Renal Apical Membrane Sodium-Hydrogen Exchange in Genetic Salt-Sensitive Hypertension

James L. Lewis, David G. Warnock

Abstract Inbred Dahl/Rapp salt-sensitive and salt-resistant rats differ in their blood pressure response to dietary salt. We studied sodium-hydrogen (Na-H) exchanger kinetics in renal brush border membrane vesicles prepared from both strains on either a 1% or 8% NaCl diet. Kinetics measurements were made with the acridine orange fluorescence quenching technique in vesicles prepared at pH 6.0. The initial Na-H exchange rate was measured using preparations with similar initial quench values. The maximal transport rate (V_max) fluorescence units per second per milligram protein (±SEM) in salt-sensitive rats on a 1% NaCl diet was significantly lower than that in salt-resistant rats (36.9±4.4 versus 51.8±5.5, respectively, P<.0005). With the 8% NaCl diet for 1 week, the V_max of salt-resistant rats decreased and became similar to that of salt-sensitive rats. The affinity for sodium (K_m, millimoles per liter ±SEM) was also lower in salt-sensitive rats than in salt-resistant rats while on a 1% NaCl diet (11.8±1.0 versus 19.6±2.3, respectively, P<.002). These values converged when both strains were fed an 8% NaCl diet for 1 week. Inhibition by 25 µmol/L amiloride was less in salt-sensitive rats than in salt-resistant rats on the 1% NaCl diet. These results show that salt-sensitive rats have lower renal apical membrane Na-H exchange activity than salt-resistant rats on a 1% NaCl diet. Salt-sensitive rats do not modulate renal apical membrane Na-H exchange in response to an 8% NaCl diet, whereas salt-resistant rats show a more physiologically appropriate response to increased dietary salt. The maladaptive response by salt-sensitive rats on a high salt diet may well contribute to the development of severe hypertension under these conditions. The differences between these two strains in sodium affinity and amiloride inhibition suggest structural differences in the renal apical membrane Na-H exchangers at the substrate and amiloride binding sites or altered cellular regulation of these transport proteins. (Hypertension. 1994;24:491-498.)

Key Words • ion exchange • rats, inbred strains • hypertension, genetic • amiloride

The sodium-hydrogen (Na-H) exchangers make up a family of integral membrane proteins present in nearly all mammalian cells. Using the energy stored in the inwardly directed sodium gradient, these transport proteins mediate the electroneutral exchange of extracellular sodium for intracellular protons. Na-H exchangers are involved in a number of cellular homeostatic processes and are thought to play a central role in the control of intracellular pH and cell volume.1-2 It has been postulated that an abnormality in cellular sodium uptake is an important factor in the development of essential hypertension.3 Since Na-H exchange mediates a large fraction of cellular sodium uptake, the role of Na-H exchangers in the development of human hypertension, particularly of the salt-sensitive variety, has been an area of intense research over the past several years. Several studies have examined platelet and leukocyte Na-H exchange as well as red blood cell sodium-lithium exchange in humans and have found enhanced exchange rates in patients with essential hypertension.4-8

In addition to this so-called housekeeping role in nonpolarized cells, Na-H exchangers play an additional role in epithelial cells by promoting vectorial transport across the epithelium. Na-H exchange occurring in the apical membrane of the renal proximal tubule results in the majority of proximal tubular sodium reabsorption as well as the reclamation of most of the filtered load of bicarbonate.1 An enhanced Na-H exchange rate in the proximal tubular apical membrane has been suggested as an additional mechanism by which increased Na-H exchange could be causally related to essential hypertension.9

The cloning, sequencing, and expression of the cDNA encoding an amiloride-sensitive form of the Na-H exchanger by Sardet et al in 198910 and the later mapping of the gene now denoted APNH to chromosome 111 have made possible further studies exploring the relation of Na-H exchange and essential hypertension on a genetic level. Despite what seems to be an intuitive link to hypertension, Lifton et al12 did not find a linkage between increased sodium-lithium exchange and alleles at the APNH locus in defined pedigrees from Utah. Furthermore, by using affected sib-pair analysis, they excluded APNH as a candidate gene for essential hypertension in these pedigrees.12

For several years, several lines of evidence, including differential amiloride sensitivities and regulation mechanisms, have suggested that there are at least two functional types of the Na-H exchanger: an amiloride-sensitive form, which is generally distributed in nonpolarized cells and restricted to the basolateral membrane in epithelial cells, and an amiloride-insensitive form, which is present primarily on the apical membrane of renal proximal tubule cells.13-15
specialized epithelia. Indeed, the recent cloning and expression of several Na-H exchanger protein isoforms have advanced this concept. Although direct tissue localization of the protein product of each cloned Na-H exchanger awaits the development of isoform-specific antibodies, Northern analysis has been done. mRNA for the amiloride-sensitive isoform cloned by Sardefet al (NHE-1) has been found in nearly all tissues. Other isoforms (NHE-2 through NHE-4) have more restricted mRNA distribution but include kidney cortex. Previous linkage studies by Lifton et al made use of probes derived from NHE-1. Therefore, these results do not directly test the relationship between hypertension or salt sensitivity and increased activity of amiloride-insensitive isoforms of the Na-H exchanger located in the apical membrane of the proximal tubule.

Direct testing of Na-H exchange rates in renal epithelia from large numbers of human subjects is not feasible, so work in this area must rely on animal models of hypertension. We hypothesized that increased Na-H exchange activity would be found in the proximal tubule apical membrane in salt-sensitive hypertension, thus leading to increased proximal sodium reabsorption. To examine this thesis, we measured Na-H exchanger activity in salt-sensitive SS/Jr (S) and salt-resistant SR/Jr (R) Dahl/Rapp rat kidney cortex brush border membrane vesicles (BBMVs). The Dahl/Rapp rats were chosen over other hypertensive rat strains because of the clear-cut salt sensitivity of the hypertension in the S strain. In addition, pure inbred stocks of each strain were available from a single source. The homogeneity of these strains was also attractive because the small size of the rat kidney relative to the amount of tissue needed for fluorescence-based vesicle studies necessitated the pooling of tissue from several rats.

Methods

Experiments were designed to compare apical membrane Na-H exchanger kinetics between S and R rats on either a low or high NaCl diet. Since vesicle preparation and fluorescent measurements can display a fairly wide day-to-day variation, a paired design was incorporated from the inception of these studies. In addition, since performing complete Na-H exchange kinetics requires a greater amount of renal BBMV than can be collected from an individual rat, renal BBMVs were pooled from groups of four to eight rats. In every instance, pairs of S and R rat groups were of equal number and age and arrived in the same shipment from the vendor. Preparation of BBMVs and fluorescence studies were carried out concurrently on each paired set, permitting paired statistical analysis as previously described.

Experimental Animals

All studies were done in agreement with The University of Alabama at Birmingham Institutional Animal Care and Use Committee guidelines. Inbred Dahl/Rapp S and R rats were used (Harlan Sprague Dawley, Inc). A total of 288 S and R rats were obtained during the years 1989 to 1991, at a time when these strains had been inbred for more than 20 generations. The resulting extremely high level of genetic homogeneity justified the pooling of samples from individual S and R rats as described above. Rats were housed at the University of Alabama at Birmingham Animal Resources Facility in a climate-controlled room and received either a standard (1% NaCl) or high salt (8% NaCl) diet (Agway Inc) for 1 week before death and vesicle preparation. The two diets differed significantly only in their NaCl content. Throughout the di-

etary manipulation period, all rats had free access to food and water.

Blood Pressure and Body Weight Measurements

Blood pressure and body weight were measured on two separate occasions during the week the rats were receiving their prescribed diets. After several days were allowed for rats to acclimate after arrival from the vendor, blood pressure was measured in conscious, restrained rats using the tail-cuff method with a temperature-controlled restraining cage and tail electrotygmanomanometer (Narco Biosystems) to familiarize the animals with the restraining cage device. At the end of the week of dietary manipulation, the rats were weighed and blood pressure was once again measured. The median of five successive measurements was recorded as systolic blood pressure as described by Chen et al.

BBMV Preparation

Renal BBMVs were prepared by modifications of the Mg aggregation technique of Booth and Kenny. After 1 week on the respective diets, rats were given an injection of pentobarbital (5 mg/100 g body wt IP) and decapitated. The kidneys were perfused by rapid injection into the distal aorta after the aorta was occluded above the renal arteries with 10 mL iced homogenizing solution containing (mmol/L) sucrose 50, Hepes/KOH 10 (pH 6.0), and EDTA/KOH 5 (pH 6.0). Throughout the next steps the tissue was kept at 5°C either by chilling on ice or in a refrigerated centrifuge. The renal cortices from four to six rats were then harvested and homogenized in 150 mL of the same buffer with a Sorvall Omnimixer (Omnin Corp International) at full speed for 4 minutes. The homogenate was centrifuged for 15 minutes at 8000 rpm with a JA-20 rotor and J2-21 refrigerated centrifuge (Beckman Instruments, Inc). The supernatant was then centrifuged at 17,000 rpm for 30 minutes. The resulting pellet was resuspended in a second sucrose solution that contained 250 mmol/L sucrose and 10 mmol/L Hepes/KOH, pH 6.0, by passage through a 22-gauge needle and centrifuged at 8000 rpm for 15 minutes. The supernatant was then centrifuged at 20,000 rpm for 25 minutes, and the resulting pellet was resuspended by passage through a 27-gauge needle. The resulting suspension was further concentrated by a fifth centrifugation at 20,000 rpm for 20 minutes, after which the supernatant was discarded and the final pellet stored at −70°C to preserve Na-H exchange activity until assays were performed.

Na-H Exchanger Assays

Na-H exchange rates were determined with a ratio spectrophotometer (excitation, 493 nm; emission, 530 nm) using the acidine orange fluorescence quenching technique as previously described. The BBMVs (which had been formed and stored at pH 6.0) were placed in an acidine orange buffer that contained 6 μmol/L acidine orange, 150 mmol/L sucrose, 150 mmol/L N-methyl-glucamine gluconate (pH 7.5), and 10 mmol/L Hepes/KOH (pH 7.5). The actual amount of BBMVs used was adjusted to always give an initial quenching of acidine orange fluorescence between 50% and 60%. This essential precaution ensured that the actual transmembrane pH gradient was always the same. Na-H exchange rates, expressed as fluorescence units (FU) per second, were determined by calculating the initial rate of the reappearance of fluorescence after the addition of sodium gluconate. Sodium gluconate was added by rapid injection using a 100-μL syringe (Hamilton) to give final concentrations of 90, 50, 25, 10, and 5 mmol/L. The initial recovery rates of fluorescence from five trials at each sodium concentration were averaged to determine the Na-H exchange rate for that sodium concentration. Amiloride inhibition was determined in BBMVs using 0, 25, 50, and 100 μmol/L amiloride at the four higher sodium concentrations listed above.
Blood Pressure and Body Weight Measurements

Systolic blood pressure was measured in all groups of awake S and R rats on two separate occasions. As shown in Table 1, none of the groups had severely elevated systolic blood pressure at the time of the study. Blood pressure was significantly higher in S than in R rats within both dietary groups. After less than 1 week on the high salt diet, S rats developed significantly higher blood pressures than S rats that remained on the 1% NaCl diet (P<.05). Body weight was determined in each rat and is also reported in Table 1. There was no significant difference in body weight between the S and R rats on either diet.

Enzyme Assays

Brush border membrane enrichment and recovery (relative to the crude homogenate) were determined by measuring leucine aminopeptidase (LAP) activity as a marker of apical membrane and protein concentration in both brush border membrane preparations and cortical homogenates. LAP activity was determined by the appearance of 4-nitroaniline at 405 nm in a Lambda 6 UV/Vis spectrophotometer (Perkin-Elmer Corp) at 0 and 6 minutes in 1 mL incubation medium containing 100 mmol/L mannitol, 20 mmol/L Hepes/Tris (pH 7.4), and 0.3 mg L-leucine 4-nitroanilide per milliliter.

Enzyme Assays

The total protein concentrations of the brush border membrane preparations were similar, as were the protein concentrations of the crude homogenates. As shown in Table 2, the percent recovery, specific activity, and enrichment of LAP activity were similar in groups of S and R rats on both a 1% and 8% NaCl diet. Even though LAP specific activity appeared to be slightly greater in BBMVs prepared from S rats compared with those from R rats, LAP enrichment was identical for both strains and unaffected by dietary NaCl content.

BBMV Na-H Exchange Studies

BBMV preparations from groups of S and R rats on either a 1% or 8% NaCl diet were used to measure Na-H exchange activity with the acridine orange fluorescent quenching technique. Initial rates of the increase in acridine orange fluorescence with addition of five incremental sodium concentrations were measured and are expressed as FU per second per milligram protein (±SEM). Fig 1 shows the kinetic parameters of Na-H exchange in BBMVs from 15 groups of S and R rats on a 1% NaCl diet. The V_{max} in BBMVs from S rats was 36.7±4.4 and in BBMVs from R rats was 51.8±5.5. This difference is highly statistically significant (P<.0005). The K_{m} for sodium in S rats (millimoles per liter sodium [±SEM]) was 11.8±1.0 and in R rats was 19.6±2.3. This difference is also statistically significant (P<.002) and reflects a lower affinity for substrate in BBMVs from R rats compared with those from S rats. There were no obvious correlations between either V_{max} or K_{m} and age over the range of 4 to 9 weeks, but power to detect a difference was limited by the low numbers of groups studied at each age.

Fig 2 shows V_{max} data for six groups of BBMVs from S and R rats on either a 1% or 8% NaCl diet. The V_{max} was 37.0±4.9 in BBMVs from S rats on a 1% NaCl diet and 53.5±9.1 in BBMVs from R rats. This difference is significant (P<.04) and similar to that shown in Fig 1. V_{max} was 34.7±6.2 in BBMVs from S rats on an 8% NaCl diet and 38.7±3.4 in BBMVs from R rats on an 8% NaCl diet. These values are not significantly different.
Two-by-two ANOVA shows a main effect for strain (F=6.58, \(P<.02\)) and a trend toward a main effect for diet (F=3.56, \(P<.079\)).

Fig 3 shows \(K_m\) data obtained in the same six groups presented in Fig 2. The \(K_m\) (millimoles per liter sodium [\(\pm\) SEM]) for sodium was 12.0 \(\pm\)2.1 in BBMVs from S rats and 19.7 \(\pm\)3.1 in BBMVs from R rats on a 1% NaCl diet. This difference is statistically significant (\(P<.02\)) and is also similar to the difference seen in Fig 1. The \(K_m\) for sodium was 13.7 \(\pm\)3.4 in S rats on an 8% NaCI diet and 11.3 \(\pm\)2.1 in R rats. As illustrated by the differences in the slopes of the two lines in Fig 3, there is an interaction between diet and strain on \(K_m\) (F=5.48, \(P<.04\)). This implies that in BBMVs from S rats Na-H exchange \(K_m\) reacts in a significantly different fashion to increases in salt intake than in BBMVs from R rats.

### Amiloride Inhibition Studies

Amiloride inhibition of Na-H exchange was studied in BBMVs from both S and R rats on a 1% NaCl diet.

Two-by-two nested ANOVA demonstrated main effects for strain and amiloride for \(V_{max}\) (F=14.48, \(P<.005\) and F=8.92, \(P<.02\), respectively). It also should be noted that there was a trend toward an interaction between strain and amiloride (F=3.58, \(P<.091\)) that did not reach statistical significance. Figs 4 and 5 illustrate these data, which are summarized in Table 3. Despite the significantly decreased \(V_{max}\) in BBMVs from R rats of roughly 30\% (\(P<.02\)), 25 \(\mu\)mol/L amiloride had no significant effect on \(K_m\), as seen with the mixed-type inhibition previously reported with the acridine orange assay.\(^{25,28}\) As in BBMVs from R rats, there was no significant effect on \(K_m\) in BBMVs from S rats. At higher amiloride concentrations of 50 and 100 \(\mu\)mol/L, the \(V_{max}\) in BBMVs from S rats was found to decrease 57\% and 60\%, respectively, whereas the \(V_{max}\) in BBMVs from R rats decreased 63\% and 76\%, respectively (data not shown). Therefore, Na-H exchange in BBMVs from S and R rats demonstrates a noncompetitive effect to amiloride. In addition, Na-H exchange in BBMVs from...
S rats is less sensitive to amiloride than Na-H exchange in BBMVs from R rats.

**Discussion**

In the present study we have demonstrated a difference in renal apical membrane Na-H exchange $V_{\text{max}}$ and $K_m$ between S and R rats on a 1% NaCl diet (Fig 1). Contrary to predictions made by the hypothesis that enhanced apical membrane Na-H exchanger activity in the kidney plays a central role in the pathogenesis of hypertension, our results show that maximal Na-H exchange rates are lower in BBMVs from hypertensive S rats than in those from R rats. The Na-H exchange $K_m$ for sodium is also lower in BBMVs from S rats than in those from R rats, reflecting a higher affinity for sodium in the hypertensive strain. However, it seems unlikely that this difference in affinity for substrate should have any major physiological effect, as the proximal tubule luminal concentration of sodium (approximately 140 mmol/L) is severalfold greater than these $K_m$ values. Thus, the proximal tubule Na-H exchanger is saturated with respect to sodium in both S and R rats, and $V_{\text{max}}$ governs the rate of apical Na-H exchange.

The relationship of these findings to sodium balance or hypertension is unclear for a number of reasons. Although glomerular filtration rate (GFR) has been shown not to differ between 5- to 7-week-old S and R rats on a low salt diet, differences in GFR and thus the filtered load of sodium presented to the proximal tubule certainly exist between S and R rats by 16 to 20 weeks of age, with significantly decreased GFR in S rats. In addition, differences between S and R rats have been described in distal nephron sodium reabsorption. Prehypertensive and hypertensive S rats have been shown to have increased reabsorption of chloride in the loop of Henle as well as alterations in more distal nephron segments in sodium and chloride handling. Thus, it is difficult to predict the effect that differences in proximal Na-H exchange will have on either the net renal handling of a salt load or the blood pressure response to a high salt diet.

Even in the absence of distal nephron adjustments to sodium reabsorption, the present association between suppressed proximal tubule Na-H exchange rates in S rats and hypertension cannot be taken to infer a causal relationship between hypertension and suppressed Na-H exchange. The developing hypertension in S rats could just as likely be responsible for suppressing Na-H exchange as the converse. Alternatively, hypertension and suppressed BBMV Na-H exchange might not be related at all but simply occur together in S rats because...
of a random chance event occurring during the initial selection and inbreeding of this line. The elimination of this possibility requires the demonstration of a statistically significant association between Na-H exchange rates and hypertension in a population with random segregation of genetic material, such as the progeny of an F2 cross between S and R rats. Nonetheless, the question of causality would exist unless an association could be found between a polymorphism in the actual gene encoding the renal apical membrane Na-H exchanger responsible for the observed kinetic differences and hypertension. A cosegregation between a polymorphism in the renal apical Na-H exchanger gene and a measurable kinetic parameter of Na-H exchange or hypertension itself would suggest a causal relationship between this genetic polymorphism and the phenotypic trait.

Although differences in the absolute number of functioning Na-H exchange proteins present in the apical membrane as well as the turnover rate of each exchanger unit can account for differences in $V_{\text{max}}$, the observed $K_m$ disparity implies either important differences in the regulation of apical Na-H exchange or structural differences influencing the substrate binding site or sites. This latter possibility implies the existence of polymorphisms in the coding sequence of the renal apical membrane Na-H exchanger gene between S and R rats. As discussed below, the different responses to high salt diet as well as the differences in amiloride inhibition between the two strains are consistent with this possibility. Determination of whether alterations in amino acid sequence are responsible for the functional differences observed between S and R rat renal Na-H exchange will have to await gene sequence information for the apical Na-H exchangers in S and R rats, the discovery of polymorphisms between them, and the measurement of Na-H exchange kinetics in individuals of an F2 population so the relationship between these polymorphisms and Na-H exchange can be determined.

As interesting as the baseline Na-H exchange rate differences between S and R rats is the response to a high salt diet. We observed a downregulation of BBMV Na-H exchange rate with a high salt diet in R rats, which was not seen in S rats (Fig 2). Decreases in apical membrane Na-H exchange rate in response to a high salt diet would decrease proximal sodium reabsorption, resulting in augmented distal nephron sodium delivery and thus allowing R rats to maintain sodium balance without necessarily a change in GFR. S rats, on the other hand, would need to increase the filtered load of sodium to maintain the same distal sodium delivery, because there appears to be no change in proximal Na-H exchange rate in response to a high salt diet. An inability of S rats to adequately modulate GFR when faced with a high salt intake could lead to positive sodium balance and an increase in blood pressure. Indeed, data suggest that young S rats increase their GFR less than R rats do when placed on an 8% NaCl diet. Although these data were obtained in younger rats than in the present studies, this may offer some insight into the pathogenesis of the accelerated hypertension seen in S rats on a high salt diet. However, rather than apical Na-H exchange activity, this observation implies that a variety of other physiological factors controlling renal blood flow and filtration rate are involved in the development of salt-sensitive hypertension in this model. Still, the failure of S rats to decrease proximal tubule Na-H exchange activity on an 8% NaCl diet could be interpreted as a maladaptive response that would contribute to renal salt retention and potentiate the hypertensive response to a high salt diet.

On the other hand, different responses to an 8% diet in Na-H exchange between S and R rats could be caused by differences in intracellular regulatory mechanisms. Mechanisms controlling Na-H exchange activity directly or indirectly, for instance, through the manipulation of intracellular pH could account for the observed responses. Differences in rat lymphocyte Na-H exchange have been attributed to changes in intracellular pH ($pH_i$). LaPointe and Battle have demonstrated that S rats have a significantly lower $pH_i$, and higher net acid excretion rate than R rats on a high salt diet. Although this raises several intriguing questions regarding differences between S and R cellular metabolism, it is unlikely that such a finding would explain the observed differences between S and R rat BBMV Na-H exchange because we used a fixed pH gradient in the present studies. In addition, since a variety of hormonal signals are known to be involved in the control of Na-H exchange activity, alterations in the elaboration of or sensitivity to the effects of hormones such as angiotensin II could alter Na-H exchange kinetics in response to a high salt diet. Angiotensin II has been shown to have more than one effect on Na-H exchange.

### Table 3. Inhibition with 25 μmol/L Amiloride in Salt-Resistant and Salt-Sensitive Rats on 1% NaCl Diet

<table>
<thead>
<tr>
<th>R</th>
<th>Amiloride, μmol/L</th>
<th>P</th>
<th>S</th>
<th>Amiloride, μmol/L</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 (n=4)</td>
<td>25 (n=4)</td>
<td>0 (n=4)</td>
<td>25 (n=4)</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>$V_{\text{max}}$ (FU/mg protein)</td>
<td>47.0±9.6</td>
<td>32.5±6.9</td>
<td>31.0±5.7</td>
<td>27.8±6.2</td>
<td>NS</td>
</tr>
<tr>
<td>Apparent $K_m$, mmol/L, Na</td>
<td>19.8±4.3</td>
<td>22.0±4.8</td>
<td>12.3±1.3</td>
<td>12.8±3.6</td>
<td>NS</td>
</tr>
</tbody>
</table>

R indicates salt-resistant rats; S, salt-sensitive rats; and FU, fluorescence units. Results were obtained from Eadie-Hofstee transformations and are expressed as mean±SEM.
Another aspect of the difference between S and R rat BBMV Na-H exchange is inhibition by amiloride. As Table 3 shows, 25 μmol/L amiloride inhibits 30% of Na-H exchange rate in BBMVs from R rats, whereas inhibition could be demonstrated in BBMVs from S rats only with higher concentrations. K_i was not altered significantly by 25 μmol/L amiloride in either strain. Renal apical Na-H exchange has shown a pattern of mixed-type inhibition with amiloride in rabbit BBMVs when measured with the acridine orange fluorescence quenching method and anions other than chloride in the external buffer. A possible explanation for the presence of a noncompetitive effect with 25 μmol/L amiloride in BBMVs from R rats but not in BBMVs from S rats is that differences exist between the amiloride binding sites of S and R rat renal apical Na-H exchangers. A second possibility involves the differential sensitivities to amiloride characteristically ascribed to the different Na-H exchanger isoforms. Chambrey et al. have recently expressed individual isoforms of the Na-H exchanger in mouse fibroblasts deficient in Na-H exchange. The IC50 for amiloride differed greatly between cells transfected with NHE-1 (6 μmol/L) and NHE-3 (450 μmol/L) cDNAs. The inhibition pattern with amiloride seen in BBMVs from R rats could be explained by the presence of an isoform that is relatively sensitive to amiloride, such as NHE-1 or -2. On the other hand, the inhibition pattern seen in BBMVs from S rats could result from a relative increase in expression of an isoform such as NHE-3 on the apical membrane of proximal tubule cells. The present study did not incorporate a measure of basolateral contamination of the BBMV preparations, and therefore another possible explanation is that the degree of basolateral contamination differed between BBMVs from S and R rats. Although possible, this seems unlikely given the parallel manner in which BBMVs from S and R rats were prepared. More work is needed to further our understanding of the mechanisms behind the observed differences in BBMV Na-H exchange but will require an experimental approach to separate the individual contributions of each NHE isoform.

Other researchers have studied Na-H exchange in Dahl rats in a variety of nonrenal tissue. LaPointe and Battle have shown that Na-H exchange in isolated vascular smooth muscle cells from hypertensive S rats is actually reduced when compared with that of cells from R rats. These findings show that suppressed Na-H exchange in S rats is present in another cell type besides renal cortical epithelia, providing evidence of a generalized epithelial cell transport defect involving the Na-H exchanger. Na-H exchange has also been studied in erythrocytes from Dahl/Rapp S and R rats by Pontremoli et al. Both blood pressure and red blood cell Na-H exchange were found to be higher in S than R rats on a high (8%) salt intake. No differences in Na-H exchange were found between S and R rats on a low (0.02%) salt diet. Despite the apparent discrepancy between these observations and our data, one cannot compare studies measuring Na-H exchange in nonpolared and polarized tissues because of the existence of different isoforms of the Na-H exchange protein and the differential expression of these various isoforms in different tissue types. Nevertheless, it is interesting to note that in these studies erythrocyte Na-H exchange was modulated by dietary manipulation only in S rats, whereas in our studies BBMV Na-H exchange was modulated only in R rats. This supports the possibility of altered differential tissue expression of NHE isoforms between S and R rats rather than a single generalized defect in cellular regulation in the S strain. In conclusion, we have shown that there are differences in apical membrane Na-H exchange in the renal cortices of the two inbred Dahl/Rapp rat strains. These differences probably do not explain or cause hypertension in S rats or prevent it in R rats; however, the inability of S rats to decrease their Na-H exchange rate in response to a high salt diet may exacerbate the development of salt-sensitive hypertension. The differences observed in the response to sodium and the inhibitory responses to amiloride and an 8% NaCl diet raise the possibility that more than one isoform of the Na-H exchanger are expressed in renal BBMVs from Dahl/Rapp rats.

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**References**


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