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Presence of a potential-sensitive Na^+ transport across renal brush-border membrane vesicles from rats of the Milan hypertensive strain

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Sodium transport was measured in brush-border membrane vesicles prepared from kidney cortex of the Milan hypertensive strain (MHS) rats and the corresponding normotensive controls. In the presence of an outwardly directed proton gradient, ^{22}Na was transiently accumulated in the vesicles. When a transmembrane electrical potential was imposed across membrane vesicles, both the accumulation ratio and the initial uptake were increased, indicating the presence of an electrogenic pathway for sodium in these membranes. The potential-dependent sodium uptake was significantly higher in MHS rats. Kinetic analysis give simple Michaelis Menten curves in the presence and in the absence of a membrane potential. In both conditions J_{max} was significantly increased in MHS rats, whereas K_m was the same for the two rat strains. Sodium uptake was inhibited by amiloride at concentrations that inhibit Na^+-H^+ exchange. The presence of the higher, potential-sensitive, sodium uptake in MHS is in agreement with studies on renal physiology which support the hypothesis that an increase in tubular sodium reabsorption may be the primary cause for the development of hypertension in this rat strain.

Introduction

In the Milan hypertensive strain (MHS) of rats the blood pressure is similar to that of the control Milan normotensive strain (MNS) rats, until the fourth week of age. From 22 to 40 days of age a marked difference in blood pressure between MHS and MNS rats develops, with a maximum difference of 40–50 mmHg systolic blood pressure [1,2]. Studies carried out at the prehypertensive and hypertensive stages of MNS and MHS rats showed that an abnormality in sodium handling by the kidney may be the cause of this type of genetic hypertension in rats. The most probable explanation for this abnormality seems to be a primary and genetically determined increase in sodium reabsorption across the tubular epithelium of MHS rats at the prehypertensive stage [1–6].

The experiments reported in this paper were designed to compare sodium transport across brush-border membranes prepared from the kidney of MNS and MHS rats at the prehypertensive stage, in order to elucidate the role of proximal tubular sodium reabsorption in the pathogenesis of hypertension in MHS rats.

Abbreviations: FCCP, carbonyl cyanide *p*-trifluoromethoxyphenyl hydrazone; Hepes, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid; Mes, 4-(morpholine)ethanesulfonic acid; MHS, Milan hypertensive strain; MNS, Milan normotensive strain.

Materials and Methods

Materials

$^{22}NaCl$ (carrier free) was obtained from Radiochemical Centre (Amersham International,

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Amersham, U.K.); valinomycin, Hepes and Mes were from Boehringer (Mannheim, F.R.G.); FCCP from Sigma (St. Louis, MO). All other reagents were analytical grade products from Merck (Darmstadt, F.R.G.).

Brush-border membrane vesicles preparation

Young male MHS rats in the prehypertensive stage (four weeks old, body weight 60–70 g), and corresponding MNS control rats were used. Brush-border membrane vesicles from kidney cortex slices were isolated by differential centrifugation, using the method described by Malathi et al. [7], with the following modifications: (i) homogenization buffer was 50 mM mannitol/5 mM Hepes-Tris (pH 7.0); (ii) to effect precipitation of nonbrush-border membranes, 10 mM $MgCl_2$ was used instead of $CaCl_2$; (iii) the pellet from the second centrifugation step and the final pellet were resuspended in 193 mM mannitol/90 mM Mes/17 mM Tris (pH 5.5). For each preparation the kidneys from 4–6 rats were used, with a yield of about 4 mg of brush-border proteins per g of kidney cortex. Protein concentration in the brush-border membrane vesicles preparations was 10–15 mg/ml. The specific activities of marker enzymes of the brush border (maltase, γ-glutamyltransferase and leucine aminopeptidase) were not statistically different between rat strains and were 10–15-fold enriched with respect to the crude homogenate. The comparison between the physiological characteristics of the preparations was performed by studying the Na^+-dependent D-glucose transport in the presence of gradients of different sodium salts, according to Kessler et al. [8]. The D-glucose uptake was very similar in both strains.

$^{22}Na^+$ uptake determination

Uptake of $^{22}Na^+$ was measured by a rapid filtration technique at 10°C. Brush-border membrane vesicles suspended in 193 mM mannitol/90 mM Mes/17 mM Tris (pH 5.5) were diluted 1 : 5 with a cocktail containing $^{22}Na^+$/160 mM mannitol/90 mM Hepes/45 mM Tris (pH 7.5); the additions are reported in the tables or figure legends; final pH was 7.2. Ionophores, when present, were added from ethanol stocks so that the ethanol concentration in the incubation mixture did not exceed 0.5%. At selected times, 50 μl samples were withdrawn from the incubation mixture, diluted with 2.0 ml of ice-cold stop solution, containing 150 mM $MgCl_2$/1 mM Hepes-Tris (pH 7.0), immediately filtered through on a prewetted cellulose nitrate filter (0.45 μm pore size. Micro Filtration Systems Dublin, CA). 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. Incubation, for times shorter than 10 s, was carried out with an automated apparatus consisting of a timer which controls both a shaker (Vibrofix VFI, Janke & Kunkel Ika Werk, Staufen, F.R.G.) and an injector (Automatic Dispensor, Oxford, Athy, Ireland). A drop of the cocktail (40 μl) and a drop of brush-border membrane vesicles suspension (10 μl) were placed on the bottom of a tube fitted into the shaker. At the start of the timer, the shaker was switched on for 0.5 s at about 2000 rpm and the two drops rapidly mixed. At the chosen incubation time, 2 ml of ice-cold stop solution were automatically injected into the test tube by the dispensor at maximum speed. The sample was then filtered as described above. The time necessary for complete mixing and stopping the reaction was 0.17 ± 0.07 s and was determined by the time-course of an enzymatic reaction as indicated by Kessler et al. [9]. Cpm values were transformed into pmoles of solute taken up by calculation of the specific radioactivity (cpm/pmole) of $^{22}Na^+$ in the incubation mixture and referred to the mg protein value in the sample. Protein determination was carried out according to Bradford [10], using a Bio-Rad kit.

Results

The experiment illustrated in Fig. 1 shows the time-course of the sodium uptake when a pH gradient ($5.5_{in}/7.2_{out}$) was imposed across brush-border membrane vesicles at 1 mM external $^{22}Na^+$. In these conditions (closed symbols), a transient accumulation of sodium higher in hypertensives was observed, which can be attributed to the activity of the Na^+-H^+ antiporter. The long stationary phase of the sodium overshoot could be due to the high capacity of the buffer inside the vesicles. By the addition of the proton ionophore FCCP, the proton permeability increased together with the intravesicular negativity. In this condition, both

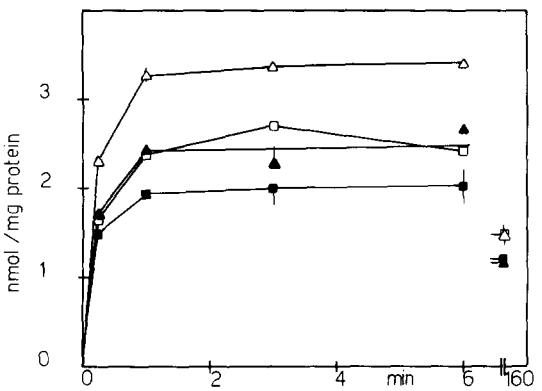

Fig. 1. Time-course of ΔpH-driven sodium uptake in brush-border membrane vesicles from kidney cortex of MNS (closed symbols) and prehypertensive MHS (open symbols) rats. The uptake was determined at 1 mM ^{22}NaCl and in the presence of a pH gradient ($5.5_{in}/7.2_{out}$). Brush-border membrane vesicles suspended in 193 mM mannitol/90 mM Mes/17 mM Tris (pH 5.5), were diluted 1 : 5 with 160 mM mannitol/90 mM Hepes/45 mM Tris (pH 7.5)/1.25 mM ^{22}NaCl in the absence (■, □) or in the presence (▲, △) of 100 μM FCCP (final concentration). Final pH was 7.2. Mean of four determinations ± S.E.; when not given, the S.E. bar was smaller than the symbol used.

the initial sodium uptake and the sodium accumulation inside the vesicles were consistently stimulated in both strains, with an effect higher in hypertensives.

In the time-course reported in Fig. 1, the first drawing was at 15 s, a time already too long for precise measure of the initial sodium influx into the vesicles. Therefore short-time measurements have been carried out, with times not exceeding 10 s. The results are reported in Fig. 2A for 1mM NaCl and in Fig. 2B for 10 mM NaCl. At 1 mM NaCl, sodium uptake increased as a linear function of time almost until 7 s under all conditions. The difference between rat strains in the presence of pH gradient but without FCCP (i.e., in the same standard conditions as for the Na^+-H^+ exchange measurement) was negligible. However, a consistent, much higher stimulation by FCCP of the initial sodium influx was observed in prehypertensives, so that the difference between the strains became highly significant. Similar results were obtained at 10 mM NaCl (Fig. 2B), but with higher uptake rates and with the linear portion of the curve shorter than at 1 mM NaCl. Therefore, for an evaluation of the initial sodium influx, incubation time must not exceed 5 s. The stimulation by FCCP was higher than at 1 mM NaCl, as was the difference between the two strains in the presence of the uncoupler.

The slope of the linear part of each uptake curve (Fig. 2) corresponded to the initial rate of sodium transport; the extrapolation of this linear part of the curve to zero incubation time gave a non-zero positive intercept on the ordinate, indicating that a portion of sodium associated with brush-border membrane vesicles was not taken up in the internal space of the vesicles, but it was bound to the membranes. Actually, it has been reported that, at equilibrium, about 40% of the sodium associated with brush-border membrane vesicles is independent of the osmolarity of the

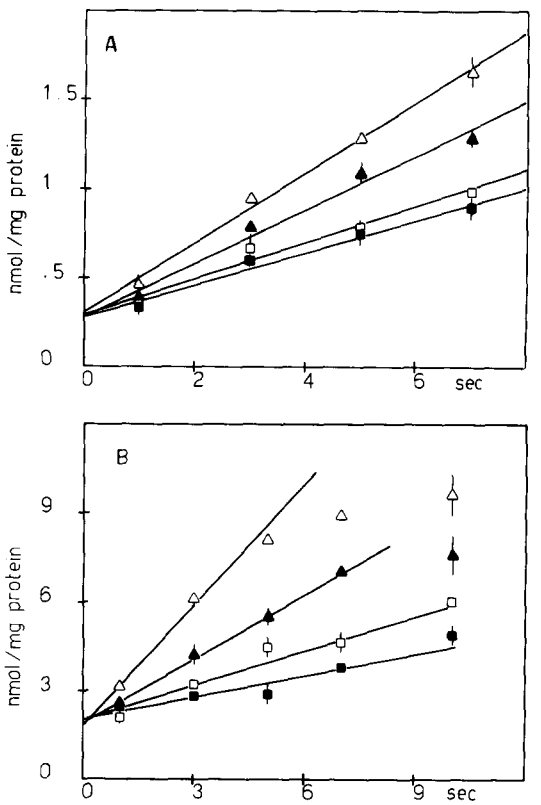

Fig. 2. Time-course of ΔpH-driven sodium uptake in brush-border membrane vesicles from kidney cortex of MNS (closed symbols) and prehypertensive MHS (open symbols) rats. The uptake was determined at 1 mM ^{22}NaCl (A) and 10 mM ^{22}NaCl (B), with a pH gradient ($5.5_{in}/7.2_{out}$), in the absence (■, □) or in the presence (▲, △) of 100 μM FCCP. The pH gradient was obtained as described in Fig. 1. Mean of four determinations ± S.E.; when not given, the S.E. bar was smaller than the symbol used.

TABLE I

INTERCEPTS AND SLOPES OF THE TIME-COURSE OF ΔpH-DRIVEN SODIUM UPTAKE IN BRUSH-BORDER MEMBRANE VESICLES FROM KIDNEY CORTEX OF MNS AND PREHYPERTENSIVE MHS RATS

Slopes are expressed as nmol/s per mg protein ± S.E. Intercepts are expressed as nmol/mg protein ± S.E. Their values were obtained by regression analysis of the linear part of the curves reported in Figs. 2A and 2B for 1 mM and 10 mM NaCl, respectively. The comparison between MNS and MHS rats was carried out with the *t*-test; n.s., not significant.

	ΔpH			ΔpH + FCCP		
	MNS rats	MHS rats	*P*	MNS rats	MHS rats	*P*
1 mM NaCl						
Slope	0.093 ± 0.010	0.102 ± 0.014	n.s.	0.151 ± 0.015	0.197 ± 0.010	< 0.05
Intercept	0.27 ± 0.05	0.29 ± 0.06	n.s.	0.28 ± 0.07	0.30 ± 0.05	n.s.
10 mM NaCl						
Slope	0.267 ± 0.045	0.422 ± 0.047	< 0.05	0.737 ± 0.025	1.236 ± 0.143	< 0.01
Intercept	1.99 ± 0.27	1.92 ± 0.28	n.s.	1.90 ± 0.11	2.10 ± 0.49	n.s.

external medium [11]. The binding value can be easily calculated from the intercept on the vertical axis [12]. Slope (initial rate) and intercept (binding) values calculated by linear regression analysis of the linear part of time courses shown in Fig. 2 are reported in Table I. From this table it appears that the binding of sodium to the membranes was the same, irrespectively of the rat strain and experimental conditions, but its value depended on the sodium concentration. The initial rates of sodium uptake, expressed as the slope of the linear part of the time courses, were significantly different in prehypertensive and normotensive rats in the presence of FCCP at both NaCl concentrations and also in the absence of FCCP at 10 mM NaCl.

TABLE II

EFFECT OF VOLTAGE CLAMP ON THE ΔpH-DRIVEN SODIUM UPTAKE IN BRUSH-BORDER MEMBRANE VESICLES FROM KIDNEY CORTEX OF MNS AND PREHYPERTENSIVE MHS RATS

Sodium uptake was determined at 10 mM ^{22}NaCl and in the presence of a pH gradient ($5.5_{in}/7.2_{out}$) ± 100 μM FCCP. The pH gradient was obtained as described in Fig. 1. In voltage clamp conditions, 50 mM potassium chloride and valinomycin (10 μg/mg protein) were added to brush-border membrane vesicles and cocktail. Uptake is expressed as nmol/3 s per mg protein. Mean of four determinations ± S.E. The comparison between MNS and MHS was carried out with the *t*-test; n.s., not significant.

	MNS rats	MHS rats	*P*
ΔpH	3.24 ± 0.41	5.73 ± 0.38	< 0.01
ΔpH + FCCP	5.53 ± 0.24	9.47 ± 0.22	< 0.001
ΔpH (volt. clamp)	4.42 ± 0.86	5.53 ± 0.44	n.s.
ΔpH + FCCP (volt. clamp)	5.77 ± 0.71	6.77 ± 0.38	n.s.

The occurrence of a higher, membrane potential-sensitive, sodium transport in prehypertensive MHS rats became more evident under voltage clamp conditions, i.e., when measured with K^+ on both sides of the vesicle and in the presence of valinomycin (Table II). Under these conditions, any membrane electrical potential difference caused by rheogenic sodium transport or by proton conductance would be compensated by a net movement of K^+, whose permeability has been increased by the ionophore valinomycin. In these conditions, the effect of the pH gradient on the sodium transport disappeared, as did the difference between the studied rat strains. The sodium uptake due to the indirect coupling between the Na^+ conductance and an intrinsic H^+ conductance of the membrane could be evaluated, by comparing the values of the uptake in the presence of pH gradient ± potassium and valinomycin (lines 1 and 3 of Table II). From the obtained data, the proton conductance appears to be negligible in both strains since the shunt of the electrical potential difference across the membrane promoted by K^+ and valinomycin did not decrease sodium uptake.

In Fig. 3A the kinetics of sodium uptake was studied with respect to the external sodium con-

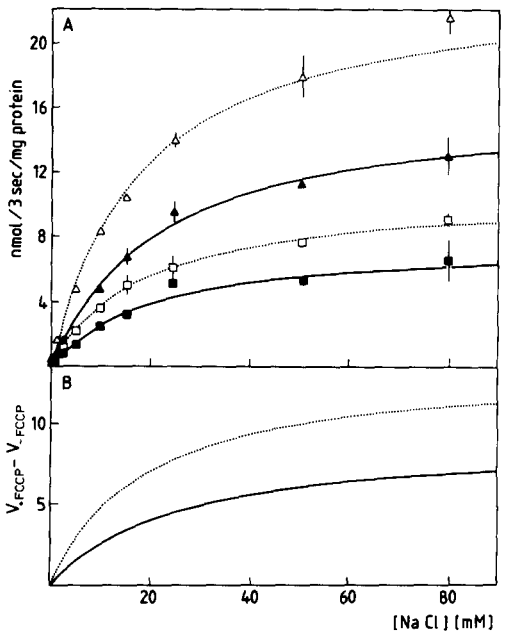

Fig. 3. Effect of external sodium concentration on the initial rate of ΔpH-driven sodium uptake in brush-border membrane vesicles from kidney cortex of MNS (closed symbols, continuous lines) and prehypertensive MHS (open symbols, dotted lines) rats. The uptake was determined at the indicated NaCl concentrations, with a pH gradient ($5.5_{in}/7.2_{out}$), in the absence (■, □) or in the presence (▲, △) of 100 μM FCCP. The pH gradient was obtained as described in Fig. 1. (A) Initial rate (nmol/3 s per mg protein) as a function of external NaCl concentration. Theoretical curves were drawn by a computer program, utilizing kinetic parameters reported in Table III. Mean of four determinations ± S.E.; when not given, the S.E. bar was smaller than the symbol used. (B) Differences between the curves obtained in the presence and in the absence of FCCP for MNS (continuous line) and MHS (dotted line) rats.

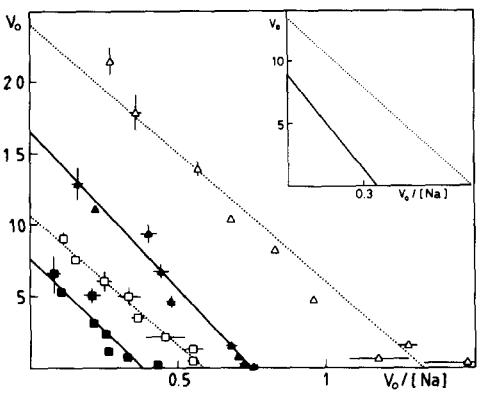

Fig. 4. Eadie-Hofstee plot of the data reported in Fig. 3A. V_0 = initial sodium uptake (nmol/3 s per mg protein). In the inset, the curves reported in Fig. 3B were linearized.

centration, in the presence or in the absence of FCCP and at 3 s of incubation. The initial rate of the sodium influx showed a saturation kinetics in the presence and in the absence of FCCP. At the chosen incubation time, no diffusional component of the uptake was evidenced. In fact, uptake values, without any correction, plotted in the Eadie-Hofstee plot reported in Fig. 4, gave straight lines with determination coefficients between 0.86 and 0.98. From the same linear regression analysis the values of $K_{0.5}$ and J_{max} were obtained (Table III). These kinetic parameters have been used to draw the hyperbolic curves in Fig. 3, which appears to fit fairly well with experimental data. By an appropriate computer program, for each rat strain the difference was obtained between the curve in the presence of FCCP and in the absence of the

TABLE III

KINETIC CONSTANTS OF THE ΔpH-DRIVEN SODIUM UPTAKE IN BRUSH-BORDER MEMBRANE VESICLES FROM KIDNEY CORTEX OF MNS AND PREHYPERTENSIVE MHS RATS

The constants were calculated from Fig. 4 for −FCCP and +FCCP conditions, and from the inset of Fig. 4 for the ΔFCCP condition. $K_{0.5}$ is expressed as mM ± S.E. J_{max} is expressed as nmol/3 s per mg protein ± S.E. The comparison between MNS and MHS rats was carried out with the *t*-test; n.s., not significant.

	$K_{0.5}$			J_{max}		
	MNS rats	MHS rats	*P*	MNS rats	MHS rats	*P*
−FCCP	19.31 ± 3.15	18.08 ± 1.01	n.s.	7.74 ± 0.82	10.68 ± 0.38	< 0.02
+FCCP	22.14 ± 1.34	18.13 ± 1.64	n.s.	16.62 ± 0.73	24.11 ± 1.52	< 0.01
ΔFCCP	25.35	18.15		8.94	13.43	

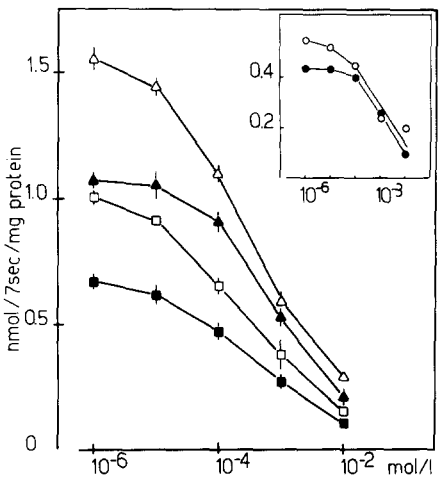

Fig. 5. Effect of amiloride on the ΔpH-driven sodium uptake in brush-border membrane vesicles from kidney cortex of MNS (closed symbols) and prehypertensive MHS (open symbols) rats. The uptake was determined at 7 s incubation and at 1 mM ^{22}NaCl, with a pH gradient ($5.5_{in}/7.2_{out}$), in the absence (■, □) or in the presence (▲, △) of 100 μM FCCP and of increasing amiloride concentrations. The pH gradient was obtained as described in Fig. 1. Mean of four determinations ± S.E.; when not given, the S.E. bar was smaller than the symbol used.

uncoupler. The differential curves are reported in Fig. 3B, their linearized form in the inset of Fig. 4, and the corresponding kinetic parameters in Table III. As is evident, the extra sodium influx promoted by electrical potential difference also showed a saturation kinetics.

The main difference induced by the potential difference generated by the H^+ diffusion concerned the J_{max}, which showed an approximate 2-fold increase. J_{max} was higher in prehypertensives than in normotensive controls in the absence of potential, but this difference increased in the presence of the uncoupler. In contrast, $K_{0.5}$ values for sodium were always the same, whether or not a potential difference was present. As regards the extra sodium influx generated by the potential difference $K_{0.5}$ for sodium was lower and J_{max} was higher in MHS than in MNS rats.

The stimulation by FCCP of sodium uptake could be due in principle (i) to a potential-sensitive pathway different from Na^+-H^+ exchange or (ii) to the same Na^+-H^+ exchanger, whose activity became sensitive to membrane potential or changed its stoichiometry Na^+/H^+ to a value higher than 1. To distinguish between these two alternatives, the sensitivity to amiloride of the sodium uptake was tested by exponentially increasing the amiloride concentration from 10^{-6} to 10^{-2} M. The data are reported in Fig. 5 and they show that in both strains the initial sodium uptake was sensitive to amiloride in the presence and in the absence of FCCP. Therefore, extra sodium uptake stimulated by the electrical potential difference was also amiloride-sensitive (Fig. 5, inset). From the curves reported in Fig. 5, rough K_i values for amiloride were evaluated, for the different experimental conditions, by aid of the Hill equation

$$\log[(v_0 - v_i)/v_i] = \log K_i + n \log[\text{amiloride}]$$

where v_0 and v_1 represent uptake rates in the absence and in the presence of amiloride. The mM K_i values obtained for MNS and MHS rats were, respectively: in the absence of FCCP, 0.83 and 0.43; in the presence of FCCP, 1.17 and 0.69; for the component stimulated by FCCP, 1.96 and 1.63.

Discussion

Many transcellular routes of sodium reabsorption in the proximal convoluted tubule have been ascertained: cotransport with organic metabolites and inorganic ions; Na^+-H^+ exchange; and simple rheogenic sodium transport [13–16]. The occurrence of the first two pathways has been demonstrated with isolated proximal tubules and with brush-border membrane vesicles from kidney cortex, whereas evidence for a simple rheogenic transport was obtained only from studies on isolated tubules. No evidence has been obtained for the occurrence in the proximal tubule of the neutral NaCl cotransport, which, on the contrary, has been demonstrated in the distal tubule. The correlation between the simple rheogenic sodium transport and some sodium-transporting pathways in brush-border membrane vesicles has not been yet clearly explained. If this pathway definitely exists also in brush-border membrane vesicles, it should appear as a potential-dependent sodium uptake into the vesicles. A pathway endowed with such characteristics has been observed in brush-border membrane vesicles using ^{22}Na as a tracer or moni-

toring acridine orange fluorescence quenching [17–20]. The electrical potential difference has been generated by potassium plus valinomycin, by a pH gradient, or by gradients of sodium salts with permeant anions such as SCN^- or Cl^-. In the reported studies the potential dependent sodium uptake has been further identified as the non-saturable and diffusible component of sodium uptake. Sabolic and Burckhardt [21] have pointed out that the conductances for various ions, sodium included, depend on the cation used for the preparation of brush-border membranes. In the Mg^{2+}-prepared brush-border membrane vesicles, a conductance for sodium was very low or absent, whereas it was present in Ca^{2+}-prepared membranes, although the total Na^+-H^+ exchange was lower in the latter preparation. When acridine orange was used as a fluorescent probe, the non-saturable component was undetectable [21].

In the Mg^{2+}-prepared brush-border membrane vesicles from MNS and MHS rats used in the present study, the proton conductance was negligible, as expected, because the addition of KCl on both sides of the vesicle in the presence of valinomycin failed to depress sodium uptake at 10 mM NaCl (Table II). In the presence of an outwardly directed proton gradient (pH $5.5_{in}/7.2_{out}$), the steady-state overshoot of sodium into the vesicles, as well as the initial rate of sodium uptake were increased by FCCP (i.e. by electrical potential difference) in both rat strains, but the effect was more evident in prehypertensive than in normotensive animals (Fig. 1 and Table I). The stimulation by electrical potential difference was higher at 10 mM than at 1 mM NaCl; therefore the kinetics of sodium transport was examined both in the absence and in the presence of the uncoupler. In both cases and strains, the kinetics followed a simple saturation curve lacking any diffusional component. This can be evaluated by the linearity of the Eadie-Hofstee plots presented in Fig. 4 or by the fit of the experimental points to the hyperbolae reported in Fig. 3. For each strain, the kinetic change observed following the addition of FCCP was mainly due to an increase in J_{max}, since $K_{0.5}$ for sodium remained unchanged. Also the differences between MNS and MHS rats could be related to a significant increase in J_{max} in MHS rats in the absence (38% increase) and in the presence of FCCP (45% increase). The component of sodium uptake dependent on electrical potential difference could be evaluated by subtracting the curve obtained in the absence of FCCP from that obtained in its presence. Also, the potential-dependent component thus calculated showed saturation (Fig. 3B), with $K_{0.5}$ lower and J_{max} higher in prehypertensives. However, it should be pointed out that the subtraction shown in Fig. 3B would not have been possible if the potential-sensitive and the potential-insensitive components of the sodium uptake had occurred through the same carrier. In contrast, if different carriers are involved, they must have similar $K_{0.5}$ values for sodium, because of the linearity of the Eadie-Hofstee plot in the presence of FCCP.

However, all the data could also be explained by supposing that the Na^+-H^+ exchange became potential sensitive, either because the rate-limiting step of the exchange was sensitive to membrane potential, or because the stoichiometry $Na^+ : H^+$ changed and became higher than 1. If this hypothesis is true, an inhibition of the Na^+-H^+ exchange would lead to a concurrent inhibition of the potential-sensitive sodium uptake. A first approach was carried out by use of amiloride, a well-known inhibitor of the Na^+-H^+ exchange [22]. As expected, by exponentially increasing the amiloride concentration, the initial rate of the Na^+-H^+ exchange gradually decreased in both rat strains, with K_i about 2-fold higher in normotensives than in prehypertensives. However, amiloride clearly affected sodium uptake also in the presence of FCCP, as well as the extra sodium uptake caused by the potential difference. These data support the hypothesis that electrical potential difference affects the Na^+-H^+ exchanger, but they are not conclusive. Actually, amiloride is not a specific inhibitor of the Na^+-H^+ exchange, since it inhibits with much lower K_i values the Na^+ channels occurring in some tight epithelia [23]. Moreover, the relative sensitivity of the Na^+-H^+ exchange to amiloride derivatives obtained by chemical modification of the guanidino group or of 5-amino nitrogen consistently differs from the relative sensitivity of the epithelial Na^+ channels to the same analogues. It is noteworthy that the lower K_i value found in the brush-border membrane vesicles prepared from prehypertensive MHS rats

is in agreement with the results obtained by studying the effect of amiloride in isolated kidneys perfused with cell-free artificial medium in vitro. Also in this preparation amiloride increased sodium excretion at a much lower concentration in kidneys of MHS rats than of MNS rats (unpublished results).

The findings described here are consistent with the experimental evidence obtained from previous studies on kidney transplantation and on renal physiology in the whole animal and in isolated preparations in vitro. These studies supported the hypothesis that the genetic difference existing between MNS and MHS rats concerns mainly the kidney, and that an increase in tubular sodium reabsorption is the primary cause of the renal dysfunction present in the prehypertensive stage and of the following rise in blood pressure in MHS rats [1–6].

The present study suggests that one of the alterations of the sodium reabsorption pathways in MHS in the prehypertensive stage occurs at the brush border of the proximal tubular cells, where a potential-dependent sodium pathway appears to be endowed with a $K_{0.5}$ similar in MNS and in MHS rats, but with a J_{max} higher in MHS rats. At present, it is not possible to distinguish whether this faster sodium uptake is due to an increase in the number of carriers per unit membrane or to an increase in the rate of the limiting step in the transport.

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