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Research Article

Characterization of D-fructose transport by rat kidney brush-border membrane vesicles: changes in hypertensive rats

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Abstract. D-fructose transport was characterized in renal brush-border membrane vesicles (BBMVs) from both spontaneously hypertensive rats (SHR) and normotensive genetic control Wistar-Kyoto (WKY) rats. Kinetic studies indicated that the maximal rate (Vmax) of D-fructose transport was significantly lower in SHR compared with WKY rats. No differences were observed in the Michaelis constant (Km) or the diffusion constant (Kd) between the two groups of animals. D-fructose inhibited its own transport, whereas the presence of D-glucose, D-galactose, phlorizin, and cytochalasin B did not inhibit the transport of D-fructose in either animal group. To explain the reduction in D-fructose transport in SHR, the density of the D-fructose transporter, GLUT5, was analyzed by Western blot. GLUT5 levels were lower in SHR, a reduction similar to that of the Vmax. Thus, there appears to be a high-affinity, low-capacity, GLUT5-type fructose carrier in the apical membranes of rat kidney cortex, and the decrease in the Vmax of D-fructose transport in renal BBMVs from hypertensive rats correlates well with a reduction in the expression of GLUT5 protein.

Key words. Fructose transport; SHR; kidney; rat; BBMV; GLUT5.

D-fructose is a natural monosaccharide found in food as a free sugar and in combination with other carbohydrates. Despite its potential benefits in glycemic control when consumed in moderate amounts [1, 2], previous studies have shown that rats fed a high-fructose diet develop an increase in blood pressure that is associated with insulin resistance, hyperinsulinemia, and hypertriglyceridemia [3].

Glucose transporters are membrane proteins that transport sugars such as glucose, galactose, and fructose. In the kidney, the expression of two Na^+-glucose cotransporters (SGLT1 and SGLT2) has been reported, being responsible for the absorption of glucose and galactose [4]. Previous studies have also demonstrated that the fructose transporter, GLUT5, is expressed in the kidney [5, 6] Specifically, in situ hybridization studies [7], together with the immunocytochemical localization of GLUT5 [8], have revealed that this protein is located in the brush-border membrane of S3 proximal tubule cells. However, the exact role of this transporter in kidney physiology and its kinetic properties in the brush-border membrane are not well characterized.

The kidney plays an important role in the pathophysiology of hypertension, and an increasing number of reports associate this disease with ion transport defects in the kidney, in particular with altered cellular sodium transport [9, 10]. We have previously demonstrated a reduction in D-glucose and D-galactose transport in renal brush-border membrane vesicles (BBMVs) from hypertensive rats, which was accompanied by modifications in Na^+ transport and in the density of sugar cotransporters,

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SGLTs [11]. However, whether the observed changes in the renal transport of D-glucose and D-galactose in hypertensive rats are specific for an Na^+-dependent sugar cotransporter or whether other sugar transporters, such as GLUT5, are also affected in the kidney of hypertensive rats is unknown.

The aims of the present work were (i) to characterize the transport of D-fructose in BBMVs isolated from rat kidney cortex and (ii) to study the activity of GLUT5 in the kidney of spontaneously hypertensive rats (SHR) and their normotensive control, Wistar-Kyoto (WKY) rats. In addition, GLUT5 protein levels were determined in BBMVs isolated from both groups of animals.

Materials 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, which were fasted for 18 h before killing.

Preparation of BBMVs

Renal cortical BBMVs were prepared from SHR and WKY by a $MgCl_2$ precipitation method, as previously described in detail [11]. The final pellets containing purified BBMVs were resuspended in the buffers 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 liquid nitrogen until use (for a period no longer than 15 days). For each preparation of BBMVs, renal cortex from two rats was used. Preparation of BBMVs was always run 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 [12] 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. [13]. The basolateral membrane marker enzyme, Na^+-K^+-ATPase, was measured according to Colas and Maroux [14]. Succinate dehydrogenase, as marker of mitochondria, and acid phosphatase, as marker of lysosomes, were also determined as previously described [15, 16]. All enzyme activities were measured at 37 °C.

Determination of transport ability and intravesicular space

Na^+-dependent D-glucose transport was measured to control the functional integrity of the isolated BBMVs and to determine the intravesicular volume. Five to 10 µl of BBMVs, loaded in a medium containing 300 mmol/l mannitol, 0.1 mmol/l $MgSO_4$, and 20 mmol/l HEPES/Tris, pH 7.4, was combined with 100 µl of incubation medium containing 100 mmol/l mannitol, 0.1 mmol/l $MgSO_4$, 20 mmol/l HEPES/Tris, pH 7.4, 0.1 mmol/L D-(^{14}C)glucose, and 100 mmol/l NaSCN. Functional integrity and the intravesicular space were estimated at 5 s and at equilibrium (30 min), respectively, at 37 °C, using the rapid filtration technique described elsewhere [11].

D-fructose transport

To measure D-fructose uptake, 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 300 mmol/l mannitol, 0.1 mmol/l $MgSO_4$, 20 mmol/L HEPES/Tris, pH 7.4, and 1 mmol/l D-(^{14}C)fructose.

For studies of the cationic dependence of D-fructose transport, the incubation medium contained 100 mmol/l mannitol, 0.1 mmol/l $MgSO_4$, 20 mmol/L HEPES/Tris, pH 7.4, 1 mmol/l D-(^{14}C)fructose, and either 100 mmol/l NaSCN or 100 mmol/l KSCN.

For the kinetic analysis of D-fructose transport, D-fructose concentrations were 0.1, 0.5, 1, 10, 50, 100, 150, and 200 mmol/l.

Inhibition studies of D-fructose transport (1 mmol/l) were carried out in the presence of D-glucose (100 mmol/l), D-galactose (100 mmol/l), cytochalasin B (50 µmol/l), phlorizin (250 µmol/l), and D-fructose (100 and 500 mmol/l). When 500 mmol/l of D-fructose was added to the incubation medium, BBMVs were loaded in a medium containing 500 mmol/l mannitol, 0.1 mmol/l $MgSO_4$, and 20 mmol/L HEPES/Tris, pH 7.4

Intra- and extravesicular media were isotonic (320 mosmol/l or 520 mosmol/l).

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 GLUT5 were carried out as previously described [17]. Immunoblotting was performed using antisera raised in rabbits against the synthesized peptide corresponding to the COOH-terminal domain of GLUT5 (residues 490–502) (kindly donated by Dr. T. Asano) di-

luted at 1:1000. In experiments carried out in parallel, membranes were incubated with the same antibody previously adsorbed with the antigenic peptide (1 mg/ml). The anti-GLUT5 antibody was detected by the enhanced chemiluminiscence (ECL) method using peroxidase-conjugated anti-rabbit IgG as a secondary antibody (1:300 dilution). After detection, the samples were measured by scanning densitometry. To reject the possibility of artifacts due to the manipulation, GLUT5 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 according to the protocol described above.

Materials

All unlabelled reagents were obtained from Sigma (Madrid, Spain), except for the reagents used in Western blot analysis, which were from Bio-Rad (Barcelona, Spain) D-(U-^{14}C)fructose, D-(U-^{14}C)glucose, anti-actin monoclonal antibody, and the ECL reagent were obtained from Amersham (Madrid, Spain). The membrane filters were obtained from Millipore (Barcelona, Spain).

Kinetic analysis

Total D-fructose fluxes from at least three independent experiments were analyzed by non-linear regression, using the Enzfitter program (Biosoft, Cambridge, UK). As errors associated with experimental fluxes were roughly proportional to their values, applying a proportional weighting to the data was considered appropriate. Kinetic parameter evaluation was made by systematically testing different model equations corresponding to one or two Michaelian components plus a linear, non-specific component.

Statistical analysis

Time- and strain-dependent differences were subjected to two-way analysis of variance (ANOVA). When significance was found, an unpaired, two-tailed Student's t test was used, and differences were considered significant at $p < 0.05$. All other comparisons were performed by the unpaired t test.

Results

Body weight and systolic blood pressure

SHR blood pressures were significantly higher (in mm Hg, mean ± SE = 163 ± 11, 180 ± 17, and 220 ± 22 at 8, 10, and 14 weeks, respectively) than those of WKY (127 ± 5, 135 ± 2, and 145 ± 20 at the same ages, respectively). Blood pressure became abnormally high in SHR at the age of 11 weeks (190 ± 18 mm Hg), and hypertension was definitively established at the age of 12 weeks (205 ± 20 mm Hg vs 140 ± 12 mm Hg for SHR and WKY rats, respectively), the values remaining constant thereafter. In contrast there were no significant differences in body weight values between either strain from 8 to 14 weeks (data not shown).

Purity of membrane vesicles

We used alkaline phosphatase activity as the marker for the brush-border membrane, and Na^+-K^+-ATPase, acid phosphatase, and succinate dehydrogenase as markers for the basolateral, lysosomal, and mitochondrial membranes, respectively. Alkaline phosphatase was 12-fold enriched in the final brush-border membranes from both experimental rats compared with the starting homogenate, and the recoveries of this enzyme were 34-40% in both rat strains. 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 (data not shown). The transport capability of the BBMVs was assessed by determining D-glucose uptake. The presence of an Na^+ gradient ($Na^+_o > Na^+_i$) induced a transient accumulation of D-glucose in both groups of animals, indicating that BBMVs isolated from both groups were functional (data not shown). In addition, no significant changes were observed in the intravesicular volume between normotensive and hypertensive rats, estimated from D-glucose distribution at equilibrium (30 min) (0.74 ± 0.001 vs 0.78 ± 0.005 µl/mg protein for SHR and WKY rats, respectively).

D-fructose uptake by BBMVs prepared from WKY and SHR kidney cortex

Figure 1 shows the time course of D-fructose uptake into BBMVs prepared from SHR and WKY rat kidney cortex.

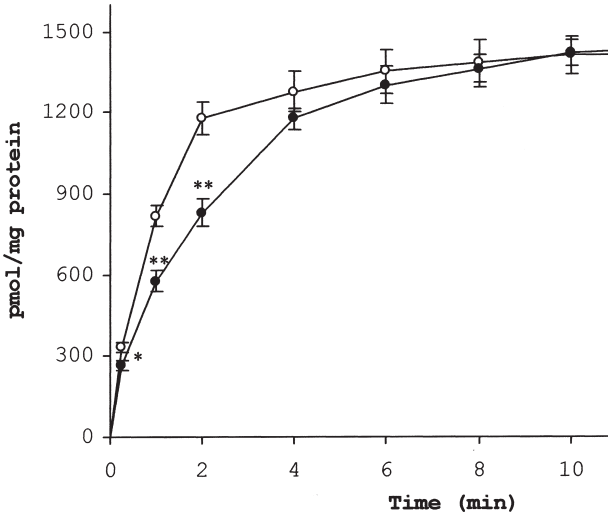

Figure 1. Time course of D-fructose uptake in BBMVs prepared from WKY (open symbols) and SHR (closed symbols) kidney cortex. Values represent means ± SE of at least ten different preparations. *$p < 0.05$; **$p < 0.01$.

Table 1. Effect of K^+ and Na^+ on D-fructose uptake in BBMVs prepared from WKY and SHR kidney cortex.

Gradient	D-fructose uptake (pmol/mg protein)					
	15 s		1 min		30 min	
	WKY	SHR	WKY	SHR	WKY	SHR
None	331 ± 18	268 ± 18	818 ± 42	575 ± 39	1408 ± 90	1452 ± 60
Na^+	334 ± 33	270 ± 30	711 ± 72	580 ± 60	1539 ± 156	1456 ± 137
K^+	320 ± 40	290 ± 25	738 ± 60	560 ± 50	1520 ± 130	1521 ± 155

Results are means ± SE of at least four different preparations.

Uptake values were always linear up to 30 s of incubation, and the equilibrium was reached at 6–8 min in both groups of animals. D-fructose uptake was significantly decreased in SHR compared to WKY rats at 15 s, 1 and 2 min. From this time, no differences were observed between the two rat groups.

To specify the functional properties of the D-fructose carrier, we analyzed D-fructose uptake (1 mmol/l) in the presence of an Na^+ and K^+ gradient. The presence of 100 mmol/l of these cations in the incubation medium did not significantly modify the sugar uptake in either rat group (table 1).

The relationship between the external D-fructose concentration and the observed uptake was measured in the range 0.1–200 mmol/l D-fructose (fig. 2). Total fluxes, in both groups of animals, could be fitted to a kinetic model comprised of one saturable component plus a non-saturable one. The calculated kinetic parameters are shown in table 2. A significant decrease in the Vmax was observed in kidney from SHR compared to WKY rats. However, no differences were noted in the Km and Kd values between the two groups of animals.

Table 2. Kinetic parameters of D-fructose uptake in BBMVs prepared from WKY and SHR kidney cortex.

Parameter	WKY	SHR
Vmax (pmol/s per mg protein)	106 ± 13	58 ± 3*
Km (mM)	12.6 ± 2.1	9.87 ± 1.26
Kd (nl/s per mg protein)	19.6 ± 0.2	22.2 ± 0.9

Vesicles were incubated for 15 s with concentrations ranging from 0.1 to 200 mM D-fructose. Values represent means ± SE of at least four different preparations (*$p < 0.05$).

D-glucose and D-galactose did not inhibit D-fructose uptake in either rat group (fig. 3). The same results were obtained with phlorizin (250 µmol/l) and cytochalasin B

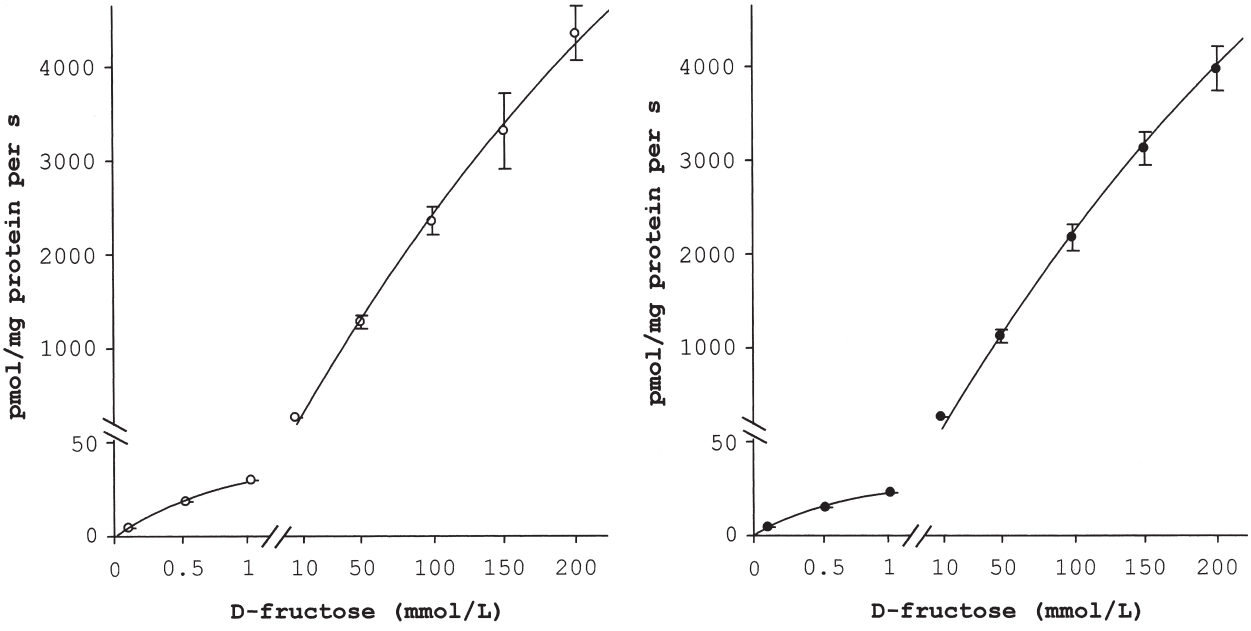

Figure 2. Kinetics of D-fructose uptake in BBMVs prepared from WKY (open symbols) and SHR (closed symbols) kidney cortex. Total fluxes were measured at 15 s incubation at the indicated D-fructose concentrations. Kinetic analysis was performed as described in Materials and methods. Values represent means ± SE of at least three independent experiments. When not given, SE bars are smaller than the symbol used.

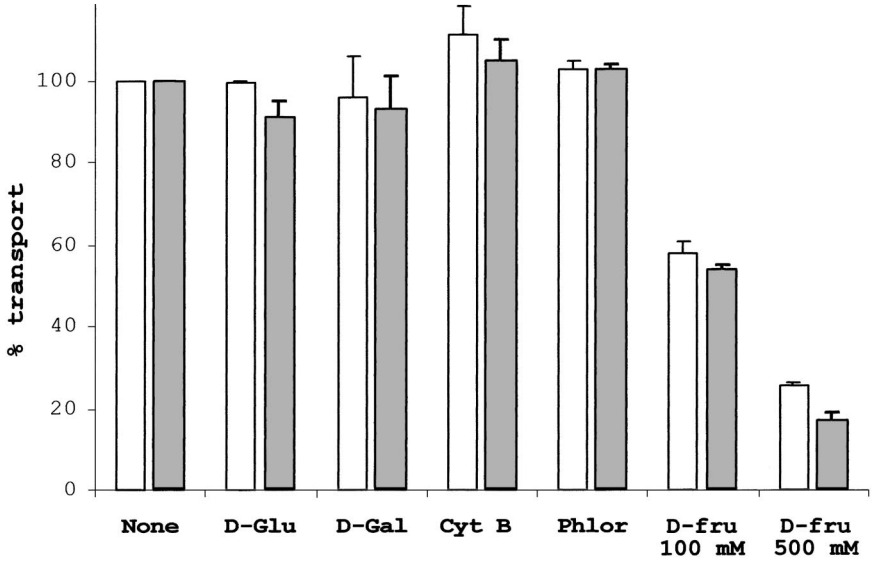

Figure 3. Effect of different substrates on the uptake of 1 mmol/l D-fructose in BBMVs prepared from WKY (open bars) and SHR (closed bars) kidney cortex. Uptake was measured at 15 s incubation, in the presence of D-glucose (100 mmol/l), D-galactose (100 mmol/l), cytochalasin B (50 µmol/l), phlorizin (250 µmol/l), and D-fructose (100 and 500 mmol/l). Values represent means ± SE of at least four different preparations, and are expressed as a percentage of the values obtained in the absence of inhibitors.

(50 µmol/l). In contrast, D-fructose transport was inhibited by the presence of high concentrations of D-fructose (40–45% at 100 mmol/l and 75–85% at 500 mmol/l in both groups of animals).

Immunoblots

Western blot analysis of BBMVs obtained from renal cortex, using an antibody against the C-terminal region of rat GLUT5, is shown in figure 4A. The antibody recognized a single band of about 51 kD in WKY (lane 3) and SHR (lane 4) that was blocked by preabsorption with the antigenic peptide (lanes 1, 2). Figure 4B shows the densitometric analysis for GLUT5 in four separate assays, indicating a reduction in the abundance of GLUT5 in membranes isolated from SHR compared to WKY rats. Figure 4C shows the immunoblot obtained when the membrane used in figure 4A was washed and incubated with anti-actin antibody. This antibody recognized a single band of 45 kD without significant abundance differences between BBMVs from WKY (lane 3) and SHR (lane 4).

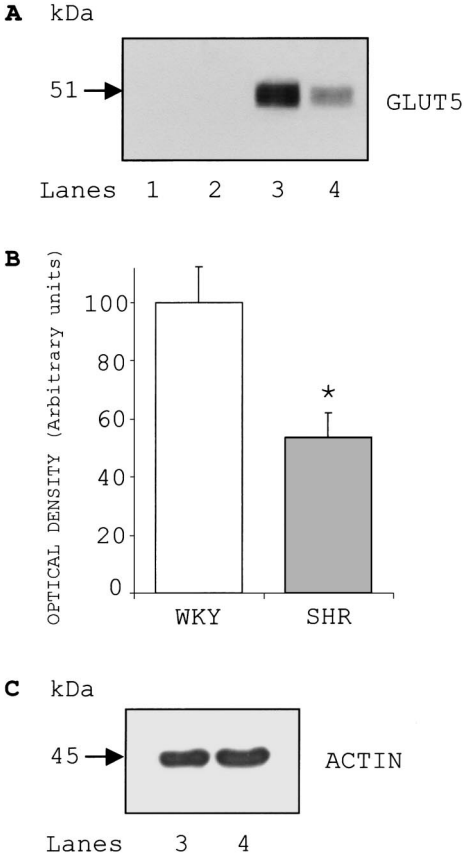

Figure 4. (*A*) Western blot analysis of GLUT5 in BBMVs obtained from renal cortex of WKY (lane 3) and SHR (lane 4). The antibody recognized an immunoreactive protein of about 51 kD. When the antibody was previously adsorbed with the antigenic peptide, no signal was detected (lanes 1, 2). (*B*) Relative abundance measured by optical densitometry. Values represent means ± SE of four separate experiments (*$p < 0.05$). (*C*) Immunoblot obtained when the membrane used in (*A*) was washed and incubated with anti-actin antibody. The antibody recognized a single band of 45 kD without significative abundance differences between BBMVs from WKY (lane 3) and SHR (lane 4).

Discussion

Although studies using Northern blot analyses identified GLUT5 mRNA expression in the rat kidney [5, 6], and results obtained by in situ hybridization [18] and immunohistochemistry [8] showed that GLUT5 was abundant in S3 proximal tubule cells at the level of apical membranes, a specific fructose transport system has not been characterized in the brush-border membrane of rat kidney. In this work, we studied the transport of D-fructose in BBMVs isolated from the kidney cortex of SHR and WKY rats, investigating the kinetic properties and substrate specificity of the fructose transporter, GLUT5.

For the kinetic analysis of D-fructose uptake by BBMVs, we used initial influx measurements over a wide concentration range, from 0.1 to 200 mmol/l. Results of GLUT5-mediated D-fructose transport revealed a 45% reduction in the maximal transport capacity (Vmax) for D-fructose in BBMVs prepared from SHR kidney cortex when compared to WKY rats (table 2). In contrast, the affinity of the D-fructose transporter was similar in SHR and WKY rats, both groups showing the same Km values. The decrease in D-fructose uptake in BBMVs isolated from SHR is not due to variations in vesicle preparations, because the purification and 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.

The Vmax value for D-fructose transport in renal BBMVs from normotensive rats, 106 pmol/s per milligram protein, is much lower than that reported in studies carried out in BBMVs from rat proximal intestine, i.e., 2675 pmol/s per milligram protein [19, 20]. This is likely due to the different substrate concentrations at these levels, since fructose levels are much higher in the intestine than in the kidney, where only a small amount of this sugar is filtered in the glomerulus.

Km values for apical D-fructose transport obtained in the present study were about 10 mM, in agreement with values obtained in rabbit [21], guinea pig [22], and hamster [23] intestine, oocytes expressing rabbit GLUT5 jejunal mRNA [24], and for human GLUT5 mRNA [25], Chinese hamster ovary cells transfected with rat GLUT5 cDNA [17], and human skeletal muscle [26]. A Km value of 110 mM was obtained by Crouzoulon and Korieh [20] in rat intestinal BBMVs. However, we have found Km values of 10–15 mM in jejunal and ileal BBMVs isolated from SHR and WKY rats (unpublished observations).

The effect of various sugars on D-fructose transport in Na^+-free medium was also examined in BBMVs from both groups of animals. We found that the transport of D-fructose across renal BBMVs is highly specific, since among the sugars examined, only D-fructose inhibited its own transport, with a similar inhibition pattern in WKY and SHR (fig. 3). Neither cytochalasin B (50 µM) nor phlorizin (250 µM) affected D-fructose transport. These observations are consistent with those of Schultz and Strecker [21], Miyamoto et al. [24], Inukai et al. [17], and Burant et al. [25]. However, our results are not in agreement with Rand et al. [5], who reported that rat GLUT5 can transport D-glucose in oocytes, although to a lower extent than D-fructose.

To examine whether the reduction in kidney D-fructose transport in SHR was related to changes in the density of GLUT5 cotransporter, we performed Western blot analyses in renal brush-border membranes. A single band of 51 kD was detected in both groups of animals, and was blocked by preabsorbing the antibody with the antigenic peptide (fig. 4A). The M_r of GLUT5 is in agreement with the study by Sugawara-Yokoo et al. [8], who demonstrated the presence of GLUT5 in apical membranes isolated from the S3 segment of proximal tubules of the rat kidney, and is within the range of previous studies: 42 kD [6] and 60 kD [27] in rat kidney, 49 kD in rabbit intestine [24], 58 kD in rat intestine [8], and 50 kD in human intestine [25].

Results of blot densitometry demonstrated that the abundance of D-fructose transporter is approximately 46% lower in BBMVs isolated from SHR kidney compared with normotensive rats (fig. 4B), a similar reduction to that observed in the maximal transport capacity for D-fructose in hypertensive rats. 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 (fig. 4C). Therefore, these results suggest that the lower kidney D-fructose transport in hypertensive rats is directly related, at least in part, to a lower number of GLUT5 transporter molecules in their brush-border membrane.

We previously demonstrated a decrease in the transport of D-glucose and D-galactose by kidney BBMVs from SHR compared to WKY rats [11], which was accompanied by a reduction in the expression of SGLT protein in these hypertensive rats. In addition, sugar transport is also diminished in the jejunum and ileum of hypertensive rats [28, 29], also paralleled by a decrease in the amount of SGLT protein (unpublished data). In the small intestine, these results were also accompanied by structural and ultrastructural alterations in the jejunum and ileum of hypertensive rats. When we analyzed the kidney from normotensive and hypertensive rats under light and electron microscopy, we did not find any modifications in hypertensive rats under light microscopy. In contrast, ultrastructural studies showed an irregular apical membrane in the kidney cortex of these rats (results not shown), which could contribute to the observed changes in kidney D-fructose transport in SHR.

In conclusion, we characterized D-fructose transport in rat kidney and found a decrease in the transport of D-

fructose in renal BBMVs prepared from SHR compared to WKY rats, which is correlated with a reduction in the density of GLUT5 transporters. These results suggest translational control of GLUT5 expression in the hypertensive rats, although changes in protein expression at a transcriptional level cannot be excluded. The cause and the mechanism underlying these functional changes in the kidney cortex of SHR are not clear, and the relevance of these findings to the pathophysiology of arterial hypertension is unknown. More studies are necessary to clarify these modifications and the physiological significance of these alterations in arterial hypertension.

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