Dietary Sodium and Inhibition of Neutral Endopeptidase 24.11 in Essential Hypertension

Donald R.J. Singer, Nirmala D. Markandu, Martin G. Buckley, Michelle A. Miller, Giuseppe A. Sagnella, and Graham A. MacGregor

Basal atrial natriuretic peptide levels and the response to exogenous atrial natriuretic peptide are influenced by dietary sodium intake. In view of interest in the therapeutic potential of elevating plasma atrial natriuretic peptide by inhibition of neutral endopeptidase 24.11, we studied the renal and hormonal effects of 200 mg of the oral endopeptidase 24.11 inhibitor candoxatril in eight patients with untreated essential hypertension on high sodium (350 mmol/day) and low sodium (10 mmol/day) diets. With endopeptidase 24.11 inhibition, plasma atrial natriuretic peptide increased more than twofold on low and high sodium diets (p<0.05). Plasma N-terminal pro-atrial natriuretic peptide increased on the high sodium intake but was unaffected by candoxatril. Urinary sodium excretion increased threefold on the low sodium and sixfold on the high sodium diet (p<0.05). The absolute increase in urinary sodium excretion during the 24 hours after treatment compared with placebo was 18±8 mmol on the low sodium and 98±34 mmol on the high sodium diet (p<0.05). Plasma renin activity was suppressed by treatment on the low but not on the high sodium diet (p<0.05). Blood pressure did not change in the 6 hours after a single dose of candoxatril. These findings show that sodium intake is a major determinant of the response to endopeptidase 24.11 inhibition. The lack of effect on N-terminal pro-atrial natriuretic peptide suggests that candoxatril does not influence cardiac secretion of atrial natriuretic peptide or catabolism of N-terminal pro-atrial natriuretic peptide, and the latter does not appear to play a role in the response to candoxatril. (Hypertension 1991;18:798–804)
insight into the mechanisms of the response to NEP inhibition, both ANF-(99-126) and N-terminal pro-ANP levels were studied.

Methods

Eight patients with established, uncomplicated essential hypertension were studied. All had been assessed in the Blood Pressure Unit for at least 2 months before the study and were well accustomed to having their blood pressure measured under standardized conditions. Average supine blood pressure at this time was 158/107±4/2 mm Hg (mean±SEM) on no treatment. There were two women and six men, seven white and one Asian, with an average age of 50.2±2.9 years (range, 38–62 years). The study was approved by the local ethical committee, and written informed consent was obtained from each subject.

In this placebo-controlled study, the effects were observed on both low and high sodium diets of a single 200-mg dose of treatment with the oral endopeptidase 24.11 inhibitor candoxatril (UK 79,300, Pfizer Central Research, Sandwich, UK). The study was conducted in a double-blind fashion and in random order, in regard to diet as well as administration of endopeptidase 24.11 inhibitor and placebo treatment. Under the supervision of the metabolic unit dietician, each subject was advised on how to take a 10 mmol sodium/day diet, which was continued throughout each of the two 7-day parts of the double-blind study. The high sodium diet (350 mmol sodium/day) was achieved by adding Slow Sodium (Ciba Laboratories) to the 10 mmol sodium/day diet. During the low sodium part of the study, subjects took equal numbers of matching placebo Slow Sodium tablets (Ciba Laboratories) in addition to their low sodium intake. On the sixth and seventh days of each dietary period, the subjects attended the Blood Pressure Unit as outpatients and received in random order 200 mg (two 100-mg capsules) of candoxatril or matching placebo. On each of the study days, subjects continued mild ambulatory activity except during the time that measurements were made. Blood pressure and heart rate were measured 1 hour before, immediately before, and 1, 2, 4, 6, and 24 hours after each treatment. Supine and standing blood pressures were measured in the same arm by semiautomatic ultrasound sphygmomanometers (Arteriosonde, Roche, Cranbury, N.J.) with attached recorders.12 Recordings were therefore observer independent. Each measurement was taken as the mean of five recordings at 1-minute intervals in each position. Mean arterial pressure was calculated by adding one third of the pulse pressure to the diastolic pressure.

Blood samples were obtained after 5 minutes' sitting rest immediately before and 1, 2, 4, 6, and 24 hours after each treatment for biochemical measurements, hematocrit, hemoglobin, immunoreactive plasma C-terminal ANP [ANF-(99-126)]9 and N-terminal pro-ANP13 by radioimmunoassay after Sep-pak extraction, renin activity,14 and aldosterone.15 Twenty-four-hour urine samples were obtained for measurement of volume, sodium, potassium, and creatinine on the fourth and fifth days of each dietary period and during the 24 hours after each single dose of endopeptidase 24.11 inhibitor or placebo. Urine samples were obtained before, every 2 hours for 6 hours after, and during the 6–24-hour period after active treatment and placebo for the above measurements.

Results are reported as mean±SEM. Statistical analysis was performed separately for each diet by two-way analysis of variance (ANOVA) for repeated measurements for the responses to endopeptidase 24.11 inhibition compared with placebo. In addition, two-way ANOVA was performed to compare the effects of the two diets on the placebo days. In the presence of unequal group variance, the data were logarithmically transformed before performing ANOVA. Where appropriate, the level of significance of differences between paired means was calculated from paired tests with the pooled error of the variance obtained from the ANOVA. Where results were not normally distributed, matched pairs of data were analyzed by the Wilcoxon signed-rank test. Results were analyzed with Northwestern University's Statistical Package for Social Sciences at the University of London computer center.

Results

Plasma Levels of Atrial Natriuretic Factor-(99–126)

Before administration of candoxatril, plasma ANP on the low sodium diet was 15.0±4.5 pg/ml and was significantly increased on the high sodium diet (31.7±9.7 pg/ml; p < 0.01). With endopeptidase 24.11 inhibition, plasma ANP increased on both diets within 1 hour and remained elevated for up to 6 hours, with a greater than twofold increase compared with placebo (Figures 1 and 2).

Plasma N-Terminal Pro-Atrial Natriuretic Peptide

Plasma N-terminal pro-ANP was significantly increased on the high sodium diet (before placebo, 422±97 pg/ml) compared with the low-sodium diet (before placebo, 212±50 pg/ml; diet effect: F=9.46, p<0.02). Endopeptidase 24.11 inhibition caused no change in the plasma levels of N-terminal pro-ANP compared with placebo on either diet (Figures 1 and 2).

Urinary Sodium, Potassium, and Water Excretion

Twenty-four-hour urinary sodium excretion before treatment on the low and high sodium diets was 22.6±5.0 mmol and 328.9±10.9 mmol, respectively. After endopeptidase 24.11 inhibition, urinary sodium excretion increased within 2 hours of the administration of candoxatril and remained elevated for at least 6 hours after treatment compared with placebo. However, there were large differences in the magnitude of
the increases in sodium excretion with endopeptidase inhibition on the high compared with the low sodium diet. On the low sodium diet, there was a maximal threefold increase in sodium excretion compared with placebo, and on the high sodium diet, there was a sixfold increase compared with placebo (Figures 1 and 2). On the low sodium diet, the cumulative difference in 24-hour urinary sodium excretion with active treatment compared with placebo was 17.7±7.9 mmol and was 98.1±34.1 mmol on the high sodium diet (p<0.05; Figure 3). The increase in urine flow with candoxatril compared with placebo on the low sodium diet was not significant by ANOVA (Figure 1). However, cu-
Cumulative urinary sodium excretion:

- Low sodium: Basal 6-24, Time (hours)
  - 0-2
  - 2-4
  - 4-6
  - 6-24

- High sodium:
  - Basal 6-24
  - Time (hours)

**FIGURE 3.** Bar graph showing cumulative increase in urinary sodium excretion with candoxatril compared with placebo on low and high sodium intake (*p<0.05 by Wilcoxon test for matched pairs on low and high sodium diet).

Cumulative 6-hour water excretion on the low sodium diet with candoxatril (1,018±144 ml) was greater than with placebo (698±113 ml; two-tailed Wilcoxon test, p=0.05). There was a significant effect on urine flow of candoxatril compared with placebo on the high sodium diet (ANOVA: F=5.44, p=0.05; Figure 2). Cumulative 6-hour water excretion on the high sodium diet was 1,064±270 ml with candoxatril and 541 ±167 ml with placebo (two-tailed Wilcoxon test, p=0.069). There was no significant difference in 24-hour cumulative water excretion after treatment on either the low or the high sodium diet. Urinary potassium excretion was not changed by endopeptidase 24.11 inhibition.

**Plasma Renin Activity and Aldosterone**

Endopeptidase 24.11 inhibition caused a significant fall in plasma renin activity on the low but not on the high sodium diet. There was a small but significant increase in plasma renin activity at 24 hours after endopeptidase 24.11 inhibition on the high sodium diet (Figure 4). Plasma aldosterone showed no significant change with endopeptidase inhibition compared with placebo except at 24 hours on the high sodium diet, when there was a significant increase compared with placebo (Figure 4).

**Blood Pressure and Heart Rate**

Average supine blood pressure on the low sodium diet before active treatment was 146/94±7/4 mm Hg and 156/101±6/3 mm Hg on the high sodium diet (p<0.05). There were no significant changes in supine or standing mean blood pressure after administration of candoxatril compared with placebo. There were no significant changes in heart rate (Table 1).

**Other Measurements (Table 1)**

There was no significant change with endopeptidase 24.11 inhibitor compared with placebo in plasma bicarbonate, chloride, calcium, phosphate, urate, bilirubin, liver or cardiac enzymes, hemoglobin, white blood cell count, or platelet count. Other variables that were measured are shown in Table 1.

**Discussion**

The administration of candoxatril, an inhibitor of endopeptidase 24.11, in patients with essential hypertension caused a twofold to threefold increase in circulating plasma levels of ANP on both a low and a high sodium intake. The increase in plasma ANP was associated with a marked increase in sodium excretion when subjects were on the low or on the high sodium intake. The twofold to threefold increase in plasma ANP with endopeptidase 24.11 inhibition in our study is an order of increase in plasma ANP similar to that reported in studies of sodium loading in normal subjects9,16,17 and in patients with essential hypertension. In the present study of patients with essential hypertension, the hormonal and renal responses to candoxatril were qualitatively similar to those previously reported by Richards et al18 in normal subjects with the same NEP inhibitor at lower doses on a sodium intake of 150 mmol/day.

In studies of normal subjects5,19 and in patients with essential hypertension,20,21 significant natriuresis occurs when this order of increase in plasma ANP is induced by infusion of low-dose exogenous ANP. Plasma ANP increased with endopeptidase 24.11 inhibition on both the low and the high sodium diet to levels within the same range as those previously reported in patients with untreated essential hypertension.2 There are no inhibitors of the actions of
ANP safe for use in humans. However, animal studies have shown that treatment with a monoclonal antibody directed against ANP blunts the natriuretic response to a sodium load.\textsuperscript{22} Our findings therefore clearly suggest that plasma ANP is playing a role in the control of sodium excretion in patients with essential hypertension, with more important effects on sodium excretion in absolute terms on a high than on a low sodium diet. When plasma ANP is increased, as it is in many patients with untreated essential hypertension, it is thus possible that this elevation in plasma ANP could contribute to a compensatory response to a defect in the ability of the kidney to excrete sodium.

The increase in sodium excretion with endopeptidase 24.11 inhibition on the high sodium diet was greater, both as a percentage and in absolute amounts, compared with that on the low sodium diet, although the relative increase in plasma ANP was the same for each diet. Thus, the increase in sodium excretion with endopeptidase inhibition is clearly dependent on sodium intake. There are three major mechanisms that may account for these differences in sodium excretion with endopeptidase inhibition on the high compared with low sodium diets. In studies in which ANP has been infused into subjects on different sodium intakes, with the same dose of infused ANP, greater natriuresis occurs on a high compared with a low sodium intake.\textsuperscript{6,23,24} Thus, one mechanism underlying the greater response on the high sodium diet is greater sensitivity to the same amount of ANP. Therefore, there are two major possible reasons for this altered sensitivity. The first and indirect mechanism is that ANP-receptor coupling in the renal-angiotensin-aldosterone system on the high sodium diet was the higher plasma ANP levels achieved.

A further important issue is the specificity of the effects of candesartan. The prodrug candesartan is metabolized to UK 73,967, a selective inhibitor of endopeptidase 24.11, and increasing evidence suggests that the rise in plasma ANP that occurs is due to inhibition of this enzyme.\textsuperscript{7} NEP inhibitors have effects on other biologically active peptides,\textsuperscript{29} including bradykinin and brain natriuretic peptide. However, evidence from this study and from previous reports suggests that the effects on ANP are of major importance in the response to the NEP inhibitor candesartan. First, treatment with antibodies to ANP blunts the renal response to ANP levels achieved.\textsuperscript{26,27} Conversely, in the present study there was the expected suppression of the renin-angiotensin-aldosterone system on the high sodium diet, which would permit a greater natriuresis to the same increase in ANP levels. A second possible mechanism is the more efficient ANP-receptor coupling on the high sodium diet. It is clear that the increase in plasma ANP levels after endopeptidase 24.11 inhibition is associated with increased urinary cyclic guanosine monophosphate levels in normal subjects.\textsuperscript{18} Further work is required to explore the possible role of changes in ANP-receptor coupling in the influence of sodium intake on the response to NEP inhibition.

A third important mechanism is that plasma ANP levels were higher on the high sodium diet. It is clear that there is a dose response for the natriuretic effects of ANP.\textsuperscript{28} In the present study and as previously reported,\textsuperscript{2,5,10} basal plasma ANP levels were higher on the high compared with the low sodium diet; in addition, as expected from the higher basal ANP levels, there was a greater increase in plasma ANP levels with NEP inhibition on the high than on the low sodium diet. Thus, one further mechanism for the greater natriuresis after NEP inhibition on the high sodium diet was the higher plasma ANP levels achieved.

### Table 1. Results of Biochemical and Hematological Measurements Before and After Treatment With Atriopeptidase Inhibitor UK 79,300 on Low and High Sodium Diets

<table>
<thead>
<tr>
<th>Measurements</th>
<th>Placebo Before +6 hr</th>
<th>Placebo Before +6 hr</th>
<th>Low sodium diet Before +6 hr</th>
<th>Low sodium diet Before +24 hr</th>
<th>UK 79,300 Before +6 hr</th>
<th>UK 79,300 Before +24 hr</th>
<th>UK 79,300 Before +6 hr</th>
<th>UK 79,300 Before +24 hr</th>
</tr>
</thead>
<tbody>
<tr>
<td>Supine mean arterial pressure (mm Hg)</td>
<td>108±3</td>
<td>110±3</td>
<td>104±4</td>
<td>111±4</td>
<td>107±4</td>
<td>117±4</td>
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<tr>
<td>Weight (kg)</td>
<td>80.7±6.4</td>
<td>80.9±6.3</td>
<td>80.6±6.3</td>
<td>80.8±6.4</td>
<td>80.6±6.4</td>
<td>80.4±6.4</td>
<td>81.6±6.3</td>
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<tr>
<td>Sodium (mmol/l)</td>
<td>137±1</td>
<td>136±1</td>
<td>137±1</td>
<td>138±1</td>
<td>136±1</td>
<td>137±1</td>
<td>139±1</td>
<td>140±1</td>
</tr>
<tr>
<td>Potassium (mmol/l)</td>
<td>3.7±0.2</td>
<td>4.1±0.2</td>
<td>3.9±0.2</td>
<td>3.7±0.2</td>
<td>3.8±0.2</td>
<td>3.8±0.2</td>
<td>3.7±0.1</td>
<td>3.8±0.1</td>
</tr>
<tr>
<td>Urea (mmol/l)*</td>
<td>5.4±0.4</td>
<td>5.2±0.6</td>
<td>5.2±0.4</td>
<td>5.1±0.4</td>
<td>4.7±0.5</td>
<td>4.8±0.5</td>
<td>4.5±0.6</td>
<td>4.6±0.5</td>
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<tr>
<td>Albumin (g/l)</td>
<td>44±1</td>
<td>45±1</td>
<td>44±1</td>
<td>44±1</td>
<td>45±1</td>
<td>45±1</td>
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<tr>
<td>Hematocrit (%)</td>
<td>39.4±1.2</td>
<td>39.4±1.8</td>
<td>38.1±1.3</td>
<td>39.9±1.2</td>
<td>39.7±1.2</td>
<td>37.9±1.2</td>
<td>39.1±1.5</td>
<td>36.9±1.4</td>
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<tr>
<td>Creatinine clearance (ml/min)</td>
<td>129±16</td>
<td>130±20</td>
<td>105±14</td>
<td>126±15</td>
<td>128±13</td>
<td>127±14</td>
<td>129±14</td>
<td>115±11</td>
</tr>
</tbody>
</table>

*Urea on low sodium diet was significantly lower after atriopeptidase inhibitor compared with placebo; F=10.6, p<0.05.
with the plasma levels of ANP achieved in the present study was comparable to the increases in urinary sodium excretion observed in low-dose ANP infusion studies, in which equivalent increases in ANP levels were achieved. Of note is that the biologic response to an increase in plasma ANP levels after NEP inhibition is likely to be greater than after the same increase in plasma ANP after ANP infusion. This is because tissue levels of ANP at sites of ANP–guanylate cyclase receptor binding and NEP activity will be greater for the same plasma ANP level achieved after NEP inhibition compared with that after ANP infusion. Third, in the study by Richards et al 18 of candoxatril in normal subjects, NEP inhibition was associated with an increase in both plasma and urinary ANP levels, as well as in urinary levels of the second messenger for ANP, cyclic guanosine monophosphate. Taken together, these findings suggest that the responses to the NEP inhibitor candoxatril are largely mediated via changes in plasma ANP metabolism.

One other possible mechanism whereby candoxatril could raise plasma ANP is by increasing the release of ANP from the heart. N-terminal pro-ANP is cosecreted with ANF-(99–126). In our study, levels of N-terminal pro-ANP as well as ANF-(99–126) were significantly greater, with similar proportional increases in the peptides on the high and on the low sodium diet; this provides evidence that the mechanism for the rise in plasma levels of the peptides with an increase in sodium intake is cosecretion from the heart. However, there was no significant change in the plasma levels of N-terminal pro-ANP with endopeptidase 24.11 inhibition on either diet. These findings indicate, first, that the metabolic pathways for N-terminal pro-ANP are different from those of the C-terminal ANF-(99–126) in that they are not affected by endopeptidase inhibition. Furthermore, the increase in plasma ANP with no change in N-terminal pro-ANP with endopeptidase inhibition strongly suggests that candoxatril is not increasing plasma ANP by stimulation of cardiac secretion of ANP; if this had occurred, an increase in N-terminal pro-ANP levels would also have been expected. Studies of animals suggest that fragments of the N-terminal pro-ANP may be biologically active. However there was no evidence in the present study for a role for changes in plasma levels of N-terminal pro-ANP in the response to candoxatril.

Both ANP infusion 3 and endopeptidase 24.11 inhibition 4 cause a decrease in renin release in normal volunteers. Our study clearly shows that in hypertensive patients, reduction in renin release, as judged by plasma renin activity, occurred on the low sodium diet when plasma renin activity was raised. On the high sodium diet after endopeptidase 24.11 inhibition, despite the associated loss of sodium, there was no change in plasma renin activity or aldosterone except at 24 hours after treatment, when there were small but significant increases. These findings suggest that the increase in ANP, while not suppressing renin release on the high sodium diet, did prevent an increase in plasma renin activity. These renal and hormonal results clearly illustrate the importance of controlling sodium intake in studies of endopeptidase 24.11 inhibition.

Interestingly, in contrast to the large differences in natriuresis with changes in sodium intake, the increase in water excretion with endopeptidase 24.11 inhibition appeared to be similar on both the low and high sodium intakes. This suggests that the mechanisms whereby alterations in sodium intake influence sodium excretion do not have a major effect on the control of water excretion.

There is considerable interest in the possible therapeutic potential of inhibitors of endopeptidase 24.11 in hypertension in view of the natriuretic, diuretic, and hormonal response to an increase in plasma ANP levels. In our study, candoxatril given as a single dose did not cause any fall in blood pressure either on a low or a high sodium diet despite the increase in sodium excretion and the lack of rise in plasma renin activity. This finding is not unexpected if one considers the analogy with thiazide diuretics, which do not cause a fall in blood pressure when given as a single dose but cause a gradual fall in blood pressure with long-term treatment. However, with thiazide diuretics, stimulation of the renin system in the longer term blunts the fall in blood pressure that occurs with the sodium and water loss. Thus, if the endopeptidase 24.11 inhibitor candoxatril causes a sustained loss of sodium from the body with continuing treatment without a compensatory increase in plasma renin activity, it is likely to be effective in lowering blood pressure. Clearly, carefully controlled, longer-term studies are needed, but our findings with a single dose of candoxatril do support the increasing evidence for a role for ANPs in the control of sodium excretion both in normal subjects and in patients with essential hypertension.

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References

1. de Wardener HE, MacGregor GA: Dahl's hypothesis that a saluretic substance may be responsible for a sustained rise in arterial pressure: Its possible role in essential hypertension. Kidney Int 1980;18:1–9

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