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Research Article

Decreased monosaccharide transport in renal brush-border membrane vesicles of spontaneously hypertensive rats

A. Mate[a], M. A. de la Hermosa[a], A. Barfull[b], I. Sánchez-Aguayo[c], J. M. Planas[b] and C. M. Vázquez[a,*]

[a]Departamento de Fisiología y Biología Animal, Facultad de Farmacia, Universidad de Sevilla, C/Tramontana s/n, E-41012 Sevilla (Spain), Fax + 34954233765, e-mail: vazquez@fafar.us.es
[b]Departamento de Fisiologia-Divisió IV, Facultad de Farmacia, Universidad de Barcelona, Avda Joan XXIII s/n, E-08028 Barcelona (Spain)
[c]Departamento de Biología Celular, Facultad de Biología, Universidad de Sevilla, Avda Reina Mercedes s/n, E-41012 Sevilla (Spain)

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Abstract. Na^+-dependent D-glucose and D-galactose transport were studied in brush-border membrane vesicles (BBMVs) from kidney cortex isolated from both spontaneously hypertensive rats (SHR) and their normotensive genetic control Wistar-Kyoto (WKY) rats. Initial rates and accumulation ratios of Na^+-dependent D-glucose and D-galactose transport were significantly lower in SHR compared with WKY, the observed decreases being similar for both substrates. To explain the reduction in sugar transport by renal BBMVs, the density of Na^+-dependent sugar cotransporters was studied in BBMVs from kidney cortex isolated from SHR and WKY rats. Phlorizin-specific binding and Western blot analysis indicated a reduction in the density of the cotransporters in SHR relative to WKY rats. This reduction was similar to those found for the initial rates and accumulation ratios for D-glucose and D-galactose in SHR. Na^+ uptake, studied using $^{22}Na^+$, was significantly increased in SHR, so the observed reduction in sugar transport could be due to disruption of the Na^+ gradient between renal BBMVs in SHR. Furthermore, a significant decrease in the activity of Na^+-K^+-ATPase was observed in SHR. In conclusion, changes in the density of the Na^+-dependent sugar cotransporter and in the Na^+ gradient across the brush-border membranes might be involved in the observed reduction in sugar transport by renal BBMVs from SHR.

Key words. Sugar transport; SHR; kidney; rat; BBMV.

Introduction

An increasing number of reports associate hypertension with ion transport defects, in particular with altered cellular sodium transport. Enhanced sodium permeability [1], increased Na^+-H^+ exchanger [2, 3] and reduced Na^+-K^+-ATPase activity [4] have been reported in various tissues from spontaneously hypertensive rats (SHR) when compared to their normotensive control, the Wistar Kyoto (WKY) rats. In the kidney in particular, increased activity of Na^+-H^+ exchanger [5, 6] and reduced Na^+-K^+-ATPase activity [7] have been observed in SHR.

Sugars are actively transported together with Na^+ by two specific carriers (sodium-coupled glucose cotransporters, SGLT1 and SGLT2) across the kidney proximal tubule in the brush-border membranes [8]. Changes in renal sodium transport observed in hypertension might result in modification of the Na^+-dependent transport of sugars in the kidney.

* Corresponding author.

Changes in glucose homeostasis in hypertension are often discussed. Essential hypertension is often associated with insulin resistance states, resulting in an increase in plasma insulin levels in SHR [9].

In the present work, we studied Na^+-dependent D-glucose and D-galactose transport in kidney cortex brush-border membrane vesicles (BBMVs) isolated from SHR and WKY rats. Possible mechanisms responsible for the changes observed are also discussed.

Material and methods

Animals. Male SHR and WKY rats were obtained at the age of 7–8 weeks from Harlan Iberica (Barcelona, Spain). Food and water were available ad libitum throughout the study. Diastolic and systolic blood pressures were measured weekly by the indirect method of tail-cuff occlusion in conscious animals, using an electrosphygmomanometer and physiograph recorder (Letica, Barcelona, Spain). The mean of three or four successive measurements was used as the estimate of blood pressure. Body weight was determined on the same day that blood pressure was measured. All experiments were carried out using 12- to 14-week-old rats, fasted for 18 h before killing.

Preparation of BBMVs. Renal cortical BBMVs were prepared from SHR and WKY rats by a $MgCl_2$ precipitation method [10] with slight modifications. Kidneys were removed and decapsulated before cutting slices from the cortex. Cortex slices were homogenized for 2 min in a buffer containing 100 mmol/l mannitol, 5 mmol/l EGTA, 2 mmol/l HEPES/Tris, pH 7.4, using a Yastral on setting 5. The homogenate was treated with 10 mmol/l $MgCl_2$ for 20 min and the mixture was then centrifuged at 1,900 *g* for 15 min. The supernatant was collected and centrifuged at 30,000 *g* for 30 min. The resulting pellet was resuspended in 30 ml of the above buffer and the Mg^{2+} precipitation and centrifugation steps were repeated. The resulting pellets were washed once in a buffer containing 300 mmol/l mannitol, 0.1 mmol/l $MgSO_4$ and 20 mmol/l HEPES/Tris, pH 7.4 and centrifuged at 30,000 *g* for 30 min. The final pellets containing purified BBMVs were resuspended in the buffer used in transport experiments, whose compositions are reported in uptake studies, and homogenized with a 25- and 29-gauge needle. The vesicles were frozen and stored in 50-µl aliquots in liquid nitrogen and used during a period of 15 days, during which, the integrity and functionality of the vesicles were not modified. For each preparation of BBMVs, renal cortex from two rats was used. BBMVs were always prepared in parallel on the same day from animals of both strains.

Protein and enzyme activity determinations. Protein determination was carried out by the method of Bradford [11] using bovine gamma-globulin as a standard. The membrane preparation was evaluated by measuring the specific activities of marker enzymes. Alkaline phosphatase was used as the marker enzyme for BBMVs and estimated by the method of Bretaudiere et al. [12]. The basolateral membrane marker enzyme, Na^+-K^+-ATPase, was measured according to Colas and Maroux [13]. Succinate dehydrogenase, as marker of mitochondria, and acid phosphatase, as marker of lysosomes, were also determined as previously described [14, 15]. All enzyme activities were measured at 37 °C.

Monosaccharide transport. The uptake of D-glucose and D-galactose was measured at 37 °C by a rapid filtration technique, as previously reported [16]. BBMVs were loaded in a medium containing 300 mmol/l mannitol, 0.1 mmol/l $MgSO_4$ and 20 mmol/l HEPES/Tris, pH 7.4. The incubation medium contained 100 mmol/l mannitol, 0.1 mmol/l $MgSO_4$, 20 mmol/l HEPES/Tris, pH 7.4, and either 0.1 mmol/l D-(^{14}C)glucose or D-(^{3}H)galactose and 100 mmol/l NaSCN or 100 mmol/l KSCN. Either Na^+-dependent D-glucose or D-galactose uptake was determined by the subtraction of the sugar uptake in the presence of a Na^+ gradient from that with no Na^+ gradient (K^+ gradient).

Intra- and extravesicular media were isotonic (320 mosmol/l), except for the experiments in which the effect of increasing osmolarity on substrate uptake was determined, where mannitol was added to the incubation medium to give the indicated osmolarity.

Phlorizin-binding measurements. For phlorizin-binding measurements, BBMV suspensions containing approximately 150 µg of protein were quickly mixed with 100 µl of the incubation medium containing either 100 mmol/l of NaSCN or KSCN and 5 µmol/l of phlorizin, and an aliquot of (^{3}H)phlorizin. Specific phlorizin binding was calculated at 5 s as that bound in the presence of Na^+ minus that bound in the presence of K^+, and the density of phlorizin-binding sites was expressed as picomoles of phlorizin bound per milligram of protein at a phlorizin concentration of 5 µM (B_5).

SDS-PAGE and Western blot analysis. Similar amounts of protein (100 µg) of BBMVs from SHR and WKY rats were solubilized in Laemmli sample buffer and resolved by 8% SDS-PAGE. Proteins were electrotransferred onto nitrocellulose membranes for 1 h at a constant voltage of 100 V. Immunoblotting and visualization of SGLT were carried out as previously described [17, 18]. Blots were incubated with a rabbit polyclonal antibody (kindly donated by Dr. M. Kasahara) raised against a synthetic peptide corresponding to amino acids 564–575 of the deduced amino acid sequence of rabbit intestinal SGLT1 [19]. In experiments carried out in parallel, nitrocellulose membranes were incubated with the same antibody which had first been adsorbed with the peptide (1 mg/ml) against which the antibody was raised (kindly provided by Dr. E. M.

Wright). The anti-SGLT1 antibody was detected by the enhanced chemiluminiscence (ECL) method using a peroxidase-conjugated anti-rabbit IgG as second antibody. After detection, the samples were measured by scanning densitometry. To reject the possibility that the results were influenced by the manipulation, SGLT1 antibody was stripped off the membranes by washing with PBS-Tween 20 for 30 min at room temperature. Membranes were later incubated with a mouse anti-actin monoclonal antibody. The process was performed according to the previously mentioned protocol [18].

Assay of Na^+ transport. BBMVs were prepared and loaded in a medium containing 100 mmol/l K gluconate, 100 mmol/l mannitol, 0.1 mmol/l $MgSO_4$ and 20 mmol/l HEPES/Tris, pH 7.4. Uptake was performed at 37 °C and initiated by incubation of vesicles with a medium comprised of 100 mmol/l mannitol, 100 mmol/l Na gluconate, 0.1 mmol/l $MgSO_4$, 20 mmol/l HEPES/Tris, pH 7.4, 45 μmol/l valinomycin and tracer $^{22}Na^+$. All transport experiments were performed in triplicate, using membrane prepared from different kidneys.

Materials. All unlabelled reagents were obtained from Sigma (Madrid, Spain), except for the reagents used to determine sucrase activity, which were from Boehringer (Mannheim, Germany) and the reagents used in Western blot analysis, which were from Bio-Rad (Barcelona, Spain). D-(U-^{14}C)glucose, D-(1-^{3}H)galactose, anti-actin monoclonal antibody and the ECL were obtained from Amersham International (Madrid, Spain). (^{3}H)phlorizin was from Itisa (Madrid, Spain). $^{22}Na^+$, as sodium chloride, was purchased from Dupont NEN (Madrid, Spain). The membrane filters were obtained from Millipore (Barcelona, Spain).

Statistical analysis. Comparison between different experimental groups was analysed by the unpaired, two-tailed Student t test, and differences were considered significant at $P < 0.05$.

Results

Body weight and blood pressure. Figure 1 shows diastolic and systolic blood pressures for SHR and WKY rats throughout the study. Both diastolic and systolic blood pressures were higher in SHR than in WKY rats. However, no significant differences were observed in the body weight of both strains from 8- to 14-week-old rats (data not shown).

Enzyme assays. Table 1 shows the specific activities of enzymes in homogenate and brush-border membranes prepared from SHR and WKY rat kidney cortex. The brush-border marker enzyme, alkaline phosphatase, was enriched 12-fold in the final brush-border membranes for both experimental rat strains compared with the starting homogenate, and the recoveries of this enzyme were high enough (34–40%) in both rat strains. Since enrichments and recoveries of alkaline phosphatase were not statistically different between preparations of BBMVs obtained from the two groups, these preparations are suitable for comparison studies in solute transport rates. Enrichments and recoveries of Na^+-K^+-ATPase, acid phosphatase and succinate dehydrogenase were low, indicating very little basolateral, lysosomal and mitochondrial contamination, respectively, in both experimental groups. No significant differences were observed in the specific activities of acid phosphatase and succinate dehydrogenase enzymes be-

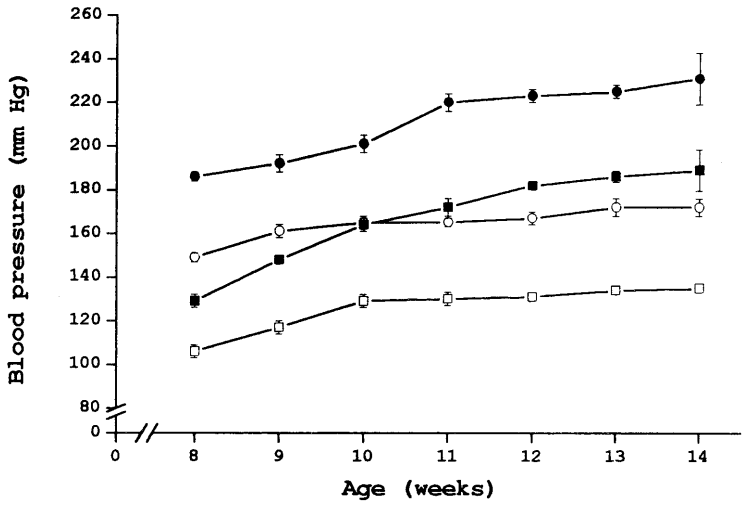

Figure 1. Diastolic (squares) and systolic (circles) blood pressure in SHR (closed symbols) and WKY rats (open symbols) throughout the study. Values represent means ± SE of at least ten animals.

Table 1. Enzyme activities in the homogenate and BBMVs prepared from WKY and SHR kidney cortex.

	Homogenate sp ac		BBMV sp ac		Enrichment		Recovery	
	WKY	SHR	WKY	SHR	WKY	SHR	WKY	SHR
Alkaline phosphatase	0.34 ± 0.03	0.24 ± 0.05	3.1 ± 0.1	2.9 ± 0.3	12 ± 1	12 ± 1	34 ± 3	40 ± 4
Na^+-K^+-ATPase	12.1 ± 0.4	6.0 ± 0.2**	7.2 ± 0.1	5.1 ± 0.3*	0.60 ± 0.01	0.83 ± 0.09	2.0 ± 0.2	2.1 ± 0.1
Acid phosphatase	47 ± 3	49 ± 1	115 ± 2	115 ± 10	2.0 ± 0.1	2.0 ± 0.1	4 ± 1	5 ± 1
Succinate dehydrogenase	25 ± 7	37 ± 11	4 ± 1	5 ± 1	0.30 ± 0.01	0.40 ± 0.01	0.20 ± 0.02	0.20 ± 0.02

Values are means $\pm$ SE for at least five different preparations. Specific activity (sp ac) is expressed in μmol p-nitrophenol formed/min per milligram protein (alkaline phosphatase), nmol p-nitrophenol formed/min per milligram protein (Na^+-K^+-ATPase and acid phosphatase), and μmol fumarate formed/min per milligram protein (succinate dehydrogenase). Enrichment = ratio of the specific activity of the brush-border membrane to that of the homogenate. Recovery = total activity in the brush-border membrane as a percentage of the total activity in the homogenate. *$P < 0.05$ and **$P < 0.01$ compared with WKY rats.

tween SHR and WKY rats. However, the specific activity of Na^+-K^+-ATPase was significantly reduced in kidney homogenate from SHR relative to WKY rats.

D-glucose and D-galactose uptake by BBMVs prepared from WKY and SHR kidney cortex. Figure 2 shows the time course of D-glucose and D-galactose uptake into BBMVs prepared from SHR and WKY rat kidney cortex. In the presence of a Na^+ gradient across the vesicle membrane, there was a transient increase (overshoot) in the intravesicular concentration of D-glucose (at 5 s) and D-galactose (at 15 s) in both groups of experimental rats. The initial rate and the magnitude of the accumulation ratio for Na^+-dependent D-glucose and D-galactose uptake were significantly decreased in SHR compared to WKY rats (see table 2). The overshoot for D-glucose and D-galactose disappeared in both groups of rats when the NaSCN gradient was replaced by a KSCN gradient. Uptake of D-glucose at equilibrium (30 min) was identical in the presence and absence of a Na^+ gradient and was not altered in SHR, indicating no difference in the size of membrane vesicles in the two animal groups (0.74 ± 0.001 μl/mg protein vs 0.78 ± 0.005 μl/mg protein for SHR and WKY rats, respectively).

Effect of medium osmolarity on Na^+-dependent D-glucose and D-galactose uptake by BBMVs prepared from WKY and SHR kidney cortex. To determine whether D-glucose and D-galactose uptake occur inside an osmotically sensitive intravesicular space, BBMVs were prepared from SHR and WKY rats, and the intravesicular space was reduced by increasing the medium osmolarity with mannitol. As shown in figure 3, D-glucose and D-galactose uptakes at equilibrium (30 min) were directly proportional to the reciprocal of medium osmolarity in WKY rats. At infinite osmolarity, there is minimal binding, indicating that Na^+-dependent sugar transport was due to transport into the intravesicular space. Although not shown, a similar relationship for D-glucose and D-galactose uptake was found in BBMVs from SHR.

Phlorizin-binding measurements. Phlorizin has long been recognized as a potent competitive inhibitor of D-glucose and D-galactose uptake at the brush-border membrane from kidney cortex [8]. Therefore, measurement of phlorizin binding indicates the density of Na^+-dependent D-sugar transporter. To establish the appropriate concentration of phlorizin to be used for phlorizin-binding studies in SHR and WKY rats, the effect of varying concentrations of phlorizin on 0.1 mM Na^+-dependent D-glucose uptake at 5 s was studied in BBMVs from SHR and WKY rats. As shown in figure 4, the IC_{50} values for phlorizin were about 1 μM, with no differences between SHR and WKY rats. These values are comparable to that previously reported [8]. The concentration of 5 μmol/l phlorizin was chosen for phlorizin-binding studies because in both groups of animals, $> 80\%$ of binding sites were occupied at this concentration. A significant decrease in the density of sugar-protectable binding sites was found in BBMVs from SHR compared to WKY rats (table 2). When the initial rates of Na^+-dependent D-glucose and D-galactose uptake were plotted against the specific phlorizin-binding-site density (B_5), a highly linear direct correlation was found (fig. 5).

Immunoblots. Figure 6A shows Western blot analysis of SGLTs in BBMVs obtained from renal cortex of WKY rats (lane 3) and SHR (lane 4). The antibody recognized a single band of about 67 kDa that was blocked by preabsorption with the antigenic peptide (lanes 1, 2). Figure 6B shows the densitometric analysis for SGLT1 of four separate assays. Figure 6C shows the hybridization signal obtained when the membrane used in figure 6A was washed and incubated with anti-actin antibody. This antibody recognized a single band of 45 kDa without significant abundance differences between BBMVs from WKY rats (lane 3) and SHR (lane 4).

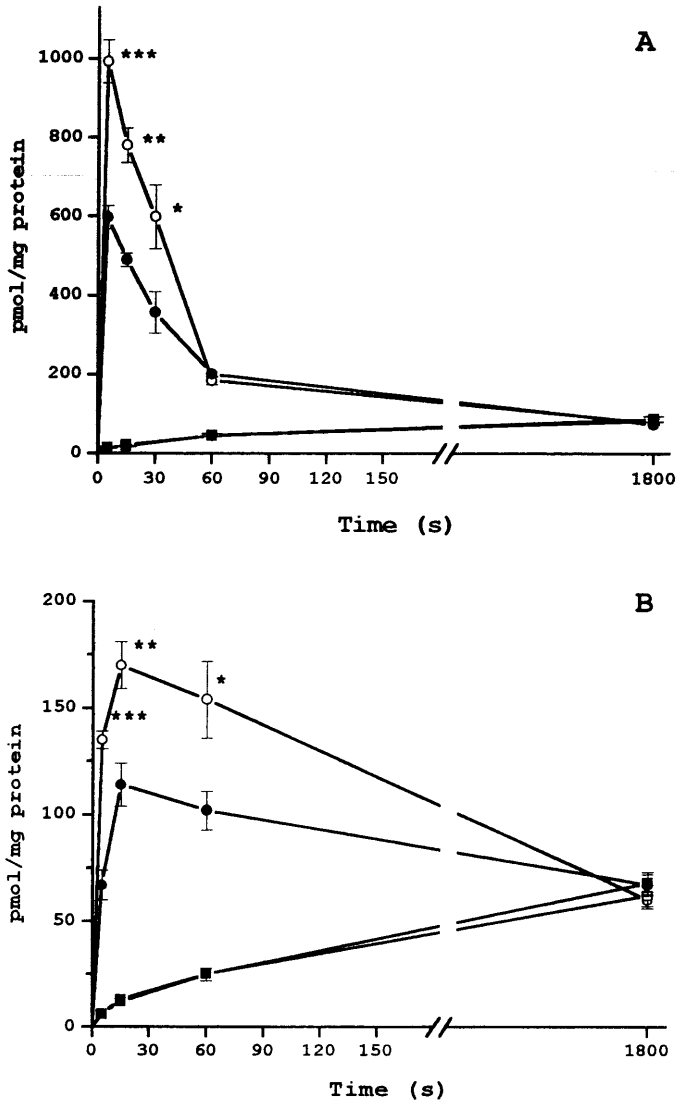

Figure 2. Time course of D-glucose (*A*) and D-galactose (*B*) uptake in BBMVs prepared from WKY (open symbols) and SHR (closed symbols) kidney cortex in the presence of a K^+ gradient (squares) or a Na^+ gradient (circles). Values represent means ± SE of at least ten different preparations. When not given, SE bars were smaller than the symbol used. *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$.

Table 2. Initial rates (IR) and accumulation ratios (AR) for Na^+-dependent D-glucose and D-galactose transport, and phlorizin binding in BBMVs prepared from WKY and SHR kidney cortex.

Rats	D-glucose		D-galactose		Phlorizin binding
	IR	AR	IR	AR	
WKY	199 ± 11	11 ± 0.9-fold	26 ± 2	2.0 ± 0.1-fold	6.3 ± 0.9
SHR	126 ± 5***	7 ± 0.4*-fold	16 ± 2*	1.3 ± 0.1**-fold	4.3 ± 0.1*

Results are means ± SE of at least ten different preparations. IR and phlorizin binding are expressed in pmol/mg protein per second. *$P < 0.05$, **$P < 0.01$ and ***$P < 0.005$ compared with WKY rats.

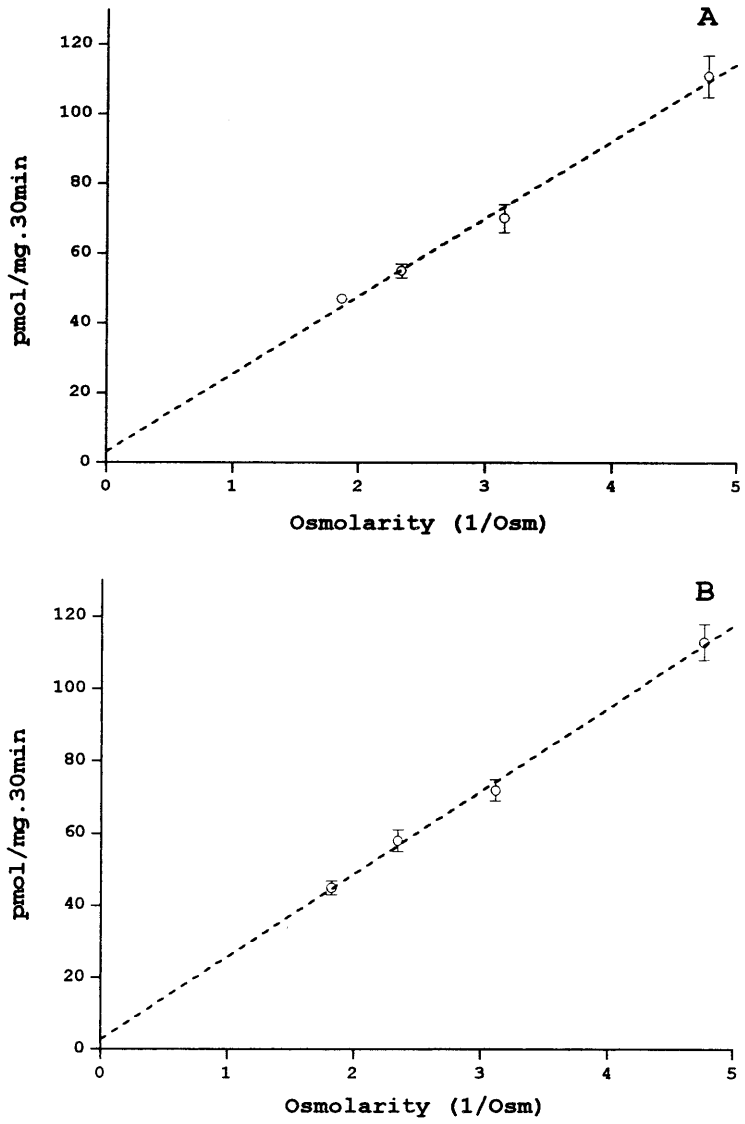

Figure 3. The effect of incubation medium osmolarity on Na^+-dependent D-glucose (*A*) and D-galactose (*B*) uptake in BBMVs prepared from WKY kidney cortex. Values represent means ± SE of at least four different preparations. Regression lines were calculated by the least-squares method. Equations and correlation coefficients (r) were: D-glucose, $y = 3.21 + 22.31x$, $r = 0.997$; D-galactose, $y = 2.81 + 22.97x$, $r = 0.999$. Similar relationships were noted for SHR.

Na^+ uptake by BBMVs prepared from WKY and SHR kidney cortex. To determine whether changes in Na^+ gradient are responsible for the observed decrease in the renal accumulation ratio of Na^+-dependent D-sugar uptake in hypertensive rats, $^{22}Na^+$ uptake into BBMVs was measured in normotensive and hypertensive rats. A significant enhancement in Na^+ uptake was found in BBMVs prepared from SHR kidney cortex at 5 and 15 s compared to WKY (table 3). A slight increase in Na^+ uptake was found at 1 min, but no significant differences between the animal groups were observed at 30 min. Because these results were accompanied by a decrease in the Na^+-dependent D-sugar uptake by BBMVs from SHR, the disruption of the Na^+ gradient due to an increase in Na^+ transport could be, in part, responsible for the observed decrease in the renal transport of monosaccharides in SHR.

Discussion

This study demonstrates that Na^+-dependent D-glucose and D-galactose uptake are significantly reduced in

BBMVs prepared from SHR kidney cortex compared to those from WKY rats. Initial rates and maximal overshoot levels of Na^+-dependent D-glucose and D-galactose uptake were significantly lower in BBMVs prepared from SHR compared with the corresponding values in WKY rats (table 2, fig. 3). The observed decrease in both initial rates and the magnitude of the overshoot in SHR were similar for D-glucose and D-galactose (1.57-fold and 1.58-fold, respectively). This decrease in Na^+-dependent D-sugar uptake in BBMVs from SHR is not due to variations in vesicle preparations, because the purification and the size of BBMVs, as measured from the enrichment of alkaline phosphatase and Na^+-dependent D-glucose uptake at equilibrium (30 min), respectively, were similar for normotensive and hypertensive rats.

These results are consistent with previous experiments by Morduchowicz et al. [5], in which a decrease in Na^+-dependent D-glucose uptake was noted in renal BBMVs prepared from SHR compared with the control WKY rats. Similarly, a decrease in the ileal transport of sugars was previously observed in our laboratory [20]. However, Parenti et al. [21] did not find modifications in D-glucose transport across renal BBMVs of Milan hypertensive rats.

To investigate whether the reduction in kidney sugar transport in SHR was paralleled by changes in the density of Na^+-dependent sugar cotransporters, the binding of phlorizin and Western blot analysis were performed. Specific phlorizin-binding sites were decreased approximately 1.58-fold in BBMVs from SHR kidney compared with normotensive rats. This ratio was similar to that of initial rates and accumulation ratios for both D-glucose and D-galactose in hypertensive rats compared to normotensive rats (table 2). Western blot analysis of SGLT1 showed a single band of 67 kDa that was blocked by preabsorbing the antibody with the antigenic peptide. Blot densitometry demonstrated that the abundance of sugar cotransporters is reduced in BBMVs isolated from kidney SHR. Western blot analysis of actin, as a control protein, was performed in parallel, and no significant differences were found between renal BBMVs from SHR and WKY rats. Therefore, these results demonstrate that lower kidney sugar transport in hypertension is in part due to a lower number of sugar transporters in hypertensive rats.

Studies of sugar transport by BBMVs were carried out with an inwardly directed Na^+ gradient. Changes in the sodium gradient by BBMVs prepared from SHR might explain the decreased sugar transport observed in hypertensive rats. To explore this possibility, $^{22}Na^+$ uptake was measured into BBMVs from SHR and WKY rats. As seen in table 3, Na^+ uptake was significantly increased in SHR at 5 and 15 s when compared to WKY rats, indicating that the observed reduction in sugar transport at 5 and 15 s in kidney from SHR could be secondary to differences in the Na^+ gradient between SHR and WKY rats. However, at 1 min, the Na^+ uptake was only slightly increased in SHR relative to WKY rats, suggesting that other factors are involved in the observed decrease in kidney sugar transport. An

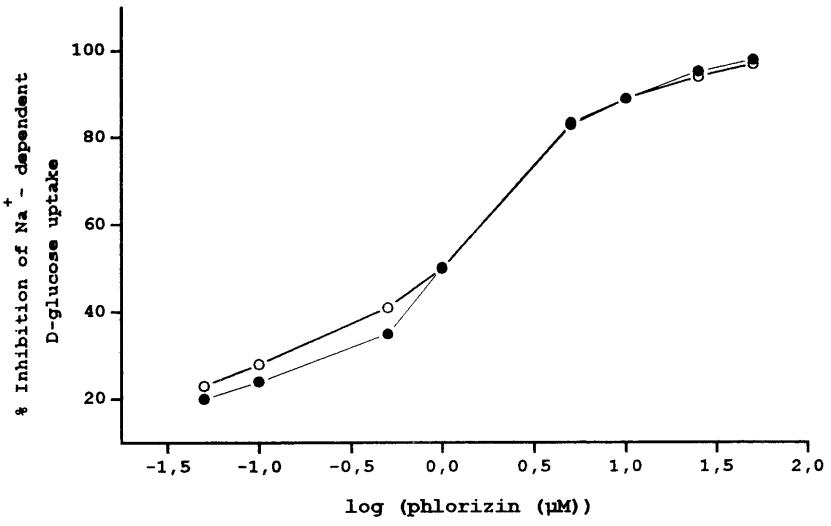

Figure 4. The effect of varying concentrations of phlorizin on 0.1 mM Na^+-dependent D-glucose uptake at 5 s in kidney cortex isolated from SHR (closed circles) and WKY rats (open circles). Data were expressed as percent inhibition of uptake without phlorizin. Values represent means ± SE of at least ten different preparations.

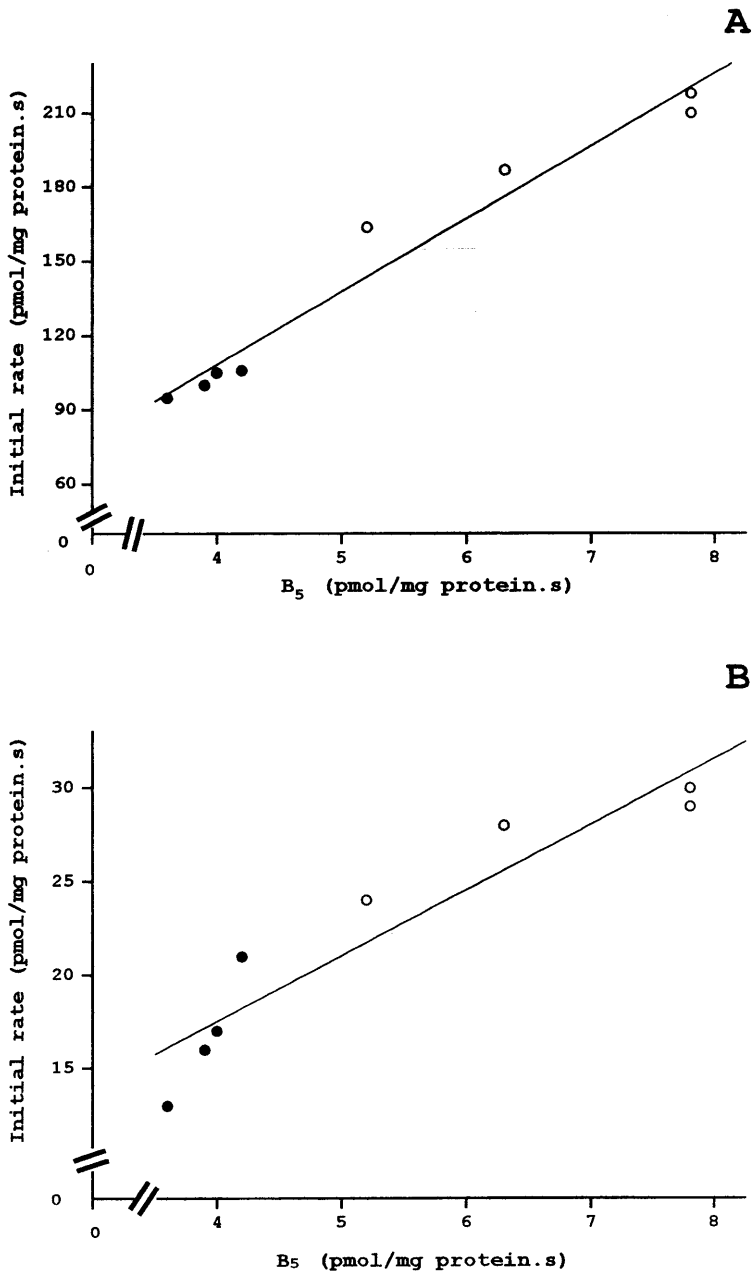

Figure 5. Relationship between initial rates of Na^+-dependent D-glucose (*A*) or D-galactose (*B*) uptake and specific phlorizin binding (B_5) in BBMVs from SHR (closed circles) and WKY rats (open circles). Equations and correlation coefficients (r) were $y = -9.74 + 29.51x$, $r = 0.980$, $P < 0.001$ for D-glucose, and $y = 3.48 + 3.51x$, $r = 0.940$, $P < 0.001$ for D-galactose.

increase in Na^+ uptake has been previously reported in renal brush-border membrane from Milan hypertensive rats [21], in the isolated perfused kidney [22], in erythrocyte cell membranes [1] and in vascular smooth muscle from SHR [23].

Other Na^+ transport pathways are altered in kidney cells from SHR that in turn might affect the Na^+ gradient across the brush-border membranes. Thus, Na^+-H^+ exchange was stimulated in the kidney cortex from SHR [5], and as previously reported in several tissues from SHR [7, 24–26] and in patients with essential hypertension [27], we found a decrease in the Na^+-K^+-ATPase activity in kidney homogenates prepared from SHR when compared to WKY rats.

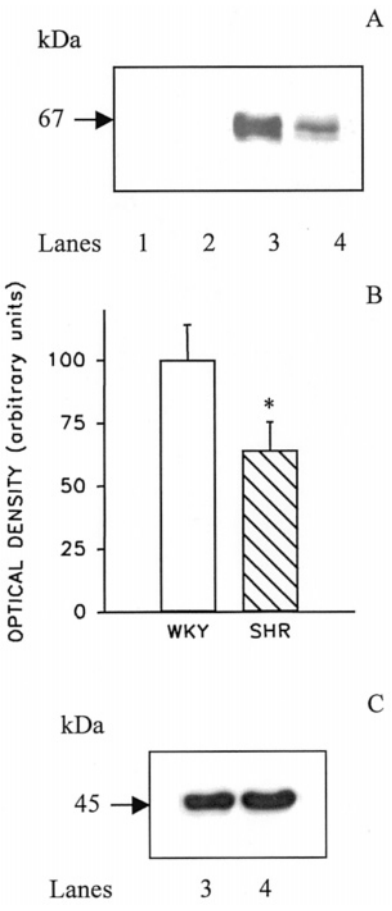

Figure 6. (*A*) Western blot analysis of SGLTs in BBMVs obtained from renal cortex of WKY rats (lane 3) and SHR (lane 4). The antibody recognized an immunoreactive protein of about 67 kDa. When the antibody was previously adsorbed with the antigenic peptide, no signal was detected (lanes 1, 2). (*B*) Relative abundance measured by optical densitometry. Values represent means $\pm$ SE of four separate experiments. *$P < 0.05$. (*C*) Hybridization signal obtained when the membrane used in (*A*) was washed and incubated with anti-actin antibody. The antibody recognized a single band of 45 kDa with no abundance differences between BBMVs from WKY rats (lane 3) and SHR (lane 4).

Table 3. $^{22}Na^+$ uptake into BBMVs prepared from WKY and SHR kidney cortex.

Rats	$^{22}Na^+$ transport (nmol/mg protein)			
	5 s	15 s	1 min	30 min
WKY	22 ± 2	41 ± 4	88 ± 7	144 ± 8
SHR	33 ± 2**	60 ± 4*	105 ± 14	147 ± 19

Results are means $\pm$ SE from at least six different preparations. *$P < 0.05$ and **$P < 0.01$ compared with WKY rats.

In conclusion, we have found a decrease in the transport of D-glucose and D-galactose in renal BBMVs prepared from SHR which paralleled a reduction in the density of Na^+-dependent sugar cotransporters, as well as changes in the Na^+ gradient across the brush-border membrane.

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