Racial Difference in the Activity of the Amiloride-Sensitive Epithelial Sodium Channel

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

Abstract—Compared with whites, blacks appear to retain additional sodium that suppresses secretion of renin and aldosterone. The epithelial sodium channel (ENaC) is an aldosterone-regulated site for sodium reabsorption. ENaC activity could be higher in blacks, contributing to sodium retention or, alternatively, lower because of reduced stimulation by aldosterone. To examine the level of ENaC activity in blacks relative to whites, blood pressure (BP) responses to amiloride (5 mg/d), an inhibitor of ENaC, were measured in 20 black and 25 white normotensive young people. After 1 week, systolic BP decreased by 3.0±9.2 (SD) and diastolic by 2.8±8.3 mm Hg in the whites, whereas systolic BP increased by 2.5±7.1 and diastolic by 3.8±8.0 mm Hg in the blacks; the racial difference in the BP response was significant for both systolic (P=0.034) and diastolic BP (P=0.010). As ENaC activity increases, renal secretion of potassium increases proportionately, and in a larger sample of subjects, the urinary potassium excretion rate was lower in the blacks (n=301) than in the whites (n=461): 3.2±0.1 versus 3.8±0.1 mmol/mmol creatinine (P=0.0001). The concentration of serum potassium was higher in the blacks (n=81) than in the whites (n=167): 4.36±0.05 versus 4.21±0.03 (P=0.012). In summary, a favorable BP response to amiloride in the whites as well as the evidence for greater retention of potassium in the blacks is consistent with blacks having less ENaC activity than whites. We suggest that increased sodium retention in blacks occurring at other nephron sites suppresses aldosterone secretion and in turn ENaC function. (Hypertension. 2002;40:903-908.)

Key Words: aldosterone ■ blacks ■ blood pressure ■ sodium ■ sodium channels

An increase in the renal reabsorption of sodium contributes to the risk of hypertension.1 Blacks retain more sodium than whites, as suggested by lower levels of plasma renin activity (PRA) in blacks2-5 and an increase in the likelihood for developing salt-sensitive hypertension.6 Where sodium retention in the kidney occurs in blacks is unknown, although one consideration has been the epithelial sodium channel (ENaC) in the cortical collecting duct, a highly regulated channel with known influences on sodium homeostasis.7 Several hypertensive syndromes stemming from single-gene mutations have as a final common mediator increased sodium reabsorption by an overactive ENaC.8 Indeed, mutations in ENaC itself can lead to a low-renin form of hypertension known as Liddle’s syndrome.9-12 We have previously reasoned that ENaC could be intrinsically more active in blacks, based on observations of a cohort of young people in whom the plasma level of aldosterone and the urinary excretion rate of aldosterone have consistently been found to be lower in the blacks than in the whites,13-15 suggestive of a more active ENaC resulting in sodium retention and secondary suppression of the renin-angiotensin-aldosterone axis. Aldosterone levels are, for example, very low in Liddle’s syndrome. Also, common molecular variations in ENaC that exist primarily in blacks have been shown to associate with hypertension and/or suppression of PRA and aldosterone levels.16,17

On the other hand, the suppressed levels of aldosterone that we observe in blacks could lower ENaC function since a principal action of aldosterone is to upregulate activity of the channel.18 If ENaC function is lower in blacks, it might indicate greater reabsorption of sodium at another site. In the present study, we addressed the question, is ENaC more or less active in blacks in comparison to whites? We explored the issue of a racial disparity in the level of ENaC function by treatment with amiloride, an inhibitor of ENaC, and comparing the blood pressure (BP) responses in blacks and whites. Subjects were from the cohort in which we had identified the lower aldosterone in blacks. In addition, since ENaC function plays an integral role in determining potassium homeostasis (sodium reabsorption by ENaC produces an electrical gradient favoring secretion of potassium), we also made measurements of potassium to further estimate the relative amounts of ENaC activity in blacks and whites.

Just as one can learn from single gene defects in family studies, so might one learn from comparisons of population groups with different susceptibilities to hypertension. A better
definition of sites for increased sodium reabsorption in blacks could have important relevance to common forms of hypertension regardless of ethnicity.

Methods

Subjects
All participants were from a cohort that included school-age children and young adults who had been followed as part of studies of BP regulation.19 For the amiloride study, members of the cohort were sent letters inviting them to participate. For the studies in which potassium excretion rates and serum potassium levels were measured, we used samples that had been collected from members of the cohort who had agreed to provide an overnight urine sample and a blood sample. Each of the participants was healthy and had a normal blood sample. Each of the participants was healthy and had a normal treatment was assessed by pill counts.

Results

Characteristics
The baseline characteristics of the 20 blacks and 25 whites who participated in the amiloride study are presented in Table 1. Age, body mass index (BMI, calculated as weight/height\(^2\) [kg/m\(^2\)]), and proportion of girls were similar in the blacks and the whites. Systolic BP was 5 mm Hg higher in the whites (marginally significant at \(P=0.075\)), and diastolic BP was 3 mm Hg higher in the whites, a difference that was not significant (\(P=0.42\)). In the blacks, PRA was lower at 7 AM (\(P=0.040\)) and marginally lower at 9 AM (\(P=0.094\)); the plasma aldosterone was also marginally lower in the blacks both at 7 AM (\(P=0.076\)) and 9 AM (\(P=0.092\)). The serum potassium concentration was similar in this group of blacks and whites, as was the serum sodium concentration; urinary potassium and urinary sodium excretion rates were also similar in the blacks and the whites.

Assay Procedures
Electrolytes were measured with a flame photometer (Instrumentation Laboratories), and urinary creatinine was measured with a Beckman-2 creatinine analyzer. Aldosterone was measured directly by radioimmunoassay with a kit from Diagnostic Products Corporation, and PRA was measured by radioimmunoassay with the Clinical Assays GammaCoat kit.

Statistical Analysis
Comparisons between blacks and whites for differences at baseline and differences in the changes before and after the administration of amiloride were made with the use of 2-sample \(t\) tests. A paired \(t\) test was used to make comparisons before and after the administration of amiloride. With respect to racial comparisons of serum potassium levels and urinary potassium excretion rates, a mixed-model ANOVA was used with subject random effects to adjust for the correlation between multiple measurements from some of the subjects.

Urine samples were collected overnight and serum samples collected in the morning for measurements of electrolytes.

Treatments With Amiloride
The subjects were admitted to the General Clinical Research Center (GCRC) the afternoon before measurements were made. They were fed an evening meal containing 10 mmol of sodium and 20 mmol of potassium. The following morning, BP was measured 3 times, with 1 to 2 minutes in between readings, using a mercury sphygmomanometer with subjects remaining recumbent. They were then fed breakfast, containing 5 mmol of sodium and 20 mmol of potassium. The following morning, BP was measured 3 times, with 1 to 2 minutes in between readings, using a mercury sphygmomanometer with subjects remaining recumbent. They were then fed breakfast, containing 5 mmol of sodium and 20 mmol of potassium. Blood samples were drawn for levels of renin activity and aldosterone while supine and after 1 hour of sitting followed by 1 hour of standing (7 and 9 AM samples) and potassium (7 AM). Then, as outpatients, the subjects took 5 mg amiloride per day for 1 week, after which they were readmitted to the GCRC, and the measurements made at baseline were repeated. Compliance with the treatment was assessed by pill counts.

Measurements of Potassium
Urine samples were collected overnight and serum samples collected in the morning for measurements of electrolytes.
levels of renin activity, aldosterone, and potassium were similar in the blacks and the whites ($P>0.25$). The plasma aldosterone increased more than PRA (level of aldosterone increased 3-fold after amiloride), most likely because of stimulation of aldosterone secretion by the increase in potassium and angiotensin II.20

Systolic and diastolic BP decreased in the whites but not in the blacks after treatment for 1 week with amiloride (Table 2). The racial differences in the BP responses were significant for both systolic ($P=0.034$) and diastolic $BP$ ($P=0.010$). The weight change after treatment was not significantly different in the blacks and the whites ($P=0.18$).

### Urinary Potassium Excretion and Serum Concentrations of Potassium

In a cross-sectional sampling of subjects from the same cohort, the urinary excretion of potassium was significantly lower in the blacks than in the whites ($P<0.0001$) (Table 3). In addition, the serum potassium concentration was significantly higher in the blacks than the whites ($P=0.012$). The racial difference in serum potassium was not the same in girls and boys: The potassium concentration was $4.40\pm0.07$ in the 39 black girls and $4.16\pm0.05$ mmol/L in the 78 white girls ($P=0.0057$); the potassium concentration was $4.32\pm0.06$ in the 42 black boys and $4.25\pm0.04$ mmol/L in the 89 white boys ($P=0.40$).

### Discussion

In the present study, a small dose of amiloride, an inhibitor of ENaC, reduced BP in the whites but not in the blacks—a difference in response between race groups that was signifi-

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**Table 2. Effects of the Amiloride Treatment**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Blacks ($n=20$)</th>
<th>Whites ($n=25$)</th>
<th>$P$ Values for Amiloride Effect</th>
</tr>
</thead>
<tbody>
<tr>
<td>Δ Weight, kg</td>
<td>$-0.3\pm1.1$</td>
<td>$0.1\pm0.8$</td>
<td>$0.30$</td>
</tr>
<tr>
<td>Δ Systolic BP, mm Hg</td>
<td>$2.5\pm7.1$</td>
<td>$-3.0\pm9.2$</td>
<td>$0.049$</td>
</tr>
<tr>
<td>Δ Diastolic BP, mm Hg</td>
<td>$3.8\pm8.0$</td>
<td>$-2.8\pm8.3$</td>
<td>$0.012$</td>
</tr>
<tr>
<td>Δ Serum potassium, mmol/L</td>
<td>$0.1\pm0.3$</td>
<td>$0.1\pm0.2$</td>
<td>$0.054$</td>
</tr>
<tr>
<td>Δ Urinary potassium excretion, mmol/mmol creatinine</td>
<td>$-0.1\pm1.2$</td>
<td>$-0.2\pm1.2$</td>
<td>$0.049$</td>
</tr>
<tr>
<td>Δ Serum sodium, mmol/L</td>
<td>$1.3\pm4.3$</td>
<td>$1.0\pm4.4$</td>
<td>$0.010$</td>
</tr>
<tr>
<td>Δ Urinary sodium excretion, mmol/mmol creatinine</td>
<td>$0.1\pm0.9$</td>
<td>$0.2\pm0.9$</td>
<td>$0.068$</td>
</tr>
</tbody>
</table>

Values are mean ± SD.
The results were consistent with the blacks having less ENaC activity to inhibit. In a larger sample of subjects, the urinary potassium excretion rate, determined in part by secretion of potassium at the collecting duct in response to sodium reabsorption by ENaC, was lower, and the serum potassium concentration was higher in the blacks than in the whites, also consistent with ENaC function being lower in the blacks. We suggest that in the blacks there was increased sodium retention at other sites that suppressed aldosterone levels, resulting in fewer ENaCs at the cell surface. In addition, with less sodium reaching the cortical collecting duct, there would be reduced numbers of sodium ions reabsorbed by ENaC, which would also lower potassium secretion.21

The racial difference in the BP response to amiloride, although significant, was modest. Conceivably, the levels after treatment reflected more a regression toward the mean (although not significant, the BP s at baseline were somewhat higher in the whites than in the blacks). Although this remains a possibility, we took precautions to limit extraneous influences on the BP. To begin with, subjects were admitted to an inpatient facility the day before with the BP measured the following morning while subjects were remaining supine; diet and level of activity were thus very similar at the baseline visit and the postamiloride visit. We administered a low dose of amiloride to minimize the chance of a nonspecific diuretic effect. Finally, the subjects who participated were young and normotensive, and thus we avoided the wider range of BP values that can occur in studies of older adults, especially those with hypertension.

Amiloride can also inhibit other sites of sodium transport but is much more inhibitory of ENaC. For example, the IC50 (μmol/L) of amiloride for ENaC has been reported to be 0.35, in comparison to 84 for the Na+/H+ exchanger, 1100 for the Na+/Ca2+ exchanger, and 1100 for Na+/K+ATPase.22 Using an HPLC method to quantify amiloride in several of the subjects, we found that the urinary levels were in the range of 2 μmol/L, below the level necessary for inhibition of the other sodium reabsorptive sites but a level that would inhibit ENaC. Amiloride could have a nonspecific diuretic effect that lowered BP more in the whites (there may have been, for example, greater consumption of sodium by the whites). However, this seems unlikely because the levels of PRA and aldosterone tended to be lower in the blacks, suggesting more sodium retention.

The lower urinary potassium excretion in blacks has been described in adults. Previous studies showed either no racial difference or lower levels of serum potassium in blacks.5,27 Variations in serum potassium between groups could, of course, stem from differences in diet or in some instance from previous treatment with diuretics. Our black subjects could have consumed a diet that was richer in potassium, something we did not assess. None of our subjects had been exposed to diuretic therapy because none were hypertensive. If indeed the blacks were retaining additional potassium by a renal mechanism, it is unclear what mechanism exists for disposal of the excess. The fecal route is one possibility, but the transport of potassium by the colon is also ENaC-dependent,28 and if ENaC activity is reduced in blacks, then fecal secretion of potassium would also be expected to be less.

We have previously argued that ENaC activity could be higher in blacks than whites, based on the observation that the ratio of urinary aldosterone to urinary potassium was lower in blacks29 (in Liddle’s syndrome this ratio is extremely low10). If ENaC activity is indeed lower in blacks, as the current results indicate, then the reduced ratio in blacks is probably driven by the lower aldosterone secretion rate. The urinary aldosterone/potassium ratio may correctly predict ENaC activity but probably only at the extreme of increased sodium reabsorption by ENaC as in Liddle’s syndrome.

There may have been definite advantages to having performed the current study in subjects who were young and healthy. The racial difference in the response to amiloride and the difference in serum potassium concentration were both small and might easily have been missed in studies limited to adults, in which age or the presence of hypertension or its treatment can be confounding influences. We have consistently observed in our young cohort lower aldosterone levels in both plasma and urine samples from the black subjects.13,15 Comparisons in adults have not always shown a similar separation of aldosterone levels between the two race groups,20 although PRA was generally noted to be lower in black adults2,4 and in some instances the aldosterone level after stimulation with angiotensin II or with upright posture31 was lower in blacks than in whites.

An important aspect of the current findings is the strong implication of non-ENaC regions as sites in which blacks retain additional sodium. Several specific locations can be suggested (Figure 2.), beginning with the proximal tubule,
where angiotensin II increases sodium reabsorption. The higher frequency in blacks of an angiotensinogen molecular variant (M235T) that leads to a higher concentration of angiotensinogen could promote intrarenal angiotensin II–stimulated sodium reabsorption in blacks more than in whites. A second potential site is the Na-K-2Cl cotransporter in the thick ascending limb of Henle. Here, sodium reabsorption is coupled to a reduced excretion of calcium. Lower calcium excretion rates have been consistently observed in blacks in comparison to whites, and thus an increase in the cotransporter’s level of activity could explain the greater reabsorption of both sodium and calcium in blacks. A third potential site is the thiazide-sensitive Na-Cl cotransporter in distal convoluted tubule. The familial syndrome of low-renin hyperkalemic hypertension known as pseudohypoaldosteronism type II (PHA II) is qualitatively similar to what we observe in blacks. It has been shown to result from mutations in serine/threonine kinases that presumably enhance the activity of the cotransporter. This site is further implicated by the fact that blacks typically respond more favorably to treatment with a thiazide diuretic. A contribution of a more active Na-Cl cotransporter would, however, be expected to be partial because hyperkalemia, a consistent phenotype in PHA II even in the absence of hypertension, does not occur in healthy blacks.

The current results do not rule out the possibility that ENaC function could be inappropriately increased in blacks in light of the positive sodium balance that may already exist. The findings suggest only that it is not ENaC alone that accounts for increased sodium retention in blacks. If greater sodium retention stems from early teleological selection pressures, then indeed all sites where sodium is reabsorbed may be more efficient in blacks.

Perspectives

Increased sodium retention by the kidney may underlie much of the risk for hypertension, but identification of the specific nephron sites involved has remained elusive. By comparing a group that on average retains more sodium (blacks) with a group that on average retains less sodium (whites), we found evidence for a difference in sodium reabsorption that was limited to a region proximal to ENaC. We, as well as others, have considered ENaC a strong candidate site for the increased sodium retention in blacks, and indeed, our strong interest in ENaC led to the current study. We were surprised at the outcome because we anticipated that amiloride would reduce BP more in the blacks. The lower aldosterone level in blacks can, however, easily explain the results. Do the findings diminish a role for ENaC? Obvious somewhat, but ENaC could still play an important role, participating with other sites.

Acknowledgments

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References


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