Kidney International, Vol. 36 (1989), pp. 576–581

Increased Na^+/H^+ antiport activity in the renal brush border membrane of SHR

GABRIEL A. MORDUCHOWICZ, DAVID SHEIKH-HAMAD, OK D. JO, EDWARD P. NORD, DAVID B.N. LEE, and NORIMOTO YANAGAWA

Division of Nephrology, Medical and Research Services, Veterans Administration Sepulveda Hospital, Sepulveda, and Division of Nephrology, Department of Medicine, University of California at Los Angeles, School of Medicine, Los Angeles, California, USA

Increased Na^+/H^+ antiport activity in the renal brush border membrane of SHR. Defect in renal salt excretion may play an important role in the pathogenesis of hypertension. We examined sodium (Na^+) uptake by brush border membrane (BBM) vesicles of young (6 week old) spontaneously hypertensive rats (SHR) and normotensive Wistar-Kyoto rats (WKY) of the same age. SHR had lower urinary Na^+ excretion (223.1 ± 9.3 vs. 266.3 ± 3.7 μEq/day/100 g, $N = 8$, $P < 0.01$) and higher systolic blood pressure (98.9 ± 1.2 vs. 82.9 ± 1.8 mm Hg, $N = 8$, $P < 0.01$) than WKY. BBM vesicle Na^+ uptake, measured by rapid filtration technique, was higher in SHR when compared to WKY (1.44 ± 0.03 vs. 1.01 ± 0.06 nmol/mg/5 sec, $N = 4$, $P < 0.01$). This increase in Na^+ influx was apparent only in the present of an outward-directed proton (H^+) gradient and was abolished by 1 mM amiloride. BBM permeability to H^+ as assessed by acridine orange quenching was not different between SHR and WKY. Kinetic analyses of the amiloride-sensitive BBM Na^+ uptake revealed a higher V_{max} (2.13 ± 0.27 vs. 0.70 ± 0.30 nmol/mg/5 sec, $N = 4$, $P < 0.01$) and a higher km for Na^+ (3.55 ± 0.32 vs. 1.23 ± 0.14 mM, $N = 4$, $P < 0.05$) in SHR. These findings thus demonstrate an intrinsic derangement in BBM Na^+ transport in young SHR which is characterized by increased Na^+/H^+ antiport activity. This alteration in antiport activity is not attributable to changes in membrane permeability to H^+, and is characterized by higher V_{max} and km. Similar reports of increased Na^+/H^+ antiport activity in other tissues of SHR suggest that a generalized membrane transport disorder may exist in this model of genetic hypertension.

Considerable evidence suggests that hypertension may be associated with altered cellular sodium (Na^+) transport [1, 2]. In patients with essential hypertension, an increased activity of the red cell transport system mediating Na^+-lithium exchange has been reported [3, 4]. While the physiological significance of this transport system is not immediately apparent, it has been suggested that another Na^+ transport system analogous to Na^+-lithium exchange, that is, Na^+/hydrogen (H^+) antiport, may be important in the pathogenesis of hypertension [5, 6]. Increased Na^+/H^+ antiport activity in vascular smooth muscle cells could elevate cellular Na^+ concentration which could reduce calcium (Ca^{++}) efflux through Na^+/Ca^{++} exchange, leading to increases in cytosolic Ca^{++} concentration and vasoconstriction [7]. On the other hand, an increased Na^+/H^+ antiport in renal proximal tubules could enhance Na^+ reabsorption requiring compensatory increase in arterial pressure to maintain salt balance [8]. Impaired Na^+ excretion can also lead to the secretion of natriuretic factors which could cause increase in peripheral vascular resistance [9, 10].

Recently a lower renal lithium clearance has been reported in patients with essential hypertension [11] and it was proposed that proximal tubular Na^+ transport, possibly through Na^+/H^+ antiport, may be increased in patients with essential hypertension. However, the site and mechanism whereby lithium is reabsorbed in human kidney is not certain [12] and it is also not clear whether the altered lithium clearance is due to intrinsic proximal tubular dysfunction or occurs only secondary to other factors such as altered renal hemodynamics.

In support of an intrinsic proximal tubular dysfunction, an increased Na^+ uptake by renal brush border membrane (BBM) vesicles isolated from Milan hypertensive rats was recently reported [13]. In the present study, we have examined the Na^+ transport function of renal BBM vesicles isolated from another model of genetic hypertension, that is, the spontaneously hypertensive rat (SHR). The results indicate that in young SHR, as compared to sex and age-matched normotensive Wistar-Kyoto rat (WKY), BBM Na^+ transport via the Na^+/H^+ antiport system was higher due to a higher turnover rate or increased functional antiporters.

Methods

Experimental animals

Five- to seven-week-old male SHR and WKY (Charles River Breeding Laboratories, Boston, Massachusetts, USA) weighing 80 to 120 g were used.

Metabolic studies and blood pressure measurements

Following two weeks of acclimatization on a standard Purina rat chow diet (0.4% Na, 0.6% K, 0.9% P and 0.4% Ca) and tap water ad libitum, rats were housed in individual metabolic cages under controlled conditions of temperature (26°C), humidity (45 to 50%) and light-dark cycle (14:10 hr) for three consecutive days. During this period, standard Purina rat chow and distilled water ad libitum were given and food intake and urine excretion were measured daily. Systolic blood pressure was measured on the fourth day between 0900 and 1100 hours in conscious rats by

Received for publication May 31, 1988
and in revised form March 2, 1989
Accepted for publication May 16, 1989

tail sphygmomanometry (Narco Biosystem, Houston, Texas, USA) with the use of a temperature-controlled restraint cage. Each measurement represented the mean of five separate readings after equilibration. The intermeasurement difference did not exceed 10 mm Hg. Rats were then sacrificed in batches of two from each group in subsequent days and blood samples collected and BBM vesicles prepared. Plasma and urinary Na^+ and creatinine were measured using an Astra autoanalyzer (Beckman Instruments, Fullerton, California, USA). Fractional excretion of Na^+ (FE_{Na}), that is, the percentage of filtered Na^+ excreted in the final urine, was calculated as:

$$FE_{Na} = \frac{U_{Na}V}{GFR \times P_{Na}} \times 100\%$$

where U_{Na} and P_{Na} are the urinary and plasma Na^+ concentrations (mEq/ml), respectively; V the urinary volume (ml/min) and GFR the glomerular filtration rate as determined from the creatinine clearance (ml/min).

BBM vesicle preparation

Purified BBM vesicles were prepared from SHR and WKY renal cortex by magnesium (Mg^{++}) precipitation method [14]. The Mg^{++}-, rather than the Ca^{++}-, precipitation method was used since the Ca^{++} precipitation method may affect BBM Na^+ permeability [15].

Measurement of BBM Na^+ uptake

Transport experiments using $^{22}Na^+$ were performed at room temperature (21 to 23°C) by a Millipore rapid-filtration procedure [16]. BBM vesicles were prepared and suspended in a medium comprised of 300 mM mannitol, 10 mM $MgSO_4$ and 10 mM Tris-16 mM HEPES, pH 7.5. $^{22}Na^+$ uptake was measured in the presence of an outward H^+ gradient induced by preincubation of vesicles for two hours at room temperature in a medium comprised of 273 mM mannitol, 10 mM $MgSO_4$, 9 mM Tris, 14 mM HEPES and 30 mM MES, pH 6.0. Uptake was initiated by incubation of vesicles with a medium comprised of 1 mM $^{22}NaCl$ (2.5 μCi/ml), 286 mM mannitol, 2 mM $MgSO_4$, 13 mM Tris, 15 mM HEPES, 6 mM MES, pH 7.5, and was stopped by an ice-cold (4°C) isosmotic solution. Uptake rate was calculated from the accumulated $^{22}Na^+$ and expressed in moles per milligram protein per unit time. Protein concentration was assayed using Coomassie Brilliant Blue G250 with bovine serum albumin as the reference protein [17]. A correction for $^{22}Na^+$ binding to the filters was made by subtracting the radioactivity measured at zero time, that is, when 2 ml of ice-cold stop solution had been added to the vesicles prior to the addition of uptake buffer. All measurements were carried out in triplicate with freshly prepared membranes. Samples from both groups of rats were prepared and uptake studies performed on the same day.

Measurement of BBM ^{14}C-glucose uptake

BBM vesicles were prepared and suspended in medium similar to that for Na^+ uptake measurements. Uptake was initiated by incubation of vesicles with a medium comprised of 100 mM NaCl, 100 mM mannitol, 5 mM HEPES-Tris, pH 7.5 and 0.1 mM [U-^{14}C]-glucose (2.5 μCi/ml). Sodium-dependent glucose uptake was determined from the difference of accumulated [U-^{14}C]-glucose in the presence or absence of sodium in the uptake solution and expressed in moles per milligram protein per unit time. All measurements were carried out in triplicate with freshly prepared membranes.

Table 1. Metabolic balance studies

	WKY (N = 8)	SHR (N = 8)	P
Body weight *g*	94.2 ± 1.7	85.3 ± 1.5	<0.01
Creatinine clearance *ml/min/100 g*	0.36 ± 0.02	0.31 ± 0.03	<0.05
Sodium intake *μEq/day/100 g*	277 ± 8.4	260 ± 13.9	>0.5
Sodium excretion *μEq/day/100 g*	266.3 ± 3.7	223.1 ± 9.3	<0.01
FE_{Na} %	1.36 ± 0.09	1.18 ± 0.05	<0.01
Blood pressure *mm Hg*	82.9 ± 1.8	98.9 ± 1.2	<0.01

Measurement of BBM proton permeability

BBM proton permeability was determined using the fluorescent dye acridine orange [18]. Briefly, membrane vesicles were pre-equilibrated in a medium composed of 273 mM mannitol, 10 mM $MgSO_4$, 9 mM Tris, 14 mM HEPES and 30 mM MES, pH 6.0 similar to that for $^{22}Na^+$ uptake studies. Ten microliters of membrane vesicles were added to a cuvette containing 980 μl of the same medium to which 1 μM acridine orange had been added. The membrane vesicles were diluted with medium to a protein concentration of 10 mg/ml. After three to five minutes of equilibration, 10 μl of 1 M Tris-base was added to the cuvette (final Tris-base concentration 10 mM) and the recovery of fluorescence followed for a further five minutes. In control experiments vesicles were pre-equilibrated with 5 mM KCl, and followed the base pulse 2 μl of nigericin (1 mg/ml in methanol) were added to the cuvette during the recovery phase. Fluorescence was monitored on a Perkin-Elmer LS-5 fluorometer (Norwalk, Connecticut, USA) at 490 nm excitation, 540 emmision with slit width set at 5 nm.

Statistical analysis

Data are presented as mean ± SE. Comparison between different experimental groups was made using an unpaired *t*-test.

Materials

$^{22}Na^+$ (carrier free) was purchased from New England Nuclear (Boston, Massachusetts, USA). Acridine orange and Coomassie Brilliant Blue G250 were obtained from Eastman Kodak (Rochester, New York, USA). Amiloride, Nigericin and all other chemicals were obtained from Sigma Chemical (St. Louis, Missouri, USA).

Results

Balance studies

Table 1 summarizes the results of balance studies. Body weight was slightly but significantly less in SHR than WKY of the same age. With similar Na^+ intake, the urinary Na^+ excretion was significantly less in SHR. This is probably due to an enhanced renal tubular Na^+ reabsorption as is indicated by the comparable GFR and the lower FE_{Na} in SHR. At this

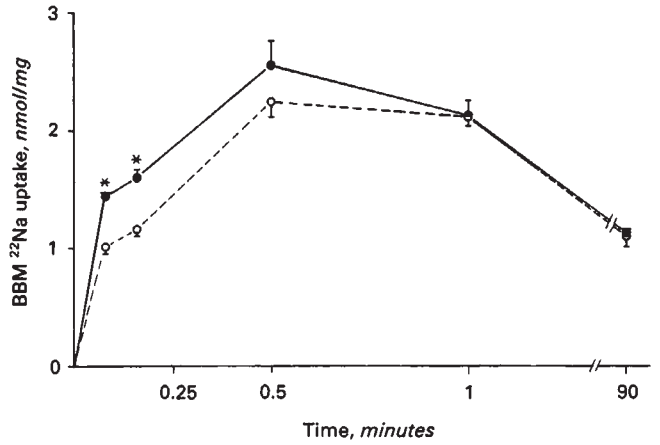

Fig. 1. *Time course of Na^+ uptake by BBM vesicles isolated from SHR (●—●) and WKY (○----○) kidneys.* BBM Na^+ uptake was performed in the presence of an outwardly directed H^+-gradient generated by two hour preincubation of vesicles at pH 6.0. Na^+ uptake at 5, 10, 30 seconds were significantly higher in SHR than in WKY. Na^+ uptake at 90 min was not different between the two groups. Values are means ± SE from four experiments. Asterisk (*) denotes significant difference ($P < 0.05$).

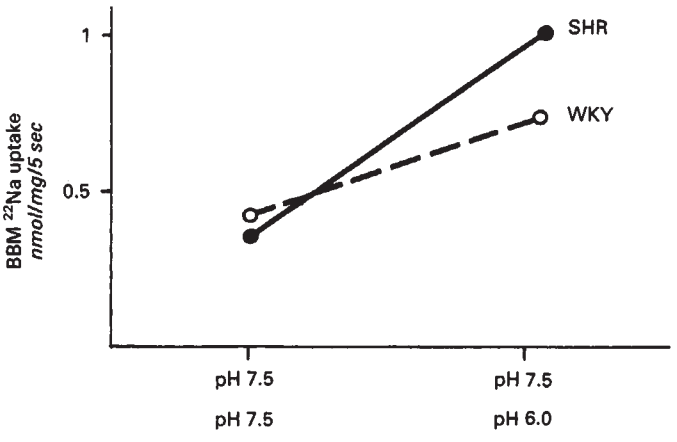

Fig. 2. *Requirement of an opposing H^+-gradient for the higher BBM Na^+ uptake in SHR.* The difference in BBM Na^+ uptake between the two groups became apparent only when an outwardly directed H^+-gradient was imposed by incubation of BBM vesicles at pH 6.0 prior to the uptake studies. Shown is the total Na^+ uptake by SHR (●) and WKY (○) BBM vesicles.

"prehypertensive" age, the blood pressure in SHR was already higher than WKY.

BBM Na^+ uptake studies

Purification of the BBM preparation, as assessed by enrichment of the activity of the BBM enzyme marker, alkaline phosphatase, was not different between SHR and WKY (8.76 ± 1.06 vs. 8.45 ± 1.19 fold, $N = 9$). The uptake of 1 mM Na^+ by BBM vesicles isolated from kidneys of SHR and WKY as a function of time is illustrated in Figure 1. In the presence of an outwardly directed H^+-gradient, the initial BBM Na^+ uptake was significantly higher in BBM isolated from SHR than WKY (1.44 ± 0.03 vs. 1.01 ± 0.06 nmol/mg/5 sec, $N = 4$, $P < 0.01$). Uptakes at one minute and after were not different between the two groups. As shown in Figure 2, the difference in Na^+ uptake between these two groups became apparent only when an outwardly directed H^+-gradient was imposed by incubation of BBM vesicles at pH 6.0 prior to the uptake studies. These results indicate the requirement of an opposing H^+-gradient for the higher BBM Na^+ uptake to be apparent in SHR and suggest that the enhanced Na^+ uptake may be mediated by Na^+/H^+ antiport. To further characterize the Na^+ transport pathway involved, the effect of Na^+/H^+ antiport inhibitor, amiloride, on Na^+ uptake was tested. As depicted in Figure 3, Na^+ uptake was inhibited by 10^{-3} M amiloride to a greater extent in SHR than in WKY, while the amiloride-insensitive component of Na^+ uptake was not different between SHR and WKY (0.53 ± 0.15 vs. 0.58 ± 0.09 nmol/mg/5 sec, $N = 8$, $P > 0.5$).[1] This

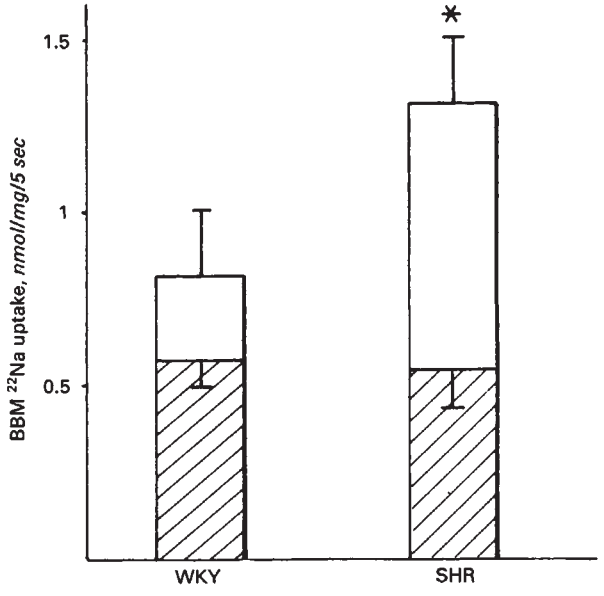

Fig. 3. *Effect of 10^{-3} M amiloride on BBM Na^+ uptake.* The higher Na^+ uptake in SHR was blunted by 10^{-3} M amiloride (shaded bars). Values are means ± SE from eight experiments. Asterisk (*) denotes significant difference ($P < 0.05$).

observation lends further support for an enhanced Na^+/H^+ antiport activity as the mechanism for the higher Na^+ uptake in SHR.

Measurement of BBM hydrogen permeability

Since changes in BBM permeability to H^+ could affect Na^+-H^+ exchange activity, the H^+ permeability of membrane vesicles from SHR and WKY was assayed with acridine orange (AO) using the base-pulse technique [21]. Acidification of the vesicle interior with respect to medium pH results in the intravesicular trapping of AO in its protonated form and is

[1] In these studies, the maximum percentage inhibition we have observed with 10^{-3} M amiloride (50 to 60%), appears to be less than that reported in previous studies [19] using rabbit renal BBM vesicles (more than 90%). This may be due to the difference in experimental animal species since in our laboratory we have obtained over 90% inhibition with 10^{-3} M amiloride in BBM vesicles isolated from rabbit kidneys (unpublished observation). A recent report also demonstrated different sensitivities to amiloride between rat and rabbit renal BBM [20].

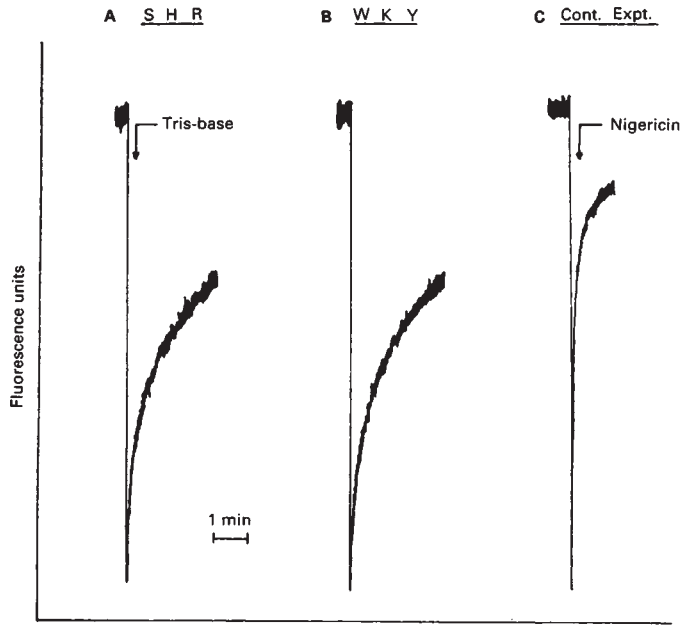

Fig. 4. *Measurement of proton permeability of BBM vesicles with acridine orange (AO).* Membrane vesicles were pre-equilibrated in pH 6.0 pre-equilibration medium similar to that for Na^+ uptake studies, to which 1 μM AO had been added. Steady-state fluorescence was continuously recorded at excitation 490 nm and emission 540 nm. After three to five min of steady-state tracing, 10 μl of 1 M Tris-base (final concentration 10 mM) was added to the cuvette (total volume 1 ml) and fluorescent tracing followed until a new steady-state had been reached. In each experiment 100 μg protein/10 μl of SHR (A) or WKY (B) membrane vesicles was used. The initial rates of proton flux were identical between the two. C. Effect of addition of 2 μl of 1 mg/ml nigericin to WKY vesicles (pre-equilibrated with 5 mM KCl) on recovery from a base pulse.

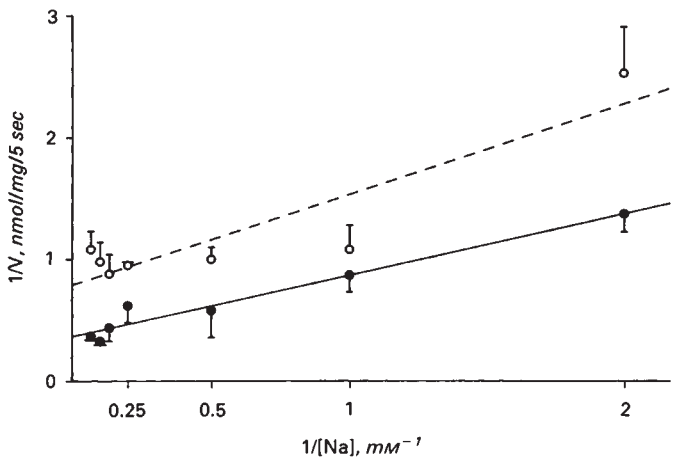

Fig. 5. *Lineweaver-Burk plot for amiloride-sensitive sodium uptake by BBM vesicles isolated from WKY (– – –) and SHR (——) under an outwardly-directed proton gradient.* Regression coefficient (r) = 0.73 for WKY and 0.98 for SHR. Values are means ± SE from four experiments.

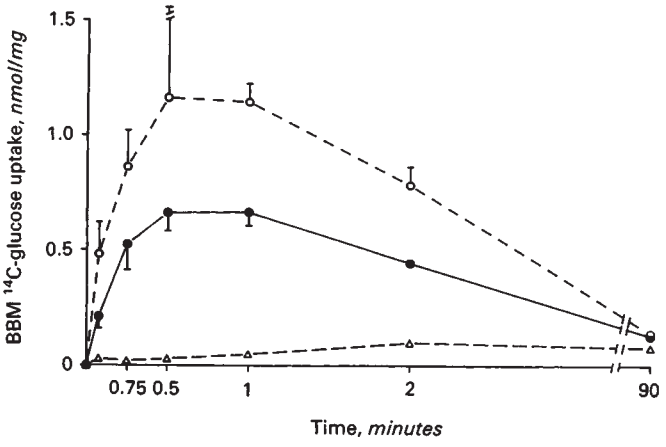

Fig. 6. *Time course of sodium-dependent glucose uptake by BBM vesicles isolated from SHR (●——●) and WKY (○----○) kidneys.* (△----△) Indicates sodium-independent glucose uptake which was not different between the two groups. Values are means ± SE from four experiments.

associated with quenching of the fluorescence signal. Recovery of AO fluorescence indicates dissipation of the pH gradient and can be used as a measure of H^+ permeability. Figure 4 depicts a representative recording of AO fluorescence. Vesicles were equilibrated at pH 6.0 in the presence of the probe. After steady-state fluorescence was recorded, 10 mM Tris-base was added to the suspension (arrow, Fig. 4) which resulted in rapid quenching of AO fluorescence. The initial rate of recovery of intravesicular pH was used as a measure of H^+ permeability. Emperical quantitation of the initial rate of fluorescence recovery by curve fitting to a quadratic function [22] gave a rate of 18.2 ± 1.5 (N = 6) arbitrary fluorescence unit per minute for WKY and 18.1 ± 1.7 (N = 6) for SHR. Since no Na^+ was used in these experiments, participation of the antiporter in H^+ efflux was presumed to be negligible. In control experiments in which vesicles were preequilibrated with 5 mM KCl, addition of the K^+-H^+ ionophore nigericin rapidly dissipated the base-induced fluorescent quench (Fig. 4C), confirming that the recovery of fluorescence was due to dissipation of a H^+ gradient. These data indicate that the higher Na^+-H^+ antiport activity of SHR microvillus vesicles does not occur as the result of altered membrane permeability to H^+.

Kinetic analyses of BBM Na^+ uptake

Figure 5 depicts the Lineweaver-Burk plot for amiloride-sensitive Na^+ uptake with Na^+ concentration varied from 1 to 15 mM by isosmotically replacing NaCl with mannitol in the uptake medium. Analysis of the data revealed a significantly higher V_{max} in SHR than WKY (3.55 ± 0.32 vs. 1.23 ± 0.13 nmol/mg/5 sec, N = 4, $P < 0.01$) and a higher km for Na^+ in SHR than WKY (2.13 ± 0.27 vs. 0.7 ± 0.30 mM, N = 4, $P < 0.05$).

BBM glucose uptake studies

To examine if the higher Na^+ uptake in SHR BBM vesicles represents an abnormality specific to Na^+/H^+ antiport system, another sodium-dependent transport system, that is, BBM glucose uptake, was compared between SHR and WKY. Sodium-dependent BBM glucose uptake as a function of time is shown in Figure 6. In contrast to Na^+/H^+ antiport system, sodium-dependent BBM glucose uptake was lower in SHR than WKY. There was no difference in sodium-independent glucose uptake between the two groups.

Discussion

Our data indicate that on a similar Na^+ intake, 6-week-old SHR excreted less Na^+ in the urine than age-matched WKY. The lower urinary Na^+ excretion rate in young "prehypertensive" SHR has been reported previously [23–25]. This has probably occurred as the result of an enhanced renal tubular Na^+ reabsorption since the calculated FE_{Na} was lower in SHR. While the nephron segments accountable for the enhanced Na^+ reabsorption in young SHR remain unclear, abnormal proximal tubule Na^+ transport has been suggested. Thus, in young SHR, an increased Na,K-ATPase activity was reported in microdissected proximal tubules [26] and a higher proximal tubule Na^+ reabsorption rate was suggested in the in vivo micropuncture study [27]. With isolated renal BBM vesicles, a higher Na^+ transport rate was found in the young Milan hypertensive rats, suggesting an altered membrane Na^+ transport property in renal proximal tubule [13].

Results of the present study also showed that the rate of Na^+ uptake by isolated BBM vesicles was higher in SHR than in WKY. It is unlikely that the higher Na^+ uptake by SHR BBM vesicles is due to variations in vesicle preparation. The purification and size of BBM vesicles, as inferred from the enrichment of alkaline phosphatase and Na^+ uptake at equilibrium, respectively, were similar between the two groups. Since solutes such as glucose, phosphate or amino acid were absent in the uptake solution, the higher BBM Na^+ uptake in SHR cannot be due to these Na^+-dependent solute transport systems. At 1 mM Na^+ concentration and in the absence of cotransportable solutes, the majority of Na^+ influx in renal BBM vesicles has been suggested to occur via Na^+/H^+ antiport system [28]. In the present study, since the higher BBM Na^+ uptake in SHR became apparent only when BBM vesicles were imposed with an outwardly directed pH gradient and was abolished by amiloride (10^{-3} M), it is likely that Na^+/H^+ antiport system is involved. Since BBM proton permeability, as determined from the recovery of AO fluorescence after basepulse, was not different between the two groups, the enhanced BBM Na^+/H^+ exchange activity in SHR does not occur as the result of altered membrane permeability to H^+ and is likely to be due to altered transport characteristic of this transport system per se.

The higher Na^+/H^+ antiport system appears to be specific since another sodium-dependent transport system, that is, sodium-dependent BBM glucose uptake, was rather lower in SHR than WKY. Although the reason for the lower glucose uptake in SHR is not clear, it is possible that an enhanced dissipation of sodium gradient across the BBM secondary to the higher Na^+/H^+ antiport activity may have contributed to such difference.

Results of kinetic studies revealed higher V_{max} and km for Na^+ in SHR than WKY. This indicates that the increased BBM Na^+ uptake in SHR is mainly due to higher turnover rate or increased functional antiporters. Since the extracellular Na^+ concentration is much higher than the km value for Na^+/H^+ antiport system, it is unlikely that the higher km value for sodium found in SHR BBM vesicles will have physiological significance.

Thus, results of BBM transport studies are consistent with the previous report of a higher proximal tubule sodium reabsorption rate in SHR and suggest that such abnormality may in part contribute to the lower urinary sodium excretion in SHR. While results of the present study showed an intrinsic derangement in renal BBM Na^+ transport in SHR, these results do not allow differentiation between genetically determined primary alterations in BBM Na^+ transport property and secondary alterations induced by in vivo humoral or neural modulations which persist in vitro. However, an increased Na^+/H^+ antiport activity has now been described in other tissues such as lymphocytes [29] and the in vitro primary cultured, vascular smooth muscle cells [30] from SHR. The fact that such abnormality persists in the cultured cells provides strong support for a genetic etiology. It is therefore possible that the increase in renal BBM Na^+/H^+ antiport activity observed in the present study may reflect a generalized membrane transport abnormality in this model of genetic hypertension. The cellular mechanism for such a transport disorder as well as its role in the development of hypertension remain to be elucidated.

Acknowledgments

This work was supported by grants from the Veterans Administration, the National Kidney Foundation of Southern California and the American Heart Association, Greater Los Angeles Affiliate.

Reprint requests to Norimoto Yanagawa, M.D., Nephrology Division (111R), Sepulveda Veterans Administration Medical Center, 16111 Plummer Street, Sepulveda, California 91343, USA.

References

1. Postnov YV, Orlov SN: Ion transport across plasma membrane in primary hypertension. *Physiol Rev* 65:904–945, 1985
2. Hilton PJ: Cellular sodium transport in essential hypertension. *N Engl J Med* 314:222–229, 1986
3. Canessa M, Adragna N, Solomon HS, Connolly TM, Tosteson DC: Increased sodium-lithium countertransport in red cells of patients with essential hypertension. *N Engl J Med* 302:772–776, 1980
4. Smith JB, Ash KO, Hunt SC, Hentschel MS, Sprowell W, Dadone MM, Williams RR: Three red cell sodium transport systems in hypertensive and normotensive Utah adults. *Hypertension* 6:159–166, 1984
5. Aronson PS: Red-cell sodium-lithium countertransport and essential hypertension. *N Engl J Med* 307:317, 1982
6. Mahnensmith RL, Aronson PS: The plasma membrane sodium-hydrogen exchanger and its role in physiological and pathophysiological processes. *Circ Res* 56:773–788, 1985
7. Blaustein MP: Sodium ions, calcium ions, blood pressure regulation, and hypertension: A reassessment and a hypothesis. *Am J Physiol* 232:C165–C173, 1977
8. Guyton AC, Coleman TG, Cowley AW, Manning RD, Norman RA, Ferguson JG: A systems analysis approach to understanding long-range arterial blood pressure control and hypertension. *Circ Res* 35:159–176, 1974
9. de Wardener HE, MacGregor GA: Dahl's hypothesis that a saluretic substance may be responsible for a sustained rise in arterial pressure: Its possible role in essential hypertension. *Kidney Int* 18:1–9, 1980
10. Blaustein MP, Hamlyn JM: Role of a natriuretic factor in essential hypertension: An hypothesis. *Ann Int Med* 98:785–792, 1983
11. Weder AB: Red-cell lithium-sodium countertransport and renal lithium clearance in hypertension. *N Engl J Med* 314:198–201, 1986
12. Boer WH, Koomans HA, Dorhout Mees EJ: Lithium clearance in mineralocorticoid escape in humans. *Am J Physiol* 252:F382–F386, 1987
13. Parenti P, Hanozet GM, Bianchi G: Sodium and glucose

transport across renal brush border membranes of Milan hypertensive rats. *Hypertension* 8:932–939, 1986
14. BIBER J, STIEGER B, HAASE W, MURER H: A high yield preparation for rat kidney brush border membranes. Different behavior of lysosomal markers. *Biochim Biophys Acta* 647:169–176, 1981
15. SABOLIC I, BURCKHARDT G: Effect of the preparation method on Na-H exchange and ion permeabilities in rat renal brush-border membranes. *Biochim Biophys Acta* 772:140–148, 1984
16. ARONSON PS, SACKTOR B: The Na gradient-dependent transport of D-glucose in renal brush border membrane vesicles. *J Biol Chem* 250:6032–6039, 1975
17. SEDMAK JJ, GROSSBERG SE: A rapid, sensitive, and versatile assay for protein using Coomassie Brilliant Blue G250. *Anal Biochem* 79:544–552, 1977
18. NORD EP, HAFEZI A, KAUNITZ JD, TRIZNA W, FINE LG: pH gradient-dependent increased Na-H antiport capacity of the rabbit remnant kidney. *Am J Physiol* 249:F90–F98, 1985
19. KINSELLA JL, ARONSON PS: Amiloride inhibition of the Na^+-H^+ exchanger in renal microvillus membrane vesicles. *Am J Physiol* 241:F374–F379, 1981
20. FRIEDRICH T, SABLOTNI J, BURCKHARDT G: Species difference between rat and rabbit renal Na-H exchanger. *Biochem Biophys Res Commun* 144:869–875, 1987
21. RABON E, CHANG H, SACHS G: Quantitation of hydrogen ion and potential gradients in gastric plasma membrane vesicles. *Biochemistry* 17:3345–3353, 1978
22. JACQUEZ JA: Tracers in the study of membrane processes, in *Membrane Physiology*, edited by TE ANDREOLI, JF HOFFMAN, DD FANESTIL, New York, Plenum Press, 1978, pp. 157–159
23. BEIERWALTES WH, ARENDSHORST WJ, KLEMMER PJ: Electrolyte and water balance in young spontaneously hypertensive rats. *Hypertension* 4:908–915, 1982
24. STERN N, LEE DBN, SILIS V, BECK FWJ, DEFTOS L, MANOLAGAS SC, SOWERS JR: Effects of high calcium intake on blood pressure and calcium metabolism in young SHR. *Hypertension* 6:639–646, 1984
25. DILLEY JR, STIER CT, ARENDSHORST WJ: Abnormalities in glomerular function in rats developing spontaneous hypertension. *Am J Physiol* 246:F12–F20, 1984
26. GARG LC, NARANG N, MCARDLE S: Na-K-ATPase in nephron segments of rats developing spontaneous hypertension. *Am J Physiol* 249:F863–F869, 1985
27. KATO S, MIYAMOTO M, KATO M, KANAMARU T, TAKAGI M, NAKANISHI N, SUGINO N: *J Hypertens* 4(suppl 3):S255–S256, 1986
28. ARONSON PS, SUHM MA, NEE J: Interaction of external H^+ with the Na^+-H^+ exchanger in renal microvillus membrane vesicles. *J Biol Chem* 258:6767–6771, 1983
29. FEIG PU, D'OCCHIO MA, BOYLAN JW: Lymphocyte membrane sodium-proton exchange in spontaneously hypertensive rats. *Hypertension* 9:282–288, 1987
30. BERK BC, VALLEGA G, MUSLIN AJ, GORDON HM, CANESSA M, ALEXANDER RW: Spontaneously hypertensive rat vascular smooth muscle cells in culture exhibit increased growth and Na^+/H^+ exchange. *J Clin Invest* 83:822–829, 1989