Blood Pressure Responses to Small Doses of Amiloride and Spironolactone in Normotensive Subjects

J. Howard Pratt, George J. Eckert, Shirley Newman, Walter T. Ambrosius

Abstract—The epithelial sodium channel (ENaC) is a principal site for sodium reabsorption and as such may participate importantly in blood pressure (BP) regulation. Amiloride, a direct inhibitor of ENaC, characteristically has mild antihypertensive properties, consistent with ENaC having more minor influences on BP regulation. Counter-regulatory influences may, however, prevent amiloride from effectively lowering BP. Aldosterone secretion is known to increase in response to the reduced sodium reabsorption that follows amiloride inhibition of ENaC, and because aldosterone upregulates ENaC function, we considered the possibility that secondary hyperaldosteronism mitigates the ability of amiloride to reduce BP. In the present study, the BP responses to amiloride (5 mg per day), spironolactone (25 mg per day), the combination of the 2 drugs, and placebo were studied in healthy normotensive subjects. Over 4 weeks of treatment, the combination of amiloride and spironolactone lowered systolic BP by 4.6±1.6 (mean±SEM) mm Hg (P=0.022) and diastolic BP by 2.2±1.2 mm Hg (P=0.30), whereas either drug alone had no significant effect on BP. The findings suggest that the 2 drugs with different modes of action—amiloride, a direct inhibitor of ENaC, and spironolactone, a mineralocorticoid receptor antagonist—may compliment each other’s ability to inhibit ENaC and thereby reduce sodium reabsorption to a point at which BP decreases. On the other hand, we cannot rule out that the BP response resulted from the greater dose of total drug. The lowering of BP with small doses of inhibitors of ENaC serves as additional evidence for the importance of ENaC to the tonic maintenance of BP. (Hypertension. 2001;38:1124-1129.)

Key Words: blood pressure ■ sodium channel ■ amiloride ■ spironolactone

The epithelial sodium channel (ENaC) in the collecting duct of the kidney is a highly regulated site for sodium reabsorption.1 Mineralocorticoids, primarily aldosterone, increase channel number,2 and most if not all mineralocorticoid-induced hypertension may be mediated by a net increase in ENaC function.3 Mutations in ENaC can result in either extreme of blood pressure such as the severe hypertension of Liddle’s syndrome4–6 or the life-threatening decrease in blood pressure (BP) that occurs in the neonatal period known as pseudohypoaldosteronism type 1.7 More minor but common molecular variations in ENaC may also affect risk for hypertension.8,9 If, indeed, ENaC is important for maintaining BP or contributing to the high BP of some individuals, then giving amiloride, a direct inhibitor of ENaC, should effectively lower BP, but in general, it is weakly antihypertensive10–13 and used primarily in combination with a thiazide diuretic for its potassium-sparing actions.11

In the patients described by Liddle et al,4 triamterene, also an inhibitor of ENaC, lowered BP but only after sodium intake was severely restricted. Sodium has been shown to interfere with the ability of amiloride to inhibit sodium conductance in vitro,14 and thus amiloride may not lower BP because modern diets are high in sodium. An alternative theory is that the secondary increase in aldosterone secretion that occurs in response to amiloride abrogates the ability of amiloride to prevent sodium reabsorption. In the present study, we examined the effect on BP of combining amiloride and spironolactone, a mineralocorticoid antagonist. Using a 2×2 factorial study design, the BP responses to amiloride alone, spironolactone alone, the combination of amiloride and spironolactone, and placebo were compared in normotensive subjects.

Methods

Subjects

The study used 20 whites and 20 blacks, age 18 to 30, from a cohort followed as part of a study of BP regulation.15 Subjects were healthy, and none took medication that could affect BP or the renin-aldosterone system, with the exception that 11 of the 22 women were taking an estrogen contraceptive. Subjects were screened for renal impairment and an elevation in serum potassium. A pregnancy test was performed in the women before they started the study and was repeated weekly for the remainder of the study period. A screening BP <110/65 mm Hg precluded participation to avoid the potential for hypotension during treatment.

Protocol

Each participant gave informed consent, which was previously approved by the Indiana University/Purdue University Committee

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TABLE 1. Characteristics of Subjects at Baseline

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Black Females (n=12)</th>
<th>Black Males (n=8)</th>
<th>White Females (n=10)</th>
<th>White Males (n=10)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, y</td>
<td>22.8±2.5</td>
<td>23.0±4.1</td>
<td>25.4±3.1</td>
<td>24.9±4.0</td>
</tr>
<tr>
<td>BMI, kg/m²</td>
<td>26.3±4.6</td>
<td>24.0±2.3</td>
<td>23.9±3.4</td>
<td>25.3±1.9</td>
</tr>
<tr>
<td>Systolic BP, mm Hg</td>
<td>112.6±6.6</td>
<td>123.4±9.0</td>
<td>113.2±6.6</td>
<td>116.7±9.3</td>
</tr>
<tr>
<td>Diastolic BP, mm Hg</td>
<td>74.3±7.6</td>
<td>76.8±6.7</td>
<td>70.1±3.7</td>
<td>71.6±11.3</td>
</tr>
<tr>
<td>PRA, ng · L⁻¹ · s⁻¹</td>
<td>0.2±0.1</td>
<td>0.2±0.2</td>
<td>0.4±0.2</td>
<td>0.3±0.2</td>
</tr>
<tr>
<td>Plasma aldosterone, pmol/L</td>
<td>199±111</td>
<td>182±81</td>
<td>415±224</td>
<td>370±178</td>
</tr>
<tr>
<td>Urinary sodium, mmol/mmol creatinine</td>
<td>10.6±6.1</td>
<td>12.1±4.8</td>
<td>9.9±7.9</td>
<td>8.1±5.9</td>
</tr>
<tr>
<td>Urinary potassium, mmol/mmol creatinine</td>
<td>1.9±1.0</td>
<td>1.9±0.8</td>
<td>2.2±1.2</td>
<td>2.1±1.1</td>
</tr>
<tr>
<td>Urinary aldosterone, nmol/mmol</td>
<td>1.0±0.5</td>
<td>0.8±0.3</td>
<td>1.6±1.1</td>
<td>1.3±0.6</td>
</tr>
<tr>
<td>Urinary aldosterone/potassium ratio, nmol/mmol</td>
<td>0.6±0.3</td>
<td>0.5±0.2</td>
<td>0.9±0.7</td>
<td>0.6±0.2</td>
</tr>
</tbody>
</table>

Values are mean±SD.

for the Protection of Human Subjects. The protocol consisted of giving every subject placebo for 1 week, followed by treatment with either amiloride (5 mg), spironolactone (25 mg), the combination of amiloride (5 mg) and spironolactone (25 mg), or placebo daily for 4 weeks. All subjects received 2 capsules that were identical in appearance. Patients were randomized, by a random-number generator, to receive one of the 4 treatments. Study personnel and subjects were kept blind to treatment allocation. BP was measured, and blood and overnight urine samples were collected at baseline and then every week for the duration of the study.

BP was measured in the right arm by a standard Hg sphygmomanometer after the subject had been in a sitting position for 5 minutes. Three readings were made, and the average was used in the analysis. Heart rate and weight were also measured.

Assay Procedures

Electrolytes were measured by a flame photometer (Instrumentation Laboratories), and urinary creatinine was measured by a Beckman-2 creatinine analyzer. Aldosterone was measured directly by radioimmunoassay (RIA) by a kit from Diagnostic Products Corp. Urine samples were first hydrolyzed overnight at pH 1, releasing free aldosterone from the 18-glucuronide, which was then assayed. The urinary aldosterone/potassium ratio was calculated as an index of ENaC activity, with lower ratios that were consistent with greater urinary aldosterone/potassium ratio, nmol/mmol

Results

Characteristics of Subjects

Table 1 depicts characteristics for each race and sex group at baseline. The proportions of blacks and whites, and females and males, were not different between the groups. The BMI ranged from 18.6 to 34.9 kg/m². Systolic BP (after 1 week of placebo) was 115.9±8.6 (SD) mm Hg, ranging from 101.3 to 136.0 mm Hg, and the diastolic BP after 1 week of placebo was 73.1±8.0 mm Hg, ranging from 48.7 to 90.0 mm Hg. The blacks were significantly younger (P=0.047) and had a significantly lower level of PRA (P=0.049) and plasma aldosterone (P<0.001) and a lower aldosterone excretion rate (P=0.025) than did the whites. The males had a significantly higher baseline systolic BP than did the females (P=0.007). With respect to the characteristics of the individuals assigned to each of the treatment groups, there were no significant differences in age, BP, BMI, level of PRA and plasma aldosterone, urinary excretion rate of electrolytes and aldosterone, or urinary aldosterone/potassium ratio (Table 2).

Responses to Treatment

We began our analysis by examining for main effects of amiloride and spironolactone on the response variables. Levels of PRA were significantly higher in the groups not taking amiloride (P=0.0063), whereas there was no main effect of adding spironolactone (P=0.22). Both amiloride (P=0.0001) and spironolactone (P=0.027) had significant main effects on the plasma aldosterone level. Increases in levels of plasma aldosterone were greater than those of PRA, possibly because of stimulation of aldosterone secretion by both angiotensin II and potassium. Amiloride had a marginally significant main effect on systolic BP (P=0.09) but not on diastolic BP (P=0.14). Spironolactone had no main effect on either systolic (P=0.69) or diastolic BP (P=0.89). Amiloride (P=0.0001) and spironolactone (P=0.0011) had significant main effects on urinary aldosterone excretion, whereas amiloride (P=0.0010) but not spironolactone (P=0.21) had a
significant main effect on the urinary aldosterone/potassium ratio. Amiloride had no effect on excretion rates of sodium (P = 0.82) or potassium (P = 0.91), nor did spironolactone (P = 0.39 for urinary sodium and P = 0.44 for urinary potassium).

The results after each week of treatment are presented in Table 3. Comparisons were made with the observations at baseline after 1 week of treatment with placebo. The changes from baseline were adjusted for baseline values, age, gender, BMI, and race, as well as for multiple comparisons. For the overall period of treatment (the mean of 4 weekly values), the serum potassium increased significantly from baseline by 0.20 ± 0.07 (SEM) mmol/L in the combination group (P = 0.0112) but not in the other treatment groups. Sodium did not change significantly in response to treatment in any of the treatment groups. Excretion rates of sodium and potassium did not change significantly in any of the treatment groups. In the combination-treatment group, the maximal changes in BP occurred in the first 2 weeks, usually the first week. None of the subjects were aware of a diuretic effect of the treatment, and no adverse effects were observed; there were no dropouts.

The average change in PRA over the 4 weeks was 0.03 ± 0.07 ng · L⁻¹ · s⁻¹ (P = 0.98) for placebo, 0.20 ± 0.07 ng · L⁻¹ · s⁻¹ (P = 0.011) for amiloride, 0.10 ± 0.07 ng · L⁻¹ · s⁻¹ (P = 0.44) for spironolactone, and 0.31 ± 0.06 ng · L⁻¹ · s⁻¹ (P = 0.0002) for the combination of amiloride and spironolactone. The average change in the plasma aldosterone concentration was −112 ± 129 pmol/L (P = 0.86) for placebo, 427 ± 111 pmol/L (P = 0.0008) for amiloride, 203 ± 114 pmol/L (P = 0.27) for spironolactone, and 633 ± 97 pmol/L (P = 0.0002) for the combination of amiloride and spironolactone.

The average change in urinary aldosterone excretion over the 4 weeks was −1.8 ± 1.0 mcg/mg creatinine (P = 0.27) with placebo, 3.9 ± 0.9 mcg/mg creatinine (P = 0.001) with amiloride, 1.9 ± 0.9 mcg/mg creatinine (P = 0.13) with spironolactone, and 6.2 ± 0.8 mcg/mg creatinine (P = 0.001) with the combination of amiloride and spironolactone. The average change in the urinary aldosterone/potassium ratio over the 4 weeks was −0.32 ± 0.32 (P = 0.78) with placebo, 0.96 ± 0.29 (P = 0.006) with amiloride, 0.30 ± 0.30 (P = 0.79) with spironolactone, and 1.10 ± 0.25 (P = 0.001) with the combination of amiloride and spironolactone.

A decline in BP was observed only in subjects who received the combination of amiloride and spironolactone (Table 3 and Figure 1), with a mean decline in systolic BP of 4.6 ± 1.6 mm Hg (P = 0.022) and a decrease in diastolic BP of 2.2 ± 1.2 mm Hg, which was not significant (P = 0.30). (Without adjusting for multiple comparisons, P = 0.005 and P = 0.084 for systolic and for diastolic BP, respectively). No significant change in body weight was observed in any of the treatment groups. Excretion rates of sodium and potassium did not change significantly in any of the treatment groups. In the combination-treatment group, the maximal changes in BP and levels of serum potassium, PRA, and plasma aldosterone occurred in the first 2 weeks, usually the first week. None of the subjects were aware of a diuretic effect of the treatment, and no adverse effects were observed; there were no dropouts.

### Table 2. Characteristics of Subjects Assigned to Each of the Treatment Groups

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Placebo Treated (n = 9)</th>
<th>Spironolactone Treated (n = 10)</th>
<th>Amiloride Treated (n = 9)</th>
<th>Spironolactone + Amiloride Treated (n = 12)</th>
<th>All Subjects (n = 40)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Black, n (%)</td>
<td>2 (22)</td>
<td>8 (80)</td>
<td>5 (56)</td>
<td>5 (42)</td>
<td>20 (50)</td>
</tr>
<tr>
<td>Female, n (%)</td>
<td>8 (89)</td>
<td>6 (60)</td>
<td>4 (44)</td>
<td>4 (33)</td>
<td>22 (55)</td>
</tr>
<tr>
<td>Age, y</td>
<td>25.2 ± 3.1</td>
<td>23.3 ± 3.7</td>
<td>24.1 ± 4.2</td>
<td>23.7 ± 3.2</td>
<td>24.0 ± 3.5</td>
</tr>
<tr>
<td>BMI, kg/m²</td>
<td>24.7 ± 3.1</td>
<td>26.9 ± 3.7</td>
<td>23.9 ± 3.4</td>
<td>24.5 ± 3.1</td>
<td>25.0 ± 3.4</td>
</tr>
<tr>
<td>Systolic BP, mm Hg</td>
<td>113.4 ± 6.3</td>
<td>118.9 ± 11.3</td>
<td>116.4 ± 9.4</td>
<td>114.9 ± 7.0</td>
<td>115.9 ± 8.6</td>
</tr>
<tr>
<td>Diastolic BP, mm Hg</td>
<td>71.2 ± 4.0</td>
<td>73.7 ± 7.2</td>
<td>76.4 ± 9.5</td>
<td>71.5 ± 9.5</td>
<td>73.1 ± 8.0</td>
</tr>
<tr>
<td>PRA, ng · L⁻¹ · s⁻¹</td>
<td>0.26 ± 0.13</td>
<td>0.29 ± 0.19</td>
<td>0.22 ± 0.20</td>
<td>0.34 ± 0.20</td>
<td>0.28 ± 0.19</td>
</tr>
<tr>
<td>Plasma aldosterone, pmol/L</td>
<td>285 ± 146</td>
<td>261 ± 207</td>
<td>288 ± 237</td>
<td>328 ± 165</td>
<td>292 ± 185</td>
</tr>
<tr>
<td>Urinary sodium, mmol/mmol creatinine</td>
<td>8.2 ± 5.5</td>
<td>9.3 ± 5.6</td>
<td>12.1 ± 6.2</td>
<td>10.6 ± 7.5</td>
<td>10.1 ± 6.3</td>
</tr>
<tr>
<td>Urinary potassium, mmol/mmol creatinine</td>
<td>1.8 ± 0.5</td>
<td>1.6 ± 0.9</td>
<td>2.6 ± 1.5</td>
<td>2.0 ± 0.8</td>
<td>2.0 ± 1.0</td>
</tr>
<tr>
<td>Urinary aldosterone, nmol/mmol creatinine</td>
<td>1.5 ± 1.2</td>
<td>1.1 ± 0.5</td>
<td>1.0 ± 0.6</td>
<td>1.1 ± 0.6</td>
<td>1.2 ± 0.7</td>
</tr>
<tr>
<td>Urinary aldosterone/potassium ratio, nmol/mmol</td>
<td>0.8 ± 0.7</td>
<td>0.8 ± 0.4</td>
<td>0.4 ± 0.2</td>
<td>0.6 ± 0.3</td>
<td>0.7 ± 0.4</td>
</tr>
</tbody>
</table>

Values are mean ± SD.

Discussion

The present study came about as a result of the paradox that specific inhibitors of ENaC, such as amiloride, have minor effects on BP, even though genetically derived modifications in ENaC function can result in severely disabled BP homeostasis (eg, Liddle’s syndrome). We hypothesized that the secondary hyperaldosteronism that follows amiloride treatment reduces its usefulness as a drug for lowering BP. To test this, we added spironolactone, a mineralocorticoid receptor antagonist that would at least partially block the response of an increase in aldosterone to upregulate ENaC function. Although there was no BP response to either drug when used alone, the combination did indeed result in a significant lowering of the systolic BP. The BP response could, of course, have also resulted from an effect of amiloride to
partially inhibit the hyperaldosteronism that accompanies spironolactone therapy. Also, whether we observed a dose effect (a response to twice as much drug) or a synergism of the 2 drugs could not be determined within the limits of the \(2 \times 2\) factorial study design. Thus, we can only speculate that the combination of drugs was complimentary, resulting in an overall greater inhibition of ENaC function than that which occurs with either drug used alone.
A rather remarkable aspect of the present study was the small daily doses of drug that lowered BP: 5 mg amiloride with 25 mg spironolactone. These doses are widely used for their potassium-sparing effect when combined with a more traditional diuretic, but larger doses have typically been used to treat hypertension. For example, amiloride lowered the BP in severely hypertensive blacks, but at a dose of 20 mg per day.\(^{10}\) Spironolactone was shown to lower BP in low-renin hypertensive patients,\(^{18–21}\) but at much higher doses (100 to 400 mg/d). Our subjects were normotensive and would be expected to be much less sensitive to BP-lowering interventions.\(^{22}\) Thus, despite the small doses of drug and the normal baseline BP, a reduction in BP was observed with the combination of amiloride and spironolactone.

The greatest decline in BP and the greatest increase in renin and aldosterone secretion and in serum potassium concentration occurred mostly in the first week. There were various degrees of neutralization of these responses in subsequent weeks, as if there was a delayed counter-regulatory influence coming into play. Other sodium reabsorbing sites may have become more active, such as the thiazide-sensitive channel, which has also been shown to be responsive to aldosterone,\(^{23}\) or the proximal tubule, in which an increase in angiotensin II promotes sodium uptake.\(^{24,25}\)

Previously, by the use of much larger population groups, we found evidence for greater ENaC activity in blacks compared with whites.\(^{8}\) The present study was not statistically powered to detect such relationships to race, and thus, assignment to treatment groups was made randomly. It was also not our intent to address the issue of treatment of clinical hypertension, although it would seem reasonable to consider the combination of a direct inhibitor with a mineralocorticoid receptor antagonist for the treatment of selected hypertensive patients. For example, in patients with primary aldosteronism, the combination of amiloride with spironolactone might allow the use of less spironolactone, thereby avoiding undesirable side effects such as gynecomastia and breast pain in men. Alternatively, amiloride increases aldosterone levels, which may be deleterious to the kidney and heart based on studies performed in animals.\(^{26–29}\) These extrarenal responses, which are mediated by the mineralocorticoid receptor, would be less likely to occur with the addition of spironolactone, offering still another advantage to the combination of drugs. Another potential “second drug” could be an inhibitor of the action of angiotensin II because it, too, would reduce the secondary increase in aldosterone secretion.

The combination of 2 drugs that more effectively inhibit ENaC obviously increases the likelihood for hyperkalemia. The serum potassium concentration increased by 0.4 mmol/L in the combination group in the first week (the mean increase over the 4 weeks was 0.2 mmol/L), with 1 subject reaching a level of 5.5 mmol/L during the first 2 weeks and lower levels in weeks 3 and 4. Individuals with any degree of renal impairment or other predisposing condition for hyperkalemia would be best advised to use the combination of amiloride and spironolactone only with careful monitoring of the potassium level.

Finally, the 2 drugs together created, albeit far from complete, a “human knockout” for ENaC. We suggest that the significant decline in BP observed provides additional support for the importance of this site in regulating BP.

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References
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