Sodium and Glucose Transport Across Renal Brush Border Membranes of Milan Hypertensive Rats

PAOLO PARENTI, GIORGIO M. HANOZET, AND GIUSEPPE BIANCHI

SUMMARY Sodium transport across luminal membranes of proximal tubules isolated from the kidney cortex of young, prehypertensive rats of the Milan hypertensive strain (MHS) and their corresponding normotensive controls, the Milan normotensive strain (MNS), was measured. A higher sodium uptake was observed in vesicles from MHS, although membrane preparations from both strains behaved similarly as far as enzyme profile and sodium-dependent glucose transport were concerned. In the presence of an inwardly directed sodium gradient, sodium uptake depended on the relative permeability of the counter ion: in the presence of 100 mM NaCl, sodium transport in MHS was 26% higher than that in normotensive controls ($p < 0.05$). Also, a significantly faster sodium uptake by membrane vesicles from MHS was observed when a pH gradient and an electrical potential difference (inside-negative) were imposed across the membrane. In this condition, sodium uptake by membrane vesicles from MHS was up to 39% higher than that in control MNS ($p < 0.01$). Therefore, the difference in sodium transport observed between preparations of luminal membrane from the proximal tubule of MNS and MHS seems to be due to a higher rheogenic sodium pathway in the MHS. The present results are in keeping with previous data showing an increased sodium transport across renal tubules of the MHS and support the hypothesis that the abnormality in sodium and water handling by kidneys from MHS can be related to an alteration in sodium transport across the luminal membrane of the proximal tubule cells. (Hypertension 8: 932-939, 1986)

KEY WORDS • Milan hypertensive strain • sodium transport • glucose transport • brush border membrane vesicles • membrane potential

Previous studies have shown that the whole kidney glomerular filtration rate was faster in rats of the Milan hypertensive strain (MHS) at the prehypertensive stage than in age-matched controls of the Milan normotensive strain (MNS) and that renal retention of sodium occurs during the development of hypertension in the MHS. Moreover, a faster glomerular filtration rate by kidney from MHS was also found in isolated preparations perfused in vitro up to 2 hours. Since renal sodium excretion under basal conditions at the prehypertensive stage both in vivo and in vitro was similar in the two strains, the calculated tubular sodium reabsorption was faster in MHS kidney. Considering the role played by these kidney abnormalities in the development of hypertension in the MHS and by the proximal tubule in sodium reabsorption, we thought it of interest to measure sodium transport across the luminal membrane isolated from the proximal tubule of kidney from MHS at the prehypertensive stage and from age-matched controls. Therefore, we obtained measurements of different sodium pathways across the luminal membranes of the proximal tubules, together with other factors aimed at characterizing the preparations. The latter studies were necessary to establish whether brush border membrane vesicles (BBMV) prepared from the two strains of rats were comparable.

Materials and Methods

Brush Border Membrane Vesicle Preparation

Prehypertensive male MHS (4 weeks old; body weight, 60-70 g) and corresponding control MNS were used. At this age the systolic blood pressure mea-
sured in the tail with an indirect method is slightly higher in the prehypertensive rats (MHS, 122 ± 7.6 mm Hg; MNS, 114 ± 5.5 mm Hg; mean ± SEM of 11 determinations; values not significantly different with t test). The animals from the two strains used for each experiment were reared contemporaneously under the same conditions. Preparation of BBMV from kidney cortex slices was always run in parallel on the same day from animals of both strains, using the differential centrifugation method described by Malathi et al., with the following modifications: 1) tissue homogenization was performed in 50 mM mannitol, 5 mM N-2-hydroxyethylpiperazine-N'-2-ethanesulfonic acid (HEPES)-tris(hydroxymethyl)aminomethane (Tris) buffer, pH 7.0; 2) MgCl₂ was used instead of CaCl₂ in the preparation of BBMV for sodium transport experiments; 3) the pellet from the second centrifugation step and the final pellet were resuspended in the buffer used in transport experiments, whose composition is reported in table and figure legends. For each preparation of BBMV, the kidneys from four to six rats were used, with a yield of about 4 mg of brush border proteins per gram of kidney cortex. Protein concentration in these preparations was 10 to 15 mg/ml.

**Transport Experiments**

Uptake of solutes by the suspension of BBMV was measured by a rapid filtration technique at room temperature. In the absence of ionic gradients or when the effect on an inwardly directed ionic gradient was studied, 1 volume of BBMV suspended in 200 mM mannitol and 10 mM HEPES-Tris buffer, pH 7.0, was mixed with 1 volume of a cocktail containing the same buffer, the labeled solute, and the additions reported in table or figure legends. When the effect of an outwardly directed ionic gradient was studied, 1 volume of BBMV suspended in a buffer containing the gradient-generating ion was mixed with 4 volumes of a cocktail containing the labeled solute and the additions reported in table or figure legends. Final radioactivity of the labeled solute was 10 to 15 µCi/ml. At selected times, 20 or 50 µl samples were withdrawn from the incubation mixture, diluted with 2.0 ml of ice-cold stop solution, and immediately filtered through a prewetted filter of cellulose nitrate (0.65 µm pore size; Sartorius, Göttingen, West Germany). The filter was washed twice with 5 ml of the stop solution, put into a vial, and counted by means of a liquid scintillation spectrometer. The time elapsed between adding the stop solution and terminating the two washings was less than 10 seconds. In this experiment, each measurement was carried out in quadruplicate. For glucose uptake experiments, the composition of the stop solution was 150 mM NaCl and 1 mM HEPES-Tris, pH 7.0; for sodium uptake experiments, MgCl₂ was substituted for NaCl. Blanks were determined by adding the cocktail containing the substrate to the stop solution. The cpm values were transformed into picomoles of solute taken up by calculating the specific radioactivity of the solute (cpm/mole) in the incubation mixture and refer to milligrams of protein in the sample. Protein determination was carried out according to the method of Bradford, using a kit from Bio-Rad (Richmond, CA, USA).

**Enzyme Activity Determination**

The membrane preparation was evaluated by measuring the specific activities of marker enzymes. Maltase activity was assayed according to the method of Semenza and Von Balthazar, with maltose used as substrate, and the glucose oxidase/peroxidase method (Boehringer test combination, Boehringer, Mannheim, West Germany). Alkaline phosphatase, leucine aminopeptidase, and γ-glutamyltransferase were determined with Boehringer kits, using p-nitrophenyl phosphate, leucine p-nitroanilide, and L-γ-glutamyl-3-carboxy-4-p-nitroanilide as substrates, respectively. Lactate dehydrogenase was assayed according to the method of Bergmeyer and Bernt. Cytochrome C oxidase was assayed according to the method of Smith. Acrylamide concentration was 4.5% in the stacking gel and varied exponentially from 0 to 30% in the running gel. Scanning of the stained gels was performed by a Perkin-Elmer Lambda 3 spectrophotometer (Beaconsfield, Buckinghamshire, England) equipped with a gel scanner.

**Electrophoresis**

Sodium dodecyl sulfate (SDS)-polyacrylamide gradient gel electrophoresis was performed in a discontinuous system, as described by Arosio et al. Acrylamide concentration was 4.5% in the stacking gel and varied exponentially from 0 to 30% in the running gel. All high molecular weight marker proteins for SDS-polyacrylamide gel electrophoresis were from Pharmacia (Uppsala, Sweden), except cytochrome C and chymotrypsinogen, which were from Boehringer. All other reagents were analytical grade products from Merck (Darmstadt, West Germany).

**Drugs**

[U-14C]Glucose, 257 mCi/mmol, and NaCl (carrier free) were obtained from Radiochemical Centre (Amersham, Buckinghamshire, England). Valinomycin, HEPES buffer, 2-(N-morpholinio)-ethanesulfonic acid, and reagents for enzyme activity measurements were from Boehringer. Carbonyl cyanide p-trifluoromethoxy-phenylhydrazone (FCCP) and ouabain were obtained from Sigma (St. Louis, MO, USA). All high molecular weight marker proteins for SDS-polyacrylamide gel electrophoresis were from Pharmacia (Uppsala, Sweden), except cytochrome C and chymotrypsinogen, which were from Boehringer. All other reagents were analytical grade products from Merck (Darmstadt, West Germany).

**Statistics**

All values in the tables are the means ± SEM of results obtained in independent experiments from triplicate or quadruplicate measurements. Statistical comparison between specific activities of marker enzymes in MNS and MHS (i.e., Table 1 vs Table 2) was performed with Student’s t test. Statistical analysis of
RESULTS

Vesicle Characteristics

Vesicles obtained from renal cortical slices of prehypertensive and normotensive rats were constituted mainly of brush border membranes. Tables 1 and 2 show that in both rat strains brush border marker enzymes were enriched by a factor higher than 10 in BBMV prepared by the Ca precipitation method, whereas marker enzymes of the other cellular compartments showed an enrichment factor lower than 1. In BBMV prepared by the Mg precipitation method, enrichment factors were substantially the same as before, except for Na⁺, K⁺-ATPase, which showed an enrichment factor higher than 1, as already described by Biber et al.15 Both specific activities and enrichments of the brush border marker enzymes were not statistically different between preparations of BBMV obtained from the two rat strains, indicating that these preparations were suitable for comparison studies in solute transport rates.

Glucose Transport

The BBMV prepared by the Ca precipitation method exhibited a typical sodium gradient coupled glucose transport (Figure 2). Both the initial rate and the overshoot values depended on the sodium counterion permeability, as described for adult rats.16 A statistical evaluation of the differences in glucose uptake between strains was performed by comparing the uptake at 15 seconds in the presence of Na thiocyanate (SCN), NaCl, and Na₂SO₄ gradients (Table 3). In all cases no difference was observed between normotensive and prehypertensive rats. Since the binding of D-glucose to BBMV is negligible,16 the internal volume of vesicles can be calculated from the equilibrium uptake value (60-minute incubation). Its values were 0.94 ± 0.06 and 1.12 ± 0.12 (n = 6) μl/mg protein for normoten-
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Sodium and prehypertensive rats, respectively. Essentially the same results were obtained with BBMV prepared by the Mg precipitation method in the presence of an inwardly directed NaSCN gradient (see Figure 2 inset).

The dependence of D-glucose uptake on the external sodium was determined by varying sodium ion concentration at 0.1 mM D-glucose and at a constant transmembrane electrical potential difference. This was accomplished by maintaining the thiocyanate ion concentration by additions of KSCN. The resulting data are reported in Figure 3. Glucose initial uptake showed saturation kinetics with respect to the sodium, and a small portion of sodium-independent transport was also evident. The \( V_{max} \) values were calculated, after subtracting the portion of the sodium-independent uptake, by a double reciprocal plot and were 1.12 nmol/5 sec/mg protein for normotensive rats and 0.98 nmol/5 sec/mg protein for prehypertensive rats. These values were used to draw the Hill plot shown in the inset in Figure 3. From this plot an \( (S)_0 \) of 43.35 ± 3.77 mM for normotensive rats and of 44.06 ± 2.48 mM for prehypertensive rats was calculated by linear regression analysis. Hill coefficients were higher than 1 in both strains (i.e., 1.42 ± 0.07 and 1.33 ± 0.04 for normotensive and prehypertensive rats, respectively). The \( (S)_0 \) and Hill coefficient values were not significantly different between the two rat strains.

**Sodium Transport**

Sodium uptake experiments were performed using vesicles prepared with MgCl\(_2\) instead of CaCl\(_2\), because experiments using acridine orange fluorescence quenching have shown that calcium increases sodium permeability in BBMV from kidney of adult rats. In the presence of an inwardly directed sodium gradient, sodium uptake depended on the sodium counterion. Table 4 shows sodium uptake in the presence of NaSCN, NaCl, and Na\(^{2+}\)SO\(_4\), at 10 and 100 mM sodium. The uptake value was increased by the relative permeabilities of the anions (SCN\(^-\) > Cl\(^-\) > SO\(_4^{2-}\)), especially at 100 mM sodium. The type of counterion and the salt concentration had an influence on the difference between the two strains. In fact, at 100 mM NaCl, sodium uptake by the prehypertensive BBMV was 26% faster than by normotensive BBMV, and this difference was statistically significant. The difference between strains was not statistically significant at 10 mM sodium or in the presence of SCN\(^-\) and SO\(_4^{2-}\).

The role of electrical potential difference on sodium transport was investigated by imposing a transmembrane electrical potential difference, inside-negative, caused by a diffusion potential of a cation from the interior of BBMV. This was accomplished either by a gradient of KCl (in > out) in the presence of valinomycin, which selectively enhances the permeability of K\(^+\), or by a pH gradient (in < out) in the presence of the uncoupler FCCP, which selectively enhances the permeability of H\(^+\). The experiments were performed at two sodium concentrations (Table 5). At 1 mM sodium, about a twofold stimulation of the sodium uptake by a pH gradient in the absence of FCCP was observed in both rat strains with respect to the control in the absence of a pH gradient (see Table 5). In fact, at this sodium concentration, sodium uptake is largely

**Figure 1.** Sodium dodecyl sulfate-polyacrylamide gradient gel disc electrophoresis of brush border membrane vesicles (BBMV) prepared by Ca precipitation from kidney cortex of MNS and prehypertensive MHS. Upper panel: gel photograph of standard proteins (Lane 1): cytochrome C (a; Mr, 12,500), apoferritin (b; Mr, 18,500), chymotrypsinogen (c; Mr, 25,000), lactate dehydrogenase (d; Mr, 36,000), catalase (e; Mr, 60,000), and albumin (f; Mr, 67,000); BBMV from MNS (Lane 2), 50 \\( \mu \)g protein; BBMV from MNS (Lane 3), 100 \\( \mu \)g protein; BBMV from MNS (Lane 4), 50 \\( \mu \)g protein; BBMV from MHS (Lane 5), 100 \\( \mu \)g protein. Lower panel shows scanning of Lanes 3 and 5.
FIGURE 2. Time course of $d$-glucose uptake in brush border membrane vesicles from kidney cortex of MNS (closed symbols) and prehypertensive MHS (open symbols). The incubation medium contained Ca-prepared brush border membrane vesicles, 200 mM mannitol, 10 mM HEPES-Tris (pH 7.0), 0.1 mM $d$-$[^{14}C]$glucose, and 100 mM NaSCN ($\bullet$, $\square$), 100 mM NaCl ($\bullet$, $\circ$), or 50 mM Na$_2$SO$_4$ ($\triangle$, $\triangle$). Inset: Typical $d$-glucose uptake by Mg-prepared brush border membrane vesicles in the presence of 100 mM NaSCN.

TABLE 3. $d$-Glucose Uptake by Brush Border Membrane Vesicles from Kidney Cortex of MNS and Prehypertensive MHS

<table>
<thead>
<tr>
<th>Salt</th>
<th>Glucose uptake (nmol/15 sec/mg protein)</th>
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<tbody>
<tr>
<td></td>
<td>MNS</td>
</tr>
<tr>
<td>NaSCN</td>
<td>0.885 ± 0.036</td>
</tr>
<tr>
<td>NaCl</td>
<td>0.638 ± 0.025</td>
</tr>
<tr>
<td>Na$_2$SO$_4$</td>
<td>0.332 ± 0.062</td>
</tr>
</tbody>
</table>

The incubation medium contained Ca-prepared brush border membrane vesicles, 200 mM mannitol, 10 mM HEPES-Tris (pH 7.0), 0.1 mM $d$-$[^{14}C]$glucose, and the indicated salt at 100 mM Na$^+$ concentration. Values are the means ± SEM of five independent experiments. Statistical analysis was carried out by a two-way analysis of variance and Tukey’s test and showed no significant difference between strains.

accounted for by the Na$^+$-H$^+$ antiport.$^{18}$ A further increase in sodium entry was obtained by the addition of FCCP, indicating the presence of a sodium pathway dependent on an electrical potential difference. The electrical potential difference induced by a potassium gradient plus valinomycin did not stimulate sodium uptake, which was always lower than that in the control phase. At 1 mM NaCl, sodium uptake by MHS was higher in all conditions than that by MNS, but these differences were not statistically significant.

At 10 mM NaCl, the pattern was qualitatively similar to that obtained at 1 mM NaCl, except that an inside-negative electrical potential difference, generated by the diffusion potential of K$^+$, stimulated sodium entry. In all conditions sodium uptake by MHS was faster than that by MNS, and this difference was statistically significant in the presence of a pH gradient with or without the addition of FCCP (37 and 39% increase).

Discussion

The main goal of this work was the preparation of BBMV from kidney of MNS and MHS to study possible differences in the sodium pathways across the luminal membrane of the proximal tubule between the two rat strains. The present results clearly demonstrate that under specific experimental conditions sodium uptake by BBMV from MHS is significantly faster than that by BBMV from MNS. On the other hand, the enzyme profile and the kinetics of the sodium-dependent $d$-glucose transport were similar in BBMV from both strains. In addition, the vesicle volume, calculated from the $d$-glucose equilibrium uptake, was similar in both strains with either Ca or Mg precipitation. Therefore, the difference in sodium transport across the brush border membrane seems to be rather selective, even though the wide spectrum of all the other transport pathways was not entirely explored.

In the presence of an inwardly directed sodium ion gradient, sodium entry was sensitive to the relative anion permeability, especially at high salt concentration (see Table 4; see also Reference 19). But the difference observed between MNS and MHS was significant only when the counterion was chloride and sodium concentration was high. This effect of chloride might suggest an involvement of an NaCl neutral symport in sodium uptake by membrane vesicles of MHS, but no evidence is yet available about the occurrence of an NaCl symport in the mammalian proximal tubule.$^{20,21}$ Therefore, more experiments are needed to assess a possible role of this pathway in the difference between MHS and MNS.

A more relevant difference in sodium transport between MHS and MNS was observed in the presence of a pH gradient and an electrical potential difference (inside-negative) across the vesicle membrane and be-
came statistically significant at high sodium concentration. It should be emphasized that a potential-driven sodium transport has been shown to be operative not only in isolated perfused proximal tubules, but also in BBMV from renal cortex. In the experimental conditions reported in Table 4, a portion of the sodium uptake is due to the activity of the Na\(^+\) - H\(^+\) antiport, which is an important pathway in sodium reabsorption by proximal tubule luminal membrane. However, the observed differences between strains could be directly ascribed to the antiport only by supposing that its activity in MHS is more dependent on the electrical potential difference than its activity in MNS. Moreover, the dependence of the difference between strains on the sodium concentration suggests that it could be related to the sodium pathway (or pathways) differing in capacity rather than in affinity for sodium.

What is the relationship between the present results and previous results obtained by comparing sodium handling by the kidney or cell membrane sodium transport in the erythrocytes of MHS and MNS? The following experimental results support the notion that sodium transport across renal tubules of prehypertensive MHS is faster than that across tubules of MNS. First, the whole kidney glomerular filtration rate measured in conscious rats is faster in MHS, whereas sodium balance studied in metabolic cages showed that, at this age, renal sodium excretion is similar in MHS and MNS. Second, the absolute amount of tubular sodium reabsorption and oxygen consumption measured in isolated kidneys perfused in vitro is greater in MHS than in MNS. Tubular sodium reabsorption may occur through both cellular and paracellular pathways. All the cellular sodium reabsorption occurs across the luminal membranes; therefore, the present findings described in isolated luminal membranes suggest that the cellular sodium reabsorption pathways are increased in MHS. The luminal membranes used in the present study were derived from proximal tubules; the results we have thus far obtained on tubular sodium reabsorption in both conscious animals and isolated kidneys do not allow us to localize the portion of the nephron...
where the enhanced sodium reabsorption occurs. However, cell volume and sodium content in proximal tubules were lower in MHS than in MNS, while no difference was detected in distal tubules. These results are in keeping with the hypothesis that proximal rather than distal tubules are more likely to be abnormal in MHS. Therefore, the faster sodium transport across BBMV is consistent with previous findings on the function of the whole kidney, either isolated or in situ.

The present results do not allow differentiation between primary, genetically determined alterations in sodium transport across the luminal membrane and secondary alterations caused by humoral, nervous, or load factors existing prior to the BBMV preparation and persisting in vitro because of changes in cell membrane composition. However, the following two observations suggest that the differences we found between the BBMV of MHS and MNS may be considered part of the phenotypic expression of cell membrane genetic differences present also at the level of the kidney and erythrocyte functions. First, the results on kidney transplantation and on kidney function obtained either before or after the development of hypertension in MHS are consistent with the hypothesis of a primary and genetically determined increase in tubular reabsorption (for details, see References 2 and 5). Second, although all the possible sodium transport pathways across kidney BBMV were not fully clarified in the present study, one pathway of sodium transport (or pathways) across the isolated luminal membrane of the proximal tubular cells. Such a difference is consistent with previously described abnormalities in sodium and water handling by kidneys of MHS.

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