patients).

All subjects were informed of the nature of the studies, and written consent was obtained. The protocol had been approved by the Institutional Review Board for the Protection of Human Subjects at the Health Science Center.

Results

Effect of Nifedipine in Normal Subjects During a High and Low Sodium Diet (Groups 1 and 2)

The baseline data in all subjects, including those of Groups 1 and 2 during a high and low sodium diet, are shown in Table 1. As shown in Figure 1, nifedipine, 20 mg given orally at 1.5 hours, significantly (p < 0.05) reduced the DBP during both the high and low sodium diet. The fall in DBP persisted for 3 hours, and the mean diastolic reduction was 3 to 4 mm Hg greater during the low sodium diet. PRA did not change significantly in either study.

Effect of Nifedipine on the Actions of ANG II During High and Low Sodium Intake in Normal Subjects (Groups 3–5)

Figure 2 shows the changes in DBP that resulted when ANG II was infused at three different rates, before and after nifedipine administration, during the high and low sodium diets. During the high sodium diet the DBPs before the first and second ANG II infusions were 66.0 ± 3.3 and 64.1 ± 3.9 mm Hg, respectively. The ANG II–induced changes in DBP were significantly (p < 0.05) reduced by nifedipine at all three ANG II infusion rates. The mean slope of the regression of DBP versus ANG II infusion rate before nifedipine administration was 1.5 ± 0.2 mm Hg/ng/kg/min, and the slope was not significantly different after nifedipine administration (1.4 ± 0.2 mm Hg/ng/kg/min). The upper two lines on the right side of Figure 2 show the ANG II–induced change in DBP before and 1 hour after nifedipine administration in seven normal subjects during the low sodium diet when ANG II was infused at 6, 9, and 12 ng/kg/min (Group 5). The DBPs before the first and second ANG II infusions were 67.1 ± 2.5 and 65.3 ± 2.0 mm Hg, respectively. Nifedipine did not significantly (p > 0.05) reduce the change in DBP at any ANG II infusion rate. The slope of the regression of DBP versus ANG II infusion rate before nifedipine administration was 1.5 ± 0.2 mm Hg/ng/kg/min, and the slope was not significantly different after nifedipine administration (1.4 ± 0.2 mm Hg/ng/kg/min).

Effect of Diltiazem on the Actions of ANG II During High and Low Sodium Intake in Normal Subjects (Groups 6 and 7)

Figure 3 shows the ANG II–induced changes in DBP before and 2 hours after diltiazem administration, during the high and low sodium diets. During the high sodium diet, the DBPs before the ANG II infusions were 72.5 ± 2.4 and 74.0 ± 2.0 mm Hg, respectively. Diltiazem administration the ANG II–induced rise in DBP was significantly (p < 0.05) reduced at the higher two ANG II infusion rates (see Figure 3, left side). The slope of the DBP versus ANG II infusion rate before diltiazem administration was 2.0 ± 0.2 mm Hg/ng/kg/min, and it was significantly (p < 0.01) reduced to 1.5 ± 0.2 mm Hg/ng/kg/min after diltiazem treatment.

During the low sodium diet, the DBPs before the ANG II infusions were 69.8 ± 2.2 and 69.4 ± 2.8 mm Hg, respectively. Diltiazem did not significantly reduce the ANG II–induced rise in DBP at any ANG II infusion rate (see Figure 3, right side). The slopes of the DBP versus ANG II infusion rate before and after diltiazem treatment were 1.3 ± 0.2 and 1.4 ± 0.2 mm Hg/ng/kg/min, respectively (not significantly different).

Effect of Calcium Infusion on the Actions of ANG II During High and Low Sodium Intake in Normal Subjects (Groups 8 and 9)

Table 2 shows the serum ionized calcium, PRA, and systolic and diastolic blood pressure responses to an infusion of calcium gluconate from Hour 3.5 to 6.5. During the last hour of calcium infusion, ANG II was again infused. The basal serum ionized calcium concentration was the same both on high and low sodium diets (see Table 2). During the infusion of calcium, the ionized calcium concentration increased similarly during both diets but there was no significant change in blood pressure. During the high sodium diet there was no significant difference in the ANG II–induced rise in DBP after calcium infusion. The slopes of DBP versus ANG II infusion rate before and after calcium infusion were 2.2 ± 0.2 and 2.3 ± 0.2 mm Hg/ng/kg/min (not significantly different).

During the low sodium diet the change in DBP during calcium infusion was not significantly different at any infusion rate of ANG II. In addition, the sensitivity of the DBP to ANG II infusion before and after calcium infusion was 1.5 ± 0.2 and 1.7 ± 0.3 mm Hg/ng/kg/min (not significantly different).
TABLE 1. Characteristics of and Protocols for Subjects

<table>
<thead>
<tr>
<th>Group</th>
<th>Na Diet</th>
<th>Drug</th>
<th>Age (yr)</th>
<th>Sex</th>
<th>Infused substance</th>
<th>Weight (kg)</th>
<th>UNa (mEq/24 hr)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>High</td>
<td>Nif</td>
<td>24 ± 3</td>
<td>4M, 4F</td>
<td>None</td>
<td>66 ± 5</td>
<td>180 ± 19</td>
</tr>
<tr>
<td>2</td>
<td>Low</td>
<td>Nif</td>
<td>22 ± 1</td>
<td>4M, 2F</td>
<td>None</td>
<td>67 ± 5</td>
<td>11 ± 8</td>
</tr>
<tr>
<td>3</td>
<td>High</td>
<td>Nif</td>
<td>26 ± 3</td>
<td>4M, 1F</td>
<td>ANG II</td>
<td>75 ± 3</td>
<td>180 ± 20</td>
</tr>
<tr>
<td>4</td>
<td>Low</td>
<td>Nif</td>
<td>21 ± 1</td>
<td>6M, 5F</td>
<td>ANG II</td>
<td>54 ± 9</td>
<td>6 ± 4</td>
</tr>
<tr>
<td>5</td>
<td>Low</td>
<td>Nif</td>
<td>22 ± 1</td>
<td>4M, 3F</td>
<td>ANG II</td>
<td>62 ± 6</td>
<td>12 ± 6</td>
</tr>
<tr>
<td>6</td>
<td>High</td>
<td>Dil</td>
<td>28 ± 3</td>
<td>8M, 1F</td>
<td>ANG II</td>
<td>79 ± 2</td>
<td>223 ± 33</td>
</tr>
<tr>
<td>7</td>
<td>Low</td>
<td>Dil</td>
<td>39 ± 3</td>
<td>4M, 4F</td>
<td>ANG II</td>
<td>66 ± 5</td>
<td>14 ± 9</td>
</tr>
<tr>
<td>8</td>
<td>High</td>
<td>Cal</td>
<td>24 ± 3</td>
<td>5M, 3F</td>
<td>ANG II</td>
<td>70 ± 3</td>
<td>153 ± 13</td>
</tr>
<tr>
<td>9</td>
<td>Low</td>
<td>Cal</td>
<td>32 ± 6</td>
<td>4M, 3F</td>
<td>ANG II</td>
<td>68 ± 4</td>
<td>25 ± 4</td>
</tr>
<tr>
<td>10</td>
<td>High</td>
<td>Nif</td>
<td>25 ± 1</td>
<td>6M, 1F</td>
<td>Nor</td>
<td>71 ± 4</td>
<td>199 ± 20</td>
</tr>
<tr>
<td>11</td>
<td>Low</td>
<td>Nif</td>
<td>22 ± 1</td>
<td>5M, 2F</td>
<td>Nor</td>
<td>68 ± 5</td>
<td>23 ± 13</td>
</tr>
</tbody>
</table>

- **Hypertensive patients**
  - Increased ANG II/DBP sensitivity
    - Low Nif 43 ± 2 3M, 2F ANG II 85 ± 3 25 ± 14
  - Normal ANG II/DBP sensitivity
    - Low Nif 46 ± 1 2M, 2F ANG II 88 ± 2 15 ± 3

All data are means ± SE. Number of subjects of each sex is shown in parentheses.

Nif = nifedipine; Dil = diltiazem; Cal = calcium infusion; Nor = norepinephrine infusion; UNa = urinary sodium; UK = urinary potassium; UCr = urinary creatinine; BP = blood pressure.

*After calcium channel blocking agent or calcium infusion.

**Figure 1.** The effect of 20 mg of nifedipine on mean (± SEM) DBP and PRA in eight normal subjects during a 200 mEq Na, 80 mEq K diet (Group 1; left side) and in six normal subjects during a 10 mEq Na, 80 mEq K diet (Group 2; right side). The levels of significance were calculated from paired t tests comparing results before and after nifedipine administration (given at Time = 1.5 hours).

**Effect of Nifedipine on the Actions of Norepinephrine During High and Low Sodium Intake in Normal Subjects (Groups 10 and 11)**

Figure 4 shows the norepinephrine-induced changes in mean blood pressure (MBP) before and 1 hour after nifedipine administration during the high (Group 10) and low (Group 11) sodium diets. During the high sodium diet, MBP before the norepinephrine infusions was 82.3 ± 3.4 before and 79.0 ± 3.1 mm Hg after nifedipine administration. After nifedipine administration the norepinephrine-induced rise in MBP was significantly (p < 0.04) reduced at all three norepinephrine infusion rates (see Figure 4, left side). The slope of the MBP versus norepinephrine infusion rate before nifedipine administration was 3.7 ± 0.6 mm Hg/µg/70 kg/min. This value was significantly (p < 0.005) re-
TABLE 1. (continued)

<table>
<thead>
<tr>
<th>U_2 (mEq/24 hr)</th>
<th>U_2 (g/24 hr)</th>
<th>PRA (ng ANG I/ml/hr)</th>
<th>BP (mm Hg)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Initial</td>
<td>Post-treatment*</td>
</tr>
<tr>
<td>60±10</td>
<td>1.4±0.1</td>
<td>0.4±0.2</td>
<td>—</td>
</tr>
<tr>
<td>37±9</td>
<td>1.0±0.2</td>
<td>2.0±0.3</td>
<td>—</td>
</tr>
<tr>
<td>59±11</td>
<td>1.4±0.1</td>
<td>0.5±0.2</td>
<td>0.4±0.1</td>
</tr>
<tr>
<td>46±19</td>
<td>1.1±0.2</td>
<td>2.0±0.4</td>
<td>5.2±1.5</td>
</tr>
<tr>
<td>44±10</td>
<td>1.4±0.2</td>
<td>1.5±0.4</td>
<td>2.4±0.5</td>
</tr>
<tr>
<td>78±7</td>
<td>2.3±0.2</td>
<td>0.4±0.2</td>
<td>0.7±0.1</td>
</tr>
<tr>
<td>49±11</td>
<td>1.9±0.2</td>
<td>3.4±1.1</td>
<td>4.4±1.3</td>
</tr>
<tr>
<td>56±7</td>
<td>1.5±0.1</td>
<td>0.3±0.1</td>
<td>0.5±0.1</td>
</tr>
<tr>
<td>49±9</td>
<td>1.7±0.2</td>
<td>2.1±0.8</td>
<td>2.4±0.6</td>
</tr>
<tr>
<td>75±10</td>
<td>2.2±0.2</td>
<td>0.5±0.2</td>
<td>0.5±0.1</td>
</tr>
<tr>
<td>64±14</td>
<td>1.9±0.2</td>
<td>0.9±0.2</td>
<td>1.4±0.3</td>
</tr>
</tbody>
</table>

43±5           | 2.3±0.3       | 2.2±0.5               | 6.3±2.2     | 128/72±5/6 | 120/70±5/6     |

71±20           | 1.9±0.1       | 1.3±0.5               | 2.4±1.0     | 128/72±5/6 | 112/75±5/4     |

**Figure 2.** Nifedipine results in normal subjects. Mean (± SEM) increases in DBP in response to ANG II infusion in five normal subjects (Group 3) during a high (200 mEq) sodium diet (left side). On the right side, 11 normal subjects (Group 4) were infused with ANG II at 0, 3, 6, and 9 ng/kg/min and seven normal subjects on a low (10 mEq) sodium diet were infused with ANG II at 0, 6, 9, and 12 ng/kg/min (Group 5; upper two lines). For the sake of clarity, lines joining the ANG II infusion rates of 0 and 6 ng/kg/min have not been connected for Group 5. Before and after refer to nifedipine administration.

**Figure 3.** Diltiazem results in normal subjects. Mean (± SEM) increases in DBP in response to ANG II infusion in nine normal subjects (Group 6) studied during a high (200 mEq) sodium diet and eight normal subjects (Group 7) during a low (10 mEq) sodium diet. Before and after refer to before and 2 hours after administration of 60 mg of diltiazem by mouth.
TABLE 2. Effect of Calcium Gluconate and ANG II Infusions on Serum Ionized Calcium, PRA, and Blood Pressure of Normal Subjects on a High Sodium Diet

<table>
<thead>
<tr>
<th>Variable</th>
<th>1.5</th>
<th>2.5*</th>
<th>3.5</th>
<th>4.5</th>
<th>5.5</th>
<th>6.5*</th>
</tr>
</thead>
<tbody>
<tr>
<td>High sodium intake (n = 8)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Serum ionized calcium (mM)</td>
<td>1.23 ± 0.02</td>
<td>1.23 ± 0.02</td>
<td>1.24 ± 0.02</td>
<td>1.38 ± 0.02</td>
<td>1.47 ± 0.02</td>
<td>1.57 ± 0.02</td>
</tr>
<tr>
<td>PRA (ng ANG I/ml/hr)</td>
<td>0.3 ± 0.1</td>
<td>0.4 ± 0.1</td>
<td>0.8 ± 0.3</td>
<td>0.5 ± 0.1</td>
<td>0.5 ± 0.1</td>
<td>0.3 ± 0.1</td>
</tr>
<tr>
<td>Blood pressure (mm Hg)</td>
<td>110/68 ± 6/2</td>
<td>129/88 ± 4/3</td>
<td>113/70 ± 5/3</td>
<td>116/70 ± 6/3</td>
<td>117/71 ± 4/3</td>
<td>134/90 ± 2/3</td>
</tr>
<tr>
<td>Low sodium intake (n = 8)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Serum ionized calcium (mM)</td>
<td>1.24 ± 0.02</td>
<td>1.24 ± 0.01</td>
<td>1.22 ± 0.01</td>
<td>1.37 ± 0.01</td>
<td>1.47 ± 0.02</td>
<td>1.56 ± 0.02</td>
</tr>
<tr>
<td>PRA (ng ANG I/ml/hr)</td>
<td>2.1 ± 0.8</td>
<td>1.7 ± 0.8</td>
<td>1.5 ± 0.3</td>
<td>2.4 ± 0.6</td>
<td>1.4 ± 0.4</td>
<td>0.6 ± 0.2</td>
</tr>
<tr>
<td>Blood pressure (mm Hg)</td>
<td>112/68 ± 4/2</td>
<td>129/81 ± 4/3</td>
<td>114/66 ± 4/2</td>
<td>117/68 ± 4/2</td>
<td>120/69 ± 4/2</td>
<td>136/85 ± 6/3</td>
</tr>
</tbody>
</table>

Values are means ± SE. Calcium gluconate was infused from Hour 3.5 to Hour 6.5. ANG II was infused from Hour 1.5 to Hour 2.5 and from Hour 5.5 to Hour 6.5.

*End of ANG II infusion.

Figure 4. Norepinephrine results in normal subjects. Mean (± SEM) increases in mean blood pressure (BP) in response to norepinephrine infusion in seven normal subjects (Group 10) during a high (200 mEq sodium) diet and seven normal subjects (Group 11) during a low (10 mEq) sodium diet. Before and after refer to before and after administration of 20 mg of nifedipine by mouth.

Figure 5 shows the ANG II-induced changes in DBP on a low sodium diet before and 1 hour after nifedipine administration, in the five hypertensive patients with increased DBP sensitivity to ANG II, four hypertensive patients with normal sensitivity, and 11 normal subjects. For those with increased DBP sensitivity to ANG II, nifedipine administration significantly (p < 0.05) reduced the rise in blood pressure at all three ANG II infusion rates, but this effect was not significantly different from that of the normal subjects on a similar diet (data not shown). This finding suggests that the increased ANG II–DBP sensitivity of these patients was not related to a lowered activity of the renin-angiotensin system, which may have been associated with an increased number (up-regulation) of ANG II vascular receptors. During intake of the low sodium diet, the two groups of hypertensive patients had similar recumbent PRAs, initial and postnifedipine blood pressures, and 24-hour urinary excretion of sodium and creatinine (see Table 1). Thus, differences in these parameters could not explain the differences in the DBP responses to ANG II in the two hypertensive groups. There was also a significant (r = −0.66, p < 0.01) linear correlation for the normal subjects between log (PRA) and initial ANG II–DBP sensitivity.

Effect of Nifedipine on the Actions of ANG II During a Low Sodium Diet in Patients with Essential Hypertension

Five of the nine patients with essential hypertension had a greater than normal ANG II–DBP sensitivity when compared with values in normal subjects having similar initial PRAs (Figure 5). In addition, the mean PRA for the five patients on a low sodium diet was not significantly different from that of the normal subjects on a similar diet (data not shown). This finding suggests that the increased ANG II–DBP sensitivity of these patients was not related to a lowered activity of the renin-angiotensin system, which may have been associated with an increased number (up-regulation) of ANG II vascular receptors. During intake of the low sodium diet, the two groups of hypertensive patients had similar recumbent PRAs, initial and postnifedipine blood pressures, and 24-hour urinary excretion of sodium and creatinine (see Table 1). Thus, differences in these parameters could not explain the differences in the DBP responses to ANG II in the two hypertensive groups. There was also a significant (r = −0.66, p < 0.01) linear correlation for the normal subjects between log (PRA) and initial ANG II–DBP sensitivity.

Figure 6 shows the ANG II–induced changes in DBP on a low sodium diet before and 1 hour after nifedipine administration, in the five hypertensive patients with increased DBP sensitivity to ANG II, four hypertensive patients with normal sensitivity, and 11 normal subjects. For those with increased DBP sensitivity to ANG II, nifedipine administration significantly (p < 0.05) reduced the rise in blood pressure at all three ANG II infusion rates, but this effect was not significantly different from that of the normal subjects on a similar diet (data not shown). This finding suggests that the increased ANG II–DBP sensitivity of these patients was not related to a lowered activity of the renin-angiotensin system, which may have been associated with an increased number (up-regulation) of ANG II vascular receptors. During intake of the low sodium diet, the two groups of hypertensive patients had similar recumbent PRAs, initial and postnifedipine blood pressures, and 24-hour urinary excretion of sodium and creatinine (see Table 1). Thus, differences in these parameters could not explain the differences in the DBP responses to ANG II in the two hypertensive groups. There was also a significant (r = −0.66, p < 0.01) linear correlation for the normal subjects between log (PRA) and initial ANG II–DBP sensitivity.
seen in either the hypertensive patients with normal sensitivity or the normal subjects. Nifedipine administration significantly (p < 0.05) reduced the sensitivity for those with increased DBP sensitivity to ANG II (from 3.8 ± 0.8 to 2.5 ± 7.6 mm Hg/ng/kg/min), but not for the hypertensive group with normal ANG II sensitivity (from 3.1 ± 0.8 to 2.1 ± 6 mm Hg/ng/kg/min) or for the normal subjects (from 1.5 ± 0.2 to 1.4 ± 0.2 mm Hg/ng/kg/min).

PRA measured in patients after stimulation by furosemide (40 mg i.v.) and standing for 2 hours was 2.0 ± 0.8 ng ANG I/ml/hr in the five patients with increased DBP sensitivity to ANG II, while that for four patients with normal ANG II sensitivity was 1.0 ± 0.3 ng ANG I/ml/hr (not significantly different). As shown in Table 1, there was no significant difference in the recumbent initial PRA between the normal subjects during a low sodium diet and the two groups of hypertensive patients. After nifedipine but before the second ANG II infusion on the high sodium diet, the PRA showed no significant differences between the normal subjects and the two groups of hypertensive patients.

Discussion

The acute cardiovascular responses to nifedipine have been studied previously. Nifedipine is a potent vasodilator that increases cardiac output and heart rate. After oral administration of nifedipine, the peak levels of plasma nifedipine in humans are achieved in 1 hour. In normal subjects eating an unrestricted diet, calcium channel blocking drugs lower systolic and diastolic blood pressure slightly but significantly (p < 0.05) and increase heart rate and forearm blood flow. The results presented in Figure 1 confirm the drug's hypotensive action. The slight increase in PRA may be secondary to inhibition of cell calcium entry since calcium can reduce renin secretion. Alternatively, the fall in blood pressure may have stimulated the increase in PRA.

In normal subjects, the ANG II-induced change in DBP and the sensitivity of the DBP to ANG II were significantly (p < 0.05) greater during the high sodium diet than during the low sodium diet, as previously reported. After nifedipine and diltiazem administration, DBP sensitivity to ANG II decreased during the
high but not during the low sodium diet. These results are consistent with those of Millar et al. Since the change in DBP during ANG II infusion was greater on a high than on a low sodium diet, it is possible that the greater blocking effects of nifedipine on the high sodium diet are simply related to the magnitude of the DBP change. This possibility seems unlikely, however, since, as shown in Figure 2, when ANG II was infused at higher rates in normal subjects on the low sodium intake, the DBP was increased to levels comparable with those induced by lower ANG II infusion rates during the high sodium diet, yet nifedipine did not have any effect on the ANG II–induced rise in DBP. There was no significant difference in the responses to the two calcium channel blocking drugs. Combining the results with the two calcium channel blocking drugs, there was a significant ($r = -0.78, p < 0.001$) inverse correlation between the baseline sensitivity to ANG II and the change in sensitivity of the DBP to ANG II induced by the calcium channel blocking drugs (Figure 7). Figure 7 shows the individual variations of initial sensitivity and indicates that there was some overlap of the results during the two diets. During the high sodium diet, calcium channel blocking drugs significantly ($p < 0.01$) reduced the sensitivity to ANG II. During the low sodium diet, calcium channel blocking drugs did not significantly ($p > 0.05$) reduce the initial sensitivity. These results suggest that 1) the observed effects are mediated by blockade of calcium movement, 2) the effect of sodium loading in increasing the sensitivity of DBP responses to ANG II is partially mediated by increased ANG II–induced extracellular to intracellular calcium movement, and 3) during the low sodium diet the DBP stimulation by ANG II may be more dependent on intracellular calcium movement than on movement of calcium from the extracellular fluid into the cells.

The results with norepinephrine infusion (see Figure 4) differed from those obtained with ANG II (see Figure 2). Nifedipine reduced the rise in MBP induced by norepinephrine during both a low and high sodium diet. The sensitivity of the MBP to norepinephrine infusion was slightly but not significantly higher during the high sodium diet. However, the nifedipine-induced reduction in MBP-norepinephrine sensitivity was significantly ($r = -0.81, n = 14, p < 0.001$) correlated with the initial sensitivity, as expressed by the following equation: change in sensitivity = -0.17 (initial sensitivity) + 0.7. This result is similar to that for ANG II–DBP sensitivity (see Figure 7) with nifedipine and suggests that subjects with increased MBP sensitivity to norepinephrine are more dependent on calcium movements, as blocked by nifedipine.

Five of nine patients with essential hypertension had a greater than normal rise in DBP during ANG II infusion while on a low sodium diet. This result is consistent with previous reports, especially in patients with low renin essential hypertension.

During intake of the low sodium diet the two groups of hypertensive patients and the normal subjects had similar recumbent PRA, and 24-hour urinary sodium excretion, before and after nifedipine or diltiazem administration (see Table 1). Thus, differences in these parameters could not explain the different ANG II–DBP responses in the two hypertensive groups. There was a significant ($p < 0.05$) correlation in all patients between the ANG II infusion rate and DBP. This linear response is similar to that reported previously. There was also a significant ($r = -0.7, p < 0.05$) correlation between the initial DBP sensitivity to ANG II in these patients and the changes in the DBP sensitivity to ANG II induced by nifedipine administration (Figure 8). This result should be considered tentative since the number of hypertensive subjects (nine) is too small to draw major conclusions. However, this relationship is very similar to that for the normal subjects during the high and low sodium diets (see Figure 7). The difference is that the hypertensive patients with increased DBP sensitivity to ANG II were eating a low sodium diet yet had significantly ($p < 0.05$) higher DBP sensitivity to ANG II than normal subjects eating a low sodium diet, yet as in normal subjects during a high sodium diet, nifedipine administration significantly ($p < 0.05$) lowered the DBP sensitivity to ANG II. There was no significant difference in the 24-hour urinary sodium excretion between the hypertensive subjects and those normal subjects on a low sodium diet (see Table 1). These results suggest that hypertensive patients who on the low sodium diet manifest an increased DBP response to ANG II have an abnormal ANG II–stimulated vascular calcium movement that is blocked by nifedipine administration.

ANG II raises blood pressure in humans primarily by increasing the peripheral vascular resistance. Calcium channel blockade may affect vascular ANG II receptor binding or affinity, but this would not explain why calcium channel blocking drugs were effective only on a high sodium diet. In addition, it has been reported that these drugs do not affect ANG II adrenal receptor binding. ANG II is a very potent vasoconstrictor, and it acts on the vascular plasma membrane receptors whose affinity is modulated by guanyl nucleotides. ANG II stimulates hydrolysis of smooth muscle phosphatidylinositol bisphosphate to form two intracellular messengers, 1,2-diacylglycerol and inositol trisphosphate. The latter transiently increases intracellular free calcium, while the former stimulates the protein kinase called C-kinase. Increased intracellular calcium stimulates the myosin light chain kinase triggering contraction of smooth muscle. The sustained slower phase of vascular smooth contraction caused by ANG II depends on extracellular calcium movements, and it is this phase that the calcium channel blocking drugs nifedipine and diltiazem would affect and that is apparently influenced by sodium intake.

Raising the external calcium concentration from 0 to 2.4 mM restores the blood pressure response to ANG II in vitro. Calcium infusion in the present studies raised ionized calcium from 1.23 ± 0.02 to 1.57 ± 0.02 mM (a 27% increase) during the high
sodium diet, and from $1.24 \pm 0.02\, \text{mM}$ to $1.50 \pm 0.02\, \text{mM}$ (a 21% increase) during the low sodium diet. These increases in serum calcium did not affect the blood pressure response to ANG II infusion (see above). In contrast to the results with calcium channel blockers, there was no different response during the two diets, suggesting that the calcium channel blockers affected the ANG II-induced calcium flux more than the increased serum calcium.

In summary, the results in normal subjects suggest that those calcium movements that are blocked by nifedipine and diltiazem play a significant role in the enhanced blood pressure response to ANG II during a high sodium diet. Since calcium channel blocking drugs act primarily by blocking extracellular to intracellular calcium movement, these results suggest that changes in sodium intake causing increased vascular sensitivity to ANG II are mediated in large part by changes in the movement of extracellular calcium into the cells. During a low sodium diet, those patients with essential hypertension and an increased response of blood pressure to infusion of ANG II had a significant ($p<0.05$) reduction in DBP sensitivity to ANG II after nifedipine administration, whereas those hypertensive patients with a normal DBP response to ANG II did not. These preliminary results in hypertensive patients suggest that the increased DBP response to ANG II may be caused by abnormal calcium movement from outside to inside the vascular cell.

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