Epithelial Sodium Channel Activity Is Not Increased in Hypertension in Whites

Emma H. Baker, A. James Portal, Teresa A. McElvaney, Alison M. Blackwood, Michelle A. Miller, Nirmala D. Markandu, Graham A. MacGregor

Abstract—Abnormal renal sodium transport causing excess reabsorption of sodium may be one mechanism that causes high blood pressure. For example, increased activity of epithelial sodium channels in the distal tubule is the cause of high blood pressure in Liddle’s syndrome, a rare familial form of hypertension. We have shown that the increase in sodium channel activity can be detected in the nose using transepithelial potential difference measurements in 1 family with Liddle’s syndrome. We therefore used nasal potential difference measurements to look for increased sodium channel activity in white patients with essential hypertension. Transnasal potential difference was measured in 42 white hypertensive (HT) subjects and 38 white normotensive (NT) subjects before and after topical application of 10⁻⁴ mol/L of amiloride. There was no difference in maximum potential between HT and NT subjects (HT, −18.8±0.9 mV; NT, −18.2±1.0 mV) (values mean±SEM; lumen-negative with respect to the submucosa). However, the postamiloride potential was significantly higher (HT, −12.6±0.7 mV; NT, −10.5±0.7 mV; P=0.015) and the change in potential in response to amiloride significantly lower (HT, 6.2±0.5 mV, 33.1±2.0%; NT, 7.7±0.6 mV, 41.9±2.0%; P=0.046 and 0.003, respectively) in HT than in NT subjects. These results suggest that sodium channel activity is not increased in whites with essential hypertension and indicate that sodium channel overactivity similar to that seen in Liddle’s syndrome is unlikely to be the cause of high blood pressure in this group. Increased postamiloride potential may reflect increased activity of chloride channels or amiloride-insensitive sodium channels. (Hypertension. 1999;33:1031-1035.)

Key Words: hypertension, essential ■ whites ■ sodium channels ■ amiloride ■ nasal mucosa ■ membrane potentials

Hypertension affects 15% to 20% of adults in the developed world, but the exact mechanisms underly-
ing the development of high blood pressure are poorly understood. In rat models of inherited hypertension, kidney cross-transplantation experiments have demonstrated that the kidney carries the genetic defect for high blood pressure, which appears to be expressed as a difficulty in excreting sodium. Circumstantial evidence suggests that this may also be true in essential hypertension in humans. The nature of such renal abnormalities has not been identified, although altered function of sodium transport processes, including Na⁺-K⁺ cotransport, Na⁺-Li⁺ countertransport, and Na⁺-H⁺ exchange, have been described in patients with essential hypertension. Abnormalities of the epithelial sodium channel were recently shown to cause Liddle’s syndrome, a rare inherited form of high blood pressure. In Liddle’s syndrome, mutations of β or γ subunits of the epithelial sodium channel increase channel activity, resulting in excessive renal sodium reabsorption and sodium retention that causes high blood pressure. This has led to speculation that increased activity of epithelial sodium channels in the renal distal tubule secondary to less severe mutations of the sodium channel could cause inappropriate sodium retention and predispose to high blood pressure in some patients with essential hypertension.

Renal sodium channel activity is inaccessible for clinical assessment. However, sodium channels with structural and physiological properties similar to renal channels are present in other tissues, including nasal epithelium. Sodium absorption through epithelial sodium channels is electrogenic and can be quantified in the nose by transmucosal electrical potential difference measurements before and after administration of amiloride, a drug that blocks sodium channels. Nasal potential difference measurements have been used in the diagnosis of cystic fibrosis. Recent studies have shown that nasal potential difference measurements are increased in affected members of 1 family with Liddle’s syndrome, in which sodium channel overactivity is responsible for the high blood pressure. We therefore used measurement of nasal transmucosal potential difference to assess sodium channel activity in untreated white patients with essential hypertension.
**Methods**

**Subjects**

Hypertensive subjects were taken from unselected referrals to the hypertension clinic by local general practitioners. Normotensive control subjects were volunteers from the local population recruited by advertisement.

All subjects had their blood pressure measured using a semiautomated ultrasonic sphygmomanometer (Arteriosonde) on at least 2 separate occasions. Subjects rested supine for 5 minutes, after which blood pressure recordings were done in triplicate using the appropriate cuff size based on the upper midarm circumference. Blood pressure values are the mean of these 3 recordings at the second visit. Subjects were defined as having high blood pressure if either their supine systolic blood pressure was >140 mm Hg or their diastolic blood pressure was >90 mm Hg. Hypertensive subjects had not received previous treatment or had been off all drug treatment for at least 2 weeks and diuretics for 4 weeks. Patients with ischemic heart disease, cerebrovascular disease, renal impairment, diabetes, a secondary cause of hypertension, or other concurrent illness were excluded. Normotensive subjects had a supine systolic blood pressure of ≤140 mm Hg and a diastolic blood pressure of ≤90 mm Hg. Potential subjects were excluded from the study if they had any evidence of acute or chronic rhinitis, asthma, or atopy or took nasal drugs or any other drug that might interfere with epithelial sodium channel activity or regulation.

Written informed consent was obtained from all subjects before entry into the study, which was approved by the Local Research Ethics Committee of Merton, Sutton, and Wandsworth. Procedures were in accordance with institutional guidelines.

**Measurements**

Each subject provided a 24-hour urine sample before nasal potential difference was measured, and this sample was analyzed for 24-hour urinary sodium, potassium, and creatinine excretion. On the day of measurement, subjects attended the Blood Pressure Unit after an overnight fast (no food or beverage other than water from midnight onward). Blood pressure, weight, and height were recorded. Smoking history was recorded to determine whether the subject had never smoked, was a current smoker, or was a former smoker. Serum sodium, potassium, creatinine, and urea were measured. Plasma was analyzed for plasma renin activity and plasma aldosterone concentrations by radioimmunoassay.

**Measurement of Nasal Potential Difference**

All nasal potential difference measurements were made by 1 of 2 operators using the same set of equipment. Operators were not blinded to the blood pressure status of the subjects. Transmucosal nasal potential difference was measured by a previously validated technique. The reference electrode consisted of a 23 gauge butterfly needle, which was inserted into the subcutaneous tissue of the forearm. The exploring electrode was an 8 gauge nasogastric tube filled with Ringer’s solution that was introduced along the inferior surface of the inferior turbinate to a distance of 7 cm. Both electrodes were connected to a high-impedance voltmeter by 1% Ringer’s agar bridges. The output of the voltmeter was recorded continuously on a chart recorder throughout the experiment. Nasal potential difference was recorded from the inferior surface of the inferior turbinate as the exploring electrode was withdrawn. The site of maximum potential difference was established, and the measurement at this site was recorded once the voltage was stable for >3 seconds. A second measurement of the maximum potential was made at the same site to ensure consistency of recording, and the mean of these 2 maximum values was taken as the potential difference (PDmax) for analysis. Values were lumen negative with respect to the submucosa. Amiloride (10^{-4} mol/L) in Ringer’s solution was then perfused onto the nasal mucosa, and after 4 minutes, the residual nasal potential difference (PDres) was remeasured during continued application of amiloride (10^{-4} mol/L) in Ringer’s solution. Nasal potential became less negative in response to amiloride application. The change in potential in response to amiloride (PDamil) was determined by calculating the difference between PDmax and PDres at the same point and was expressed both in millivolts and as a percentage of the total potential (PDamil%).

**Statistical Analysis**

Group values are expressed as mean±SEM for data with a normal distribution and as median and interquartile range for plasma renin activity and aldosterone concentration, which are not normally distributed. Differences between groups were tested using 2-sample t tests for normally distributed variables. Differences in plasma renin activity and plasma aldosterone concentrations were tested using the Mann-Whitney U test. Distributions of PDmax, PDres, and PDamil were found to be skewed to the left, and these variables were normalized by log transformation before differences between groups were tested with 2-sample t tests. Logistic regression analyses were used to determine whether the relationship identified between logs of potential difference values and blood pressure status could be explained by observed differences in gender distribution, obesity, and aldosterone concentrations between the groups and by age. Two-tailed probability values of <0.05 were considered significant.

**Results**

Baseline characteristics of the 2 groups are shown in Table 1. Mean blood pressure was 159.3/101.0±2.8/1.9 mm Hg in hypertensive subjects and 124.6/75.3±1.4/1.1 mm Hg in normotensive subjects. Hypertensive subjects were significantly heavier than normotensive subjects and had a higher body mass index (BMI). The groups also comprised significantly different proportions of male and female subjects. Serum and urinary electrolyte concentrations and plasma renin activity did not differ significantly between normotensive and hypertensive subjects (Table 2). Plasma aldosterone was significantly higher in hypertensive subjects (415 pmol/L; range, 279 to 565.5) than in normotensive subjects (316 pmol/L; range, 188 to 458; P=0.027).

Comparisons of nasal potential difference measurements between subjects with and without high blood pressure are shown in the Figure. The maximum nasal potential difference was −18.2±1.0 mV in normotensive subjects and...
The residual potential after application of amiloride was significantly greater in normotensive (7.7 ± 0.6 mV) than in hypertensive subjects (6.2 ± 0.5 mV; P = 0.046). The ratio of amiloride-sensitive potential in normotensive subjects to that in hypertensive subjects was 1.27 (95% CI, 1.004 to 1.61).

The proportion of maximum potential difference sensitive to amiloride was significantly greater in the normotensive (41.9 ± 2.0%) than in the hypertensive group (33.1 ± 2.0%; P = 0.003).

Logistic regression analysis showed that log-transformed residual potential was significantly related to blood pressure status (normotensive or hypertensive) (P = 0.021) and that this relationship remained significant after adjustment for gender, age, BMI, and aldosterone concentration (P = 0.043).

Logistic regression analysis showed that the relationship between log-transformed amiloride-sensitive potential and blood pressure status reached borderline significance (P = 0.052). Adjustment for gender, age, BMI, and aldosterone concentration increased the significance of the relationship (P = 0.026).

Logistic regression analysis showed that the relationship between the proportion of maximum potential difference sensitive to amiloride and blood pressure status was significant (P = 0.005). Adjustment for gender, age, BMI, and aldosterone concentration increased the significance of the relationship (P = 0.002).

**Discussion**

Measurement of nasal potential difference is an established technique that can be used to estimate the rate and type of ion transport across nasal epithelium. In vitro isotope flux studies have shown that the predominant active ion transport across airway epithelium is sodium absorption from lumen to blood, which is thought to occur through epithelial sodium channels because it is largely blocked by amiloride. Sodium absorption through sodium channels generates a lumen-negative potential difference that can be measured as PDmax. In the presence of amiloride, which blocks epithelial sodium channels, sodium absorption is inhibited. This alters the electrochemical gradient for chloride across the nasal epithelium and is thought to induce chloride secretion through apical chloride channels, such as the cystic fibrosis transmembrane regulator (CFTR). The transnasal potential difference is therefore not abolished in the presence of amiloride but can be measured as a residual potential difference (PDres), at least part of which may reflect chloride channel activity. The change in potential difference after amiloride application, measured as absolute PD (PDamil) or as the percentage of inhibition of PD (PDamil%), is of some use in evaluating the rate and type of ion transport across nasal epithelium.

In our study, PDmax did not differ between hypertensive and normotensive subjects. We previously used measurements of nasal potential difference to detect abnormal sodium channel activity in hypertension. In 1 family with Liddle’s syndrome, affected members had a significantly higher PDmax than an unaffected member and nonrelated unaffected

**TABLE 2. Biochemical Characteristics of White Hypertensive and Normotensive Subjects**

<table>
<thead>
<tr>
<th>Variable</th>
<th>Normotensive Subjects</th>
<th>Hypertensive Subjects</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plasma Na, mmol/L</td>
<td>140.6 ± 0.4 (n = 37)</td>
<td>139.7 ± 0.4 (n = 42)</td>
</tr>
<tr>
<td>Plasma K, mmol/L</td>
<td>4.12 ± 0.04 (n = 35)</td>
<td>4.20 ± 0.05 (n = 41)</td>
</tr>
<tr>
<td>Urine volume, mL</td>
<td>1796 ± 138.5 (n = 38)</td>
<td>1867 ± 101.9 (n = 41)</td>
</tr>
<tr>
<td>Urine Na, mmol/24 h</td>
<td>157.7 ± 9.4 (n = 36)</td>
<td>176.2 ± 9.0 (n = 41)</td>
</tr>
<tr>
<td>Plasma aldosterone, pmol/L</td>
<td>316 (188–458) (n = 36)</td>
<td>415 (279–565.5) (n = 41)</td>
</tr>
<tr>
<td>Plasma renin activity, nmol/L per h</td>
<td>0.60 (0.35–1.26) (n = 28)</td>
<td>0.79 (0.55–1.19) (n = 38)</td>
</tr>
</tbody>
</table>

Values are mean ± SEM for normally distributed data and median (interquartile range) for plasma renin activity and plasma aldosterone concentration. n indicates the number of patients for whom a biochemical result was available. *P = 0.027 versus normotensive subjects, Mann-Whitney U test.

Nasal potential difference measurements fell less in response to amiloride in hypertensive than in normotensive white subjects. Residual potential difference after amiloride application was higher in hypertensive than in normotensive subjects. *P = 0.046 and **P = 0.015 vs normotensive subjects.
control subjects. The finding that PDmax is not increased in white subjects with essential hypertension compared with normotensive control subjects therefore suggests that increased sodium channel activity is not a common cause of high blood pressure in whites with essential hypertension.

The effect of amiloride on nasal potential difference, however, did differ between hypertensive and normotensive subjects. The absolute and percentage fall in potential difference in response to amiloride was significantly smaller and PDamil was significantly larger in hypertensive subjects. Because PDamil is of some use in evaluating the rate of sodium absorption, these findings also suggest that sodium absorption through epithelial sodium channels is not increased in white hypertensive subjects. However, because PDmax is no lower in hypertensive than in normotensive subjects, it is unlikely that a reduction in PDamil is due to reduced sodium absorption. The higher PDres and lower PDamil values could both possibly be explained by an increase in chloride secretion, which might reflect increased activity of chloride channels such as CFTR in hypertensive subjects. Increased CFTR activity could be associated with abnormal sodium and chloride balance, leading to high blood pressure. Chloride ions are secreted through CFTR in the airways in the presence of amiloride, but chloride ions can also be absorbed through CFTR, particularly in the sweat ducts and possibly in the renal tubules. Increased CFTR activity could therefore lead to chloride and hence sodium and water retention and a rise in blood pressure. Indirect evidence of a role of CFTR in blood pressure regulation comes from a study in which investigators found significantly lower blood pressure in a group of young adults with cystic fibrosis than in age- and gender-matched controls. This association of CFTR underactivity with low blood pressure supports the possibility that increased CFTR activity could lead to high blood pressure.

A further possible explanation for the finding of higher PDres and lower PDamil in hypertensive whites is that some of the sodium absorption in hypertensive subjects could be amiloride insensitive. This might suggest increased expression of sodium channels other than the epithelial sodium channel in hypertensive subjects, which could lead to increased sodium absorption and contribute to the development of high blood pressure. Ion flux studies, however, have shown that there is little or no amiloride-resistant sodium absorption in human airway.

These interpretations assume that altered ion transport in the nasal mucosa reflects changes in ion transport elsewhere, particularly in the renal tubule.

Epithelial sodium channels comprising α, β, and γ subunits have been identified in nasal as well as renal epithelia. Cultured cells from both tissues, patch-clamp experiments have identified channels selective for sodium and sensitive to blockade by amiloride that are thought to be assembled from these α, β, and γ subunits. It is therefore likely that mutations of sodium channel subunits sufficient to cause high blood pressure would affect channel activity not only in the renal tubule but also in the nasal epithelium. Our finding that both PDmax and PDamil were increased in 3 brothers with Liddle’s syndrome compared with their unaffected sister and 40 normotensive subjects supports this conjecture and suggests that nasal potential difference measurements do reflect abnormalities in renal sodium channels.

It is possible that other differences between the normotensive and hypertensive subjects may have influenced the results of this study. Nasal potential difference measurements are known to be reduced by increasing age and cigarette smoking. In our study, there was no difference in age or smoking habits between the 2 groups. Variables that did differ between the groups (BMI and gender distribution) did not account for the relationship between PDres or PDamil and blood pressure status. It is also possible that differences in nasal potential difference between hypertensive and normotensive subjects might be explained by differences in volume status between the groups. If hypertensive subjects had expanded volume, this would result in suppression of plasma renin activity and plasma aldosterone. Sodium channel activity, at least in the kidney and colon, is stimulated by aldosterone and is reduced if aldosterone is suppressed. Pmax and PDamil might therefore be reduced in hypertensive subjects with volume expansion and low plasma aldosterone. However, it is unlikely that differences in volume status account for our findings of reduced PDamil seen in hypertensive subjects, particularly since plasma aldosterone concentrations were higher in hypertensive than in normotensive subjects. These higher aldosterone concentrations would have been expected to be associated with increased rather than decreased sodium channel activity. In addition, the relationships between PDres and PDamil and blood pressure status remained significant after adjustment for aldosterone, gender, age, and BMI. Furthermore, there is some evidence that respiratory sodium channels are insensitive to regulation by aldosterone. In subjects exposed for 2 weeks to high concentrations of spironolactone, an aldosterone receptor antagonist, nasal PD did not change.

We have shown that epithelial sodium channel activity is not increased in whites with high blood pressure compared with normotensive subjects as assessed by nasal potential difference measurements. Sodium channel overactivity similar to that seen in Liddle’s syndrome is therefore unlikely to be a frequent cause of high blood pressure in whites.

Acknowledgments

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References


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