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Increased Na^+-H^+ exchanger activity in the ileal brush-border membrane of spontaneously hypertensive rats

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Abstract. In the present study, we have examined the intestinal Na^+ transport, through the Na^+-H^+ exchanger, in ileal brush-border membrane vesicles (BBMV) isolated from spontaneously hypertensive rats (SHR), and normotensive Wistar Kyoto (WKY) rats as a control group. Na^+ uptake into ileal BBMV was stimulated in the presence of a proton gradient (pH 5.5 inside/pH 7.5 outside) in SHR and WKY rats, resulting in a transient accumulation (overshoot) in both groups of rats. No overshoot was observed in the absence of a pH gradient. The magnitude of the accumulation was significantly higher in SHR than in WKY rats. Uptake of Na^+ at equilibrium was identical in the presence and the absence of a proton gradient and was not changed in SHR. The use of amiloride inhibited pH gradient-driven Na^+ uptake in a dose-dependent manner with a Ki of 90 μM and 100 μM for SHR and WKY rats, respectively. The relationship between proton gradient-driven Na^+ uptake and external Na^+ concentration was saturable and conformed to Michaelis-Menten kinetics in both SHR and WKY rats. Lineweaver-Burk analysis of the pH gradient-driven Na^+ uptake indicated values of V_{max} that were significantly increased in SHR compared to WKY rats (11.4 ± 0.55 nmol/mg/8 s vs. 4.96 ± 0.78 nmol/mg/8 s for SHR and WKY rats, respectively). In contrast, similar K_m values for Na^+ were found between SHR and WKY rats (4.0 ± 0.2 mM vs. 4.9 ± 0.6 mM for SHR and WKY rats, respectively). These studies show derangement in ileal BBMV Na^+ transport of SHR, which is characterized by increased Na^+-H^+ exchanger activity.
Key words. Brush-border membrane vesicles; hypertension; SHR; WKY; Na^+-H^+ exchanger.

The cellular cation metabolism has been extensively studied with regard to the pathogenesis of essential hypertension. Considerable evidence associates hypertension with altered cellular sodium transport, including the inhibition of active sodium transport [1], increased Na^+-Li^+ countertransport [2], reduced Na^+-K^+-ATPase [3] and increased Na^+-H^+ exchange [4–15].

Studies from different laboratories have shown that the activity of the Na^+-H^+ exchanger is enhanced in platelets [4, 5], lymphocytes [6, 7] and red blood cells [8] of patients with essential hypertension [9], and in different cell types of hypertensive rats compared with those from normotensive controls [10–15].

In the present study, we have examined the intestinal Na^+ transport, through the Na^+-H^+ exchanger, in ileal brush-border membrane vesicles (BBMV) isolated from SHR and WKY rats.

Materials and methods

Animals. Male SHR and WKY rats were obtained at the age of 8–9 weeks from Letica (Barcelona, Spain). They were fed a commercial synthetic diet (Letica, Barcelona, Spain) for 2–3 weeks. Animals had free access to food and water. All experiments were performed on 10–12-week-old rats, which were fasted for 18 h before killing.

Brush-border membrane vesicles (BBMV) preparation. Brush-border membrane vesicles (BBMV) were isolated from the ileal mucosa by the $MgCl_2$ precipitation method [16] with slight modifications. The mucosal scrapings were homogenized for 3 min in 100 mM mannitol, 2 mM HEPES/Tris, pH 7.1, using a Waring blender at maximal speed.

The homogenate was treated with 10 mM $MgCl_2$ and centrifuged at $3000 \times g$ for 30 min. The following steps were taken as previously described [16]. All isolation steps were carried out on ice. Aliquots of BBMV were stored under liquid nitrogen until analysis.

Enzyme measurements. Isolated BBMV were checked by determination of the specific activity of the brush-border membrane marker enzyme sucrase by the method of Dahlquist [17], and the determination of basolateral membrane marker enzyme Na^+-K^+-ATPase according to Kinne et al. [18]. Protein was measured by a Coomassie Brilliant Blue protein assay using bovine gamma-globulin as standard [19].

Uptake studies. The uptake of Na^+ was measured at 25 °C by a rapid filtration technique [16]. BBMV were loaded with a medium containing 300 mM mannitol,

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Table 1. Marker enzyme activities.

	Sucrase		Na^+-K^+-ATPase	
	WKY	SHR	WKY	SHR
Homogenate	87 ± 6.0	53 ± 6.0*	1.8 ± 0.2	2.10 ± 0.30
BBMV	1453 ± 173	648 ± 46**	0.6 ± 0.1	0.50 ± 0.08
Enrichment	18 ± 1.7	18 ± 3.5	0.3 ± 0.03	0.25 ± 0.05
Recovery	22 ± 1.2	21 ± 2.7	0.3 ± 0.04	0.40 ± 0.05

Marker enzyme specific activities were measured in homogenate and in final ileal BBMV of SHR and WKY rats, and expressed in nmol glucose formed · mg protein^{-1} · min^{-1} (sucrase) and μmol Pi released · mg · protein^{-1} · h^{-1} (Na^+-K^+-ATPase). Enrichment = ratio of the specific activities of the BBMV to that of the homogenate. Recovery = total activity in the BBMV as a percentage of the total activity in the homogenate. *$p < 0.05$ and **$p < 0.01$ compared with WKY rats.

50 mM potassium gluconate and 20 mM MES/Tris, pH 5.5. The assay was initiated by the addition of 100 μl of the incubation medium to 4–6 μl of a suspension of BBMV (100–150 μg of protein). The uptake was halted by the addition of an ice-cold stop solution. The BBMV were separated from the incubation medium by filtering on a 0.22 μm pore size Millipore filter. The filter was washed with 5 ml of stop buffer. The radioactivity retained on the filter was measured using a scintillation counter. The uptake at time zero was measured and subtracted from the total radioactivity of each sample. The incubation medium contained 300 mM mannitol, 50 mM potassium gluconate, 0.1 mM sodium gluconate, 45 μM valinomycin, tracers of $^{22}Na^+$ (0.6 μCi/assay), and either 20 mM MES/Tris (pH 5.5) or 20 mM HEPES/Tris (pH 7.5). The stop solution contained 300 mM mannitol, 50 mM potassium gluconate and 20 mM MES/Tris (pH 5.5). Valinomycin, a K^+ ionophore, was added in the presence of equimolar potassium concentrations ($K_{in} = K_{out}$) to voltage clamp the membrane vesicles and prevent any voltage-driven sodium uptake.

All experiments were performed in triplicate using membrane prepared from different intestines.

Determination of intravesicular D-(U-^{14}C)glucose space. As a measure of vesicular volume, the D-glucose volume of distribution was determined. Five to 10 μl of BBMV loaded with a medium containing 300 mM mannitol, 0.1 mM $MgSO_4$ and 20 mM HEPES/Tris (pH 7.4), were combined with 100 μl of incubation medium containing 100 mM mannitol, 0.1 mM $MgSO_4$, 20 mM HEPES/Tris (pH 7.4), 0.1 mM ^{14}C-D-glucose and 100 mM NaSCN. The intravesicular space was calculated at equilibrium (30 min) at 25 °C using the rapid filtration technique described above [16].

Materials. ^{22}Na (253 mCi/mg) and D-(U-^{14}C)glu-cose (283 mCi/mmol) were purchased from Dupont NEN. Membrane filters (Millipore GSWP02500) were obtained from Millipore Products Division, U.K. All other chemicals were supplied by Sigma.

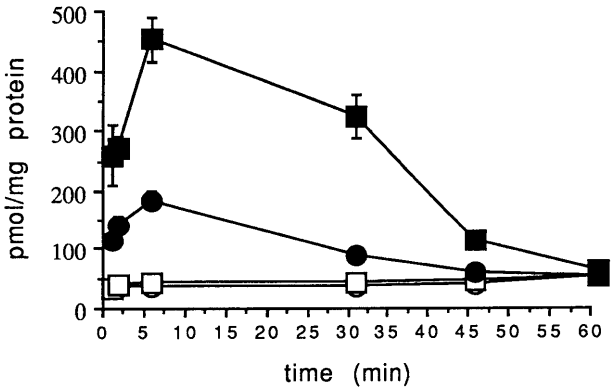

Figure 1. The time course of 0.1 mM Na^+ uptake in ileal brush-border membrane vesicles isolated from SHR and WKY rats. The preparation of the vesicles and the assay of Na^+ transport activity are described in Materials and methods. (○) and (□) indicate the uptake in the presence of no pH-gradient for WKY and SHR respectively (7.5/7.5). (●) and (■) indicate the uptake in the presence of an outwardly directed pH-gradient (pHin/pHout = 5.5/7.5) for WKY and SHR respectively. Values represent means ± SE of at least six different preparations. When not given, SE bars were smaller than the symbol used.

Statistical analysis. Comparison between different experimental groups was performed by the unpaired Student's t-test. The level of significance was $p < 0.05$.

Results

Purity of membrane vesicles

We used sucrase activity as a marker for the brush-border membrane and ouabain-sensitive Na^+-K^+-ATPase activity as a marker for the basolateral membrane. Table 1 shows the marker enzyme-specific activities in homogenate and in final ileal BBMV of SHR and WKY rats. The brush-border marker enzyme, sucrase, was enriched 18-fold for both strains compared to initial homogenate. The recovery of sucrase was high (21–22%) in both groups of animals. In contrast, there was a decrease in Na^+-K^+-ATPase specific activity in BBMV of SHR and WKY rats compared to initial homogenate, and this enzyme was not enriched compared to initial homogenate in either group of rats. The recovery of this enzyme was low in SHR and WKY rats (0.3–0.4%).

^{22}Na uptake in ileal BBMV isolated from SHR and WKY rat

Figure 1 shows the time course of Na^+ uptake into BBMV prepared from SHR and WKY rat ileum. In the presence of an outwardly directed H^+ gradient, there was a transient increase in the intravesicular concentration of Na^+ and overshoot at 5 min, in both groups of animals. The magnitude of the accumulation of Na^+ was significantly higher in SHR compared with WKY rats (9.6 ± 0.8-fold vs. 4.3 ± 0.3-fold for SHR and

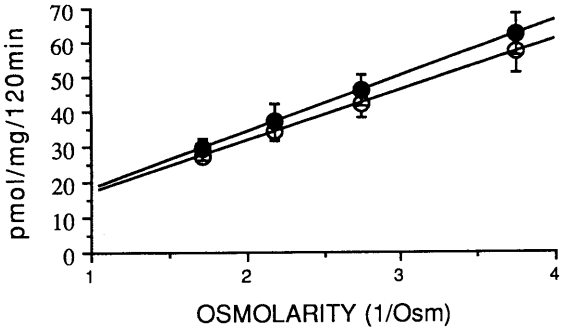

Figure 2. The effect of the osmolarity of the incubation medium on Na^+ uptake by ileal brush-border membrane vesicles isolated from SHR (●) and WKY (○) rats. Brush-border membrane vesicles were suspended in 300 mM mannitol, 50 mM potassium gluconate and 20 mM MES/Tris, pH 5.5. The incubation medium contained 50 mM potassium gluconate, 0.1 mM sodium gluconate, 45 μM valinomycin, tracers of $^{22}Na^+$ (0.6 μCi/assay), 20 mM HEPES/Tris, pH 7.5, and mannitol in concentrations to give the indicated osmolarity. Values represent means ± SE of at least six different preparations. Correlation coefficient values were 0.999 and 0.998 for SHR and WKY rats, respectively.

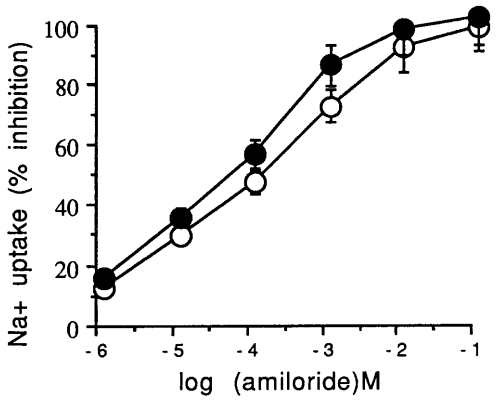

Figure 3. The effect of varying concentrations of amiloride on Na^+-H^+ exchanger in ileal BBMV isolated from SHR (●) and WKY (○) rats. Vesicles contained 300 mM mannitol, 50 mM potassium gluconate and 20 mM Mes/Tris, pH 5,5. Uptake buffer contained 300 mM mannitol, 50 mM potassium gluconate, 20 mM Hepes/tris, pH 7.5, 45 μM valinomycin, 0.1 mM sodium gluconate, tracers of $^{22}Na^+$ (0.6 μCi/assay) and various concentrations of amiloride. Data were expressed as percent inhibition of uptake without amiloride. Values are means ± SE for at least five different preparations.

WKY rats, respectively, $p < 0.01$). The Na^+ uptake rate was significantly increased in SHR (15 ± 3.4 pmol · $mg^{-1} \cdot s^{-1}$ vs. 5.5 ± 0.8 pmol · $mg^{-1} \cdot s^{-1}$ for SHR and WKY rats, respectively, $p < 0.025$). No overshoot was observed in the absence of a pH-gradient in any groups. Uptake of Na^+ at equilibrium (120 min), was identical in the presence and the absence of a pH-gradient, and it was not modified by hypertension.

Effect of medium osmolarity on Na^+ uptake

In order to know whether Na^+ uptake is into osmotically sensitive intravesicular space or due to binding, membrane vesicles were prepared from SHR and WKY rats and intravesicular space was decreased by increasing the medium osmolarity with mannitol. As shown in figure 2, there is a linear relation between media 1/osmolarity and Na^+ uptake at equilibrium (120 min) in both groups of rats in the presence of a pH gradient. At infinite osmolarity there is minimal binding for SHR and WKY rats, indicating that Na^+ uptake is due to the transport into intravesicular space.

Effect of varying amiloride concentrations on Na^+-H^+ exchanger

Figure 3 depicts the effect of varying concentrations of amiloride on 0.1 mM Na^+ uptake at 1 min under an outwardly directed pH-gradient (pHin/pHout = 5.5/7.5) in ileal BBMV from SHR and WKY rats. At 1 mM amiloride concentration, Na^+ uptake was inhibited by $84 \pm 8\%$ and $70 \pm 7\%$ in SHR and WKY rats, respectively. The Ki values for amiloride were 90 μM and 100 μM for SHR and WKY rats, respectively. These values are comparable to that previously reported [20].

Since high concentrations of amiloride could have a deleterious effect on the integrity of the BBMV, 0.1 mM Na^+ uptake at equilibrium was measured under a pH-gradient in the presence of 100 mM amiloriode in both groups of rats. Na^+ uptake was the same in the presence (44 ± 3.7 pmol/$mg^{-1} \cdot 120$ min vs. 40 ± 0.4 pmol/$mg^{-1} \cdot 120$ min for SHR and WKY rats, respectively) and absence (48 ± 7 pmol/$mg^{-1} \cdot 120$ min vs. 40 ± 1.3 pmol/$mg^{-1} \cdot 120$ min for SHR and WKY rats, respectively) of amiloride, indicating that amiloride did not affect the integrigy of membrane vesicles.

Kinetic analyses of Na^+ uptake

To assess whether alterations in Na^+ transport observed in SHR can be attributed to changes in maximal transport capacity (V_{max}) and/or in carrier affinity (K_m), transport kinetics were determined. The initial rates of Na^+ uptake were measured at 8s in the presence and absence of a proton gradient. Values were calculated from the pH gradient-driven uptake. As shown in figure 4, the proton gradient-driven Na^+ uptake was saturable and conformed to Michaelis-Menten kinetics in both SHR and WKY rats. Lineweaver-Burk plots of results obtained from SHR and WKY rats are shown in figure 5. V_{max} values of SHR were significantly greater than the values of their respective control WKY rats (11.4 ± 0.55 nmol · $mg^{-1} \cdot 8$ s^{-1} vs. 4.96 ± 0.78 nmol · $mg^{-1} \cdot 8$ s^{-1} for SHR and WKY rats respectively, $p < 0.001$). However, similar K_m values for Na^+ were found between SHR and WKY rats (4.0 ± 0.22 mM vs. 4.9 ± 0.60 mM for SHR and WKY rats, respectively).

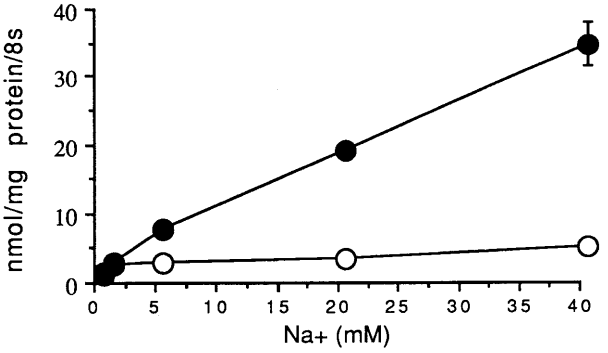

Figure 4. Kinetics of Na^+/H^+ exchanger by ileal brush-border membrane vesicles isolated from WKY (○) and SHR (●). pH gradient-dependent Na^+ uptake was measured at 8 s. Uptake buffer contained increasing concentrations of Na gluconate (0.1–40 mM) isosmotically substituted with mannitol. Each point represents means ± SE of at least six different preparations. When not given, SE bars were smaller than the symbol used.

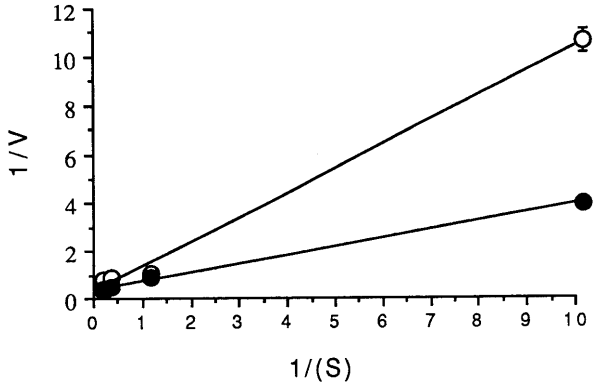

Figure 5. Lineweaver-Burk plot for pH gradient driven-Na^+ uptake by ileal brush-border membrane vesicles isolated from WKY (○) and SHR (●). Each point represents means ± SE of at least six different preparations. When not given, SE bars were smaller than the symbol used. Correlation coefficient (r) = 0.96 for WKY and 0.97 for SHR.

Discussion

Our data indicate that an outwardly directed proton gradient increased Na^+ uptake in BBMV prepared from ileum of SHR compared to WKY rats. The proton gradient produces a transient accumulation in the Na^+ transport in ileal BBMV of SHR and WKY rats, the magnitude of the accumulation being significantly higher in SHR than in WKY rats (see fig. 1). Over the last decade, evidence for the increased Na^+/H^+ exchange activity in erythrocytes, leucocytes, platelets, lymphocytes, kidney cortex and excitable cells from hypertensive rats and patients with essential hypertension has been reported from several laboratories [4, 6, 8, 10, 14, 15]. To our knowledge this study is the first observation of the increased activity of this carrier in membrane fractions isolated from ileum of SHR.

It is unlikely that the higher pH gradient-driven Na^+ uptake of SHR BBMV is due to variations in vesicle preparations, since the purification and size of BBMV, as measured by the enrichment of sucrase, and D-glucose uptake at equilibrium (30 min) (0.69 ± 0.09 µl · mg protein^{-1} vs. 0.76 ± 0.1 µl · mg protein^{-1}, for SHR and WKY rats respectively), were similar for both groups of animals (see table 1).

Results of kinetic studies revealed a 2.3-fold higher V_{max} for pH gradient-driven Na^+ uptake in SHR than in WKY rats. However, the affinity of the transporter for Na^+ was found to be similar in SHR and WKY rats, since the apparent K_m, obtained from the Lineweaver-Burk plot, was unchanged in both groups of animals (see fig. 5).

V_{max} for Na^+ has been described as unchanged in SHR lymphocytes [21] and myoblasts [22], but most studies agree that V_{max} of the Na^+/H^+ exchanger is increased in different cell types of the SHR [8, 12, 23]. Our results indicate that the markedly higher Na^+/H^+ exchanger V_{max} in ileal BBMV of SHR compared with WKY rats could be due to quantitative differences in the amount of antiporter protein or/and to an increase in the turnover of the Na^+/H^+ transporter.

Four isoforms of the Na^+/H^+ antiporter have now been described (NHE1–4). The isoforms vary widely in their sensitivity to amiloride and amiloride analogues. NHE1 and NHE2 are about equally sensitive to amiloride (Ki of 1–3 µM) [24] and NHE3 was identified as the isoform that is highly resistant to inhibition by amiloride or amiloride derivatives. Thus Ki of amiloride for NHE3 was about 100 µM when expressed in AP-1 ovary cells [25] and 39 µM when expressed in PS120 cells [26]. In addition, western analysis with an anti-NHE2 polyclonal antibody has shown that NHE2 is expressed in brush-border membranes isolated from rat small intestine, whereas NHE1 seems to be located on basolateral membranes [27]. In rat, NHE3 mRNA is also found in jejunum and ileum [28]. In addition, the isoforms can also be distinguished by their kinetic properties. The extracellular Na^+ dependence of the NHE3 isoform has been shown, with apparent K_{Na} values of about 4 mM [25]. Therefore, it is reasonable to postulate that the NHE3 isoform is the one present in our BBMV preparations, with the same isoform present in the two vesicle populations.

Membrane lipid composition and fluidity are also known to affect membrane-bound transport proteins [9, 29], including the Na^+/H^+ exchanger [30, 31]. Previous studies in our lab have shown an increased membrane fluidity together with an enhancement in the ratio of cholesterol/phospholipid in the brush-border membrane from SHR, compared to WKY rats [32]. These differences in membrane lipid composition and fluidity noted between SHR and WKY rats might also be responsible for the increase in the activity of Na^+/H^+ exchanger observed in hypertension.

Abnormalities of a number of transport systems have been described in essential hypertension [33–35], but

it is not clear whether altered membrane transport in SHR is due to extracellular factors or represents a basic plasma membrane abnormality as a metabolic consequence of altered tension status. In that case, it is possible that the increase in intestinal BBMV Na^+ transport observed in the present study may reflect a generalized membrane transport abnormality in this model of genetic hypertension.

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