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Angiotensin II-dependent increased expression of Na^+-glucose cotransporter in hypertension

Rocío Bautista,[1] Rebeca Manning,[1] Flavio Martinez,[2] Maria del Carmen Avila-Casado,[3] Virginia Soto,[3] Armando Medina,[3] and Bruno Escalante[1]

[1]*Department of Molecular Biomedicine, Centro de Investigación y Estudios Avanzados del Instituto Politécnico Nacional, Mexico City 07360;* [2]*Department of Pharmacology, Facultad de Medicina, Universidad Autónoma de San Luis Potosi, San Luis Potosí 78000; and* [3]*Department of Pathology, Instituto Nacional de Cardiología Ignacio Chávez, Mexico City 14080, Mexico*

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Bautista, Rocío, Rebeca Manning, Flavio Martinez, Maria del Carmen Avila-Casado, Virginia Soto, Armando Medina, and Bruno Escalante. Angiotensin II-dependent increased expression of Na^+-glucose cotransporter in hypertension. *Am J Physiol Renal Physiol* 286: F127–F133, 2004. First published September 23, 2003; 10.1152/ajprenal.00113.2003.—Glucose uptake is increased in hypertension. Thus we investigated Na^+-glucose cotransporter (SGLT2) activity and expression in proximal tubules from renovascular hypertensive rats. Sham-operated rats, aortic coarctation rats, and aortic coarctation rats treated with either ramipril (2.5 mg·kg^{-1}·day^{-1} for 21 days) or losartan (10 mg·kg^{-1}·day^{-1} for 21 days) were used. Na^+-dependent glucose uptake was measured in brush-border membrane vesicles (BBMV). V_{max} in BBMV from hypertensive rats was greater compared with those from normotensive rats (3 ± 0.2 vs. 1.5 ± 0.1 nmol·mg protein^{-1}·min^{-1}) without a change in K_m. Renal immunostaining was greater, and Western blot analysis and RT-PCR showed a higher expression of SGLT2 in hypertensive rats than in normotensive rats (1,029 ± 71 vs. 5,003 ± 292, 199 ± 15 vs. 95 ± 10, and 1.4 ± 0.2 vs. 0.3 ± 0.1 arbitrary units, respectively). In rats treated with either ramipril or losartan, V_{max} decreased to 2.1 ± 0.3 and 1.8 ± 0.4 nmol·mg protein^{-1}·min^{-1}, respectively, as well as did the intensity of immunostaining and levels of protein and mRNA. We suggest that in renovascular hypertension, angiotensin II induced SGLT2 via the AT_1 receptor, which was evidenced at both the functional and expression levels, probably contributing to increased absorption of Na^+ and thereby to the development or maintenance of hypertension.

renovascular hypertension; sodium reabsorption; ANG II type 1 receptor; glucose uptake

AN ACUTE INCREASE IN ARTERIAL pressure in the normotensive rat produces a natriuretic and diuretic response, associated with decreased Na^+ reabsorption in the proximal tubule (6). However, in spontaneously hypertensive rats (SHR), the natriuresis and diuresis observed with a further increase in blood pressure are blunted, suggesting that a functional resetting of sodium reabsorption has occurred and that this event sustains the hypertension (23). Indeed, several reports have associated hypertension with altered Na^+ transport. These reports have described inhibition of active Na^+ transport (12) and increased passive membrane permeability for Na^+ (2) in essential hypertension. Stimulation of the Na^+/H^+ exchanger has been shown in SHR compared with in its normotensive control, Wistar-Kyoto rats (WKY) (19). Furthermore, it has been reported that Na^+ uptake is affected in hypertensive Dahl salt-sensitive rats (DS) (13). Thus changes in the activity of several Na^+ transporters have been described, suggesting that alterations in the Na^+ channel (13), Na^+/H^+ antiporter (24), and Na^+/Ca^{2+} exchanger (20) as well as Na^+-K^+-ATPase activities (22) might be involved in altered Na^+ transport in hypertension. Moreover, initial observations by Parenti et al. (21) reported that Na^+-dependent glucose transport was faster in Milan hypertensive rats compared with normotensive rats. This report has been further supported by recent observations which suggested that the increased glucose transport could be related to increased transporter density in renal tissue from DS rats (14) or jejunal tissue from SHR (30). These data might suggest that some of the changes in cellular Na^+ transport observed in hypertension might result from a modification of Na^+-dependent glucose transport. A question that arises is which or what mechanism(s) is involved in the regulation of Na^+ transporters during the development of hypertension. ANG II represents a natural candidate, because the prohypertensive activity of ANG II through its effects on renal blood flow, renin release, and vasoconstrictor effects is well established (7). Furthermore, epithelial Na^+ transport can be modulated by a direct effect of ANG II on renal epithelial cells (9, 25). Recently, we have shown in renovascular hypertension an ANG II-dependent mRNA regulation of prohypertensive mediators (11, 16). Thus we have hypothesized that, in renovascular hypertension, increased ANG II activity is associated with an upregulation of Na^+-glucose cotransporter mRNA, thereby increasing Na^+-glucose reabsorption. Transport of glucose is carried out by the Na^+-glucose cotransporters (SGLTs), two molecules of Na^+ and one of glucose through SGLT1 or one molecule of Na^+ and one of glucose through SGLT2. SGLT1 is strongly expressed in the small intestine and at lower levels in the kidney, whereas SGLT2 expression is restricted to the kidney cortex and represents the major reabsorption pathway for glucose in the kidney (10). Therefore, the aim of the present study was to evaluate whether in hypertensive rats by aortic coarctation, there are increased activity and expression of the Na^+-glucose cotransporter and if this event is related to the ANG II system. Thus we evaluated Na^+-glucose cotransporter activity by measuring Na^+-dependent glucose uptake in the presence or absence of phlorizin, an inhibitor of the Na^+-glucose cotransporter, and by measuring protein and mRNA of the cotransporter in sham-operated and aortic coarctation rats. ANG II participation was evaluated by either inhibiting its synthesis

Address for reprint requests and other correspondence: B. Escalante, Dept. of Molecular Biomedicine, Centro de Investigacion y de Estudios Avanzados del IPN, Avenida Instituto Politécnico Nacional 2508, Colonia San Pedro Zacatenco, México City 07360, México (E-mail: bescalan@mail.cinvestav.mx).

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with the angiotensin-converting enzyme inhibitor ramipril or blocking the ANG II type 1 receptor (AT_1) with losartan.

MATERIALS AND METHODS

Chemicals were purchased from Sigma. Losartan was kindly provided by Merck Sharp & Dhome and ramipril by Aventis.

Aortic coartation. Aortic coarctation was performed according to published procedures (16). We used male Wistar rats (weighing 300–350 g). The animals were divided into the following groups: normotensive (sham operated), hypertensive (aortic coarctation), and hypertensive treated with either ramipril (an angiotensin-converting enzyme inhibitor; 2.5 $mg \cdot kg^{-1} \cdot day^{-1}$ for 21 days) or losartan (an AT_1-receptor antagonist; 10 $mg \cdot kg^{-1} \cdot day^{-1}$ for 21 days). Systolic blood pressure measurements were made 21 days after surgery (16). The kidneys were quickly perfused, and the right kidney was removed to isolate brush-border membrane vesicles (BBMV) and perform immunohistochemical studies, Western blot analysis, and RT-PCR analysis.

BBMV preparation. The preparation of BBMV was carried out by two-step $MgCl_2$ precipitation, as previously described (17). Each BBMV preparation required the use of 10 animals to obtain enough BBMV for one uptake experiment. Briefly, the renal cortices from 10 normotensive, 10 hypertensive, 10 ramipril-treated, or 10 losartan-treated rats were minced and suspended in a hypotonic buffer (10 mM mannitol, 2 mM Tris-H_2SO_4, pH 7.4) and homogenized for 2 min with a polytron (Ultra-Turrax T-25, Janke& Kunkel, IKA Labortechnik) at 20,500 rpm. Samples of the initial homogenate and the final suspension were obtained for protein and enzyme determination. The initial homogenate was subjected to a series of $MgCl_2$ (10 mM) precipitations and centrifugation (Sorval RC-5B, DuPont Instruments) steps. Between steps, the pellet was resuspended and homogenized with a Dounce glass pestle homogenizer (10 strikes). At the end of the procedure, the vesicles were suspended in the intravesicular buffer (100 mM mannitol, 100 mM KCl, 20 mM HEPES/Tris), frozen, and stored in liquid nitrogen until required. Protein content was determined according to the Bradford method (3). The purity of the BBMV was monitored by measuring the specific activities of leucine aminopeptidase and alkaline phosphatase (a typical brush-border marker enzyme) and Na^+-K^+-ATPase (a basolateral marker enzyme) according to Haase et al. (8) and Berner and Kinne (1), respectively. Enrichment factors were calculated by the relationship between enzymatic activity in the vesicles and the initial homogenate.

Transport experiments. The transport of D-glucose was measured at room temperature by a rapid filtration technique (29). The protocols were as follows.

1) The time course of [^{3}H]glucose uptake was estimated in the presence of a 100 mmol/l outside-inside KCl or NaCl gradient. Glucose uptake was initiated by mixing 10 μl (3 mg/ml) of the BBMV preparation with 50 μl of uptake medium containing ^{3}H-labeled (10 μCi/ml, Amersham Life Science) glucose, 100 mM mannitol, 100 mM KCl or NaCl, and 10 mM HEPES/Tris. The reaction was terminated after 5, 15, 30, 60, and 300 s by diluting the 60 μl of reaction mixture with 1 ml of ice-cold stop solution (300 mM mannitol, 80 mM Na_2SO_4, 10 mM Tris-H_2SO_4, 0.3 mM phlorizin, pH 7.4), which was then filtered immediately through wet Millipore filters (0.65-μm pore size; DAWP-013) and kept under suction. The filters were washed twice with 1 ml of ice-cold stop solution and dissolved in 5 ml of scintillation fluid (Aquasol-2, NEF-952, NEN), and the experiments were performed in triplicate. The radioactive material in the filters was measured using a liquid scintillation spectrophotometer (Beckman LS 6500), and all uptakes values were corrected for nonspecific filter binding of radiolabeled [^{3}H]glucose in the absence of BBMV (29). Na^+-glucose cotransporter-dependent [^{3}H]glucose uptake was measured as the difference between glucose uptake in the presence and the absence of phlorizin (0.3 mM; a competitive inhibitor of the transporter). The results are expressed in picomoles per milligram per second.

2) The kinetics of [^{3}H]glucose uptake (Na^+dependent) were measured at 15 s and calculated by subtracting the uptake in the absence of phlorizin from that in the presence of phlorizin (0.3 mM). The uptake buffer contained increasing concentrations of D-glucose (0.025–20 mM). The results are expressed in picomoles per milligram per second.

Na^+-glucose cotransporter immunoblotting. Immunoblotting analysis was used to identify the Na^+-glucose cotransporter in BBMVs. Blots were then incubated overnight at 4°C with SGLT2 antibody (Alpha Diagnostic International) diluted in blocking solution (1:1,000). Blots were stained for horseradish peroxidase activity using the enhanced chemiluminescence detection system (ECL kit, Amersham Pharmacia Biotech, Piscataway, NJ) (26). After detection, samples were measured by densitometry with a Kodak electrophoresis documentation and analysis system (EDAS 290).

Immunohistochemistry. Kidneys ($n = 6$) from normotensive, hypertensive, and hypertensive rats treated with either ramipril or losartan were fixed in 4% paraformaldehyde and subsequently embedded in paraffin. Kidney sections (3 μm) were incubated with SGLT2 antibody (Alpha Diagnostic International) diluted 1:10. The immunoreactive signal was detected with an streptavidin-biotin-immunoperoxidase reaction (LSAB$^+$ kit, Dako) and visualized by exposure to diaminobenzidine. Expression of SGLT2 was evaluated by computer image analysis according to a previously published method (27). We analyzed 20 noncrossed fields (770 × 58 μm, enlarged ×40) per biopsy, using light microscopy with a Olympus B ×51 microscope (Olympus American, Melville, NY) covering at least 80% of the cortical part of the core captured with a digital video camera. Each picture was processed on a computer and analyzed using Image-Pro and Photoshop 7, an image-processing software (Adobe Systems, San Jose, CA). Using the capabilities of color recognition by this software, we selected a specific brownish color for positive areas. After selection, these areas were quantified (pixel unit) using the histogram function of the software. For each field, the number of positive areas was expressed as a fraction of the tubule-interstisium area (positive areas divided by the overall field area). Finally, for each biopsy, the fractional amount of expression of antibody was obtained by averaging the values obtained from 20 fields examined.

RNA isolation and RT-PCR. Renal cortex was obtained from all four groups, and total RNA was isolated by the TRIzol method (GIBCO BRL, Life Technologies). Total RNA (2 μg) was converted to cDNA using a Superscript II RNAse H-Reverse Transcriptase Kit (GIBCO BRL, Life Technologies). PCR was performed with the PerkinElmer Gene Amp 2400 PCR system for 35 cycles at 94°C for 1 min, 64°C for 1 min, and 72°C for 1 min followed by a 10-min extension at 72°C. The primers utilized were 5′-ccaatagaggcacagttggtgg-3′ (sense) and 5′-cgtaaatgttccacaacgg-3′ (antisense) for SGLT2 and 5′-ggatttggccgtattggcc-3′ (sense) and 5′-catgtcagatccacaacgg-3′ (antisense) for GAPDH (28, 31). The final PCR products were 388 and 715 bp in size, respectively. The bands were analyzed with a Kodak electrophoresis documentation and analysis system (EDAS 290).

Statistics. All results are expressed as means ± SE. Significance was determined by Student's *t*-test or by ANOVA for cases with multiple comparisons. Significance is considered as $P < 0.05$.

RESULTS

Systolic blood pressure was higher in hypertensive rats than in normotensive rats. Thus blood pressure in normotensive rats was 109 ± 11 mmHg ($n = 50$), whereas in hypertensive rats blood pressure was 156 ± 15 mmHg ($n = 50$). Furthermore, inhibition of the angiotensin-converting enzyme with ramipril or blockade of the AT_1 receptor with losartan restored blood

Table 1. *Enzyme activities in the homogenate and BBMVs prepared from normotensive, hypertensive, or hypertensive rats treated with either ramipril or losartan*

	Specific Activity, μmol·mg protein^{-1}·min^{-1}		Enrichment
	Homogenate	BBMV	BBMV/Homogenate
Alkaline phosphatase			
Normotensive	75±20	841±35	11±0.9
Hypertensive	76±18	856±30	11±1.1
Hypertensive treated with ramipril	79±14	870±47	11±0.8
Hypertensive treated with losartan	77±17	856±37	11±1.0
Leucine aminopeptidase			
Normotensive	6±0.5	70±8	11±1.4
Hypertensive	5±0.3	57±10	11±1.6
Hypertensive treated with ramipril	6±0.4	68±11	11±1.4
Hypertensive treated with losartan	6±0.4	65±10	11±1.3
Na^+-K^+-ATPase			
Normotensive	44±12	6±0.8	0.14±0.04
Hypertensive	50±15	5±0.3	0.10±0.02
Hypertensive treated with ramipril	48±16	8±0.5	0.16±0.03
Hypertensive treated with losartan	47±14	6±0.5	0.13±0.03

Values are means ± SE of 5 independent determinations. BBMV, brush-border membrane vesicles.

pressure values close to normotensive values of 119 ± 9 (n = 50) and 90 ± 10 mmHg (n = 50), respectively.

Purity of membrane vesicles. Table 1 shows the specific activities of enzymes in homogenate and BBMV from normotensive, hypertensive, ramipril-treated hypertensive, or losartan-treated hypertensive rats. The brush-border markers alkaline phosphatase and leucine aminopeptidase were enriched 11-fold in the final BBMV in all four experimental groups compared with the initial homogenate. Because enrichment of the brush-border markers was not different among preparations of BBMV obtained from all four groups, these preparations are suitable for comparison in glucose transport. In contrast, there was a decrease in Na^+-K^+-ATPase specific activity in the BBMV for all four groups compared with the initial homogenate. Enrichment of this marker was very low (0.13), indicating very little basolateral contamination in the BBMV preparations.

Glucose uptake in kidney cortex BBMV. Figure 1 shows the time course of glucose uptake into BBMV prepared from normotensive and hypertensive rat kidney cortex. In the presence of a Na^+ gradient across the vesicle membrane, there was a transient increase in the intravesicular concentration of glucose (15 s). The magnitude of the accumulation ratio and the initial rate of glucose uptake were significantly higher in the BBMV from hypertensive rats compared with the BBMV from normotensive rats. Moreover, treatment of the hypertensive rats with either ramipril or losartan decreased the maximal value (15 s) of glucose accumulation in the BBMV (Fig. 2). Elimination of the Na^+ gradient by the imposition of a K^+ gradient resulted in elimination of the transient increase in glucose (Fig. 1). Furthermore, inhibition of the Na^+-glucose cotransporter with phlorizin decreased maximal intravesicular

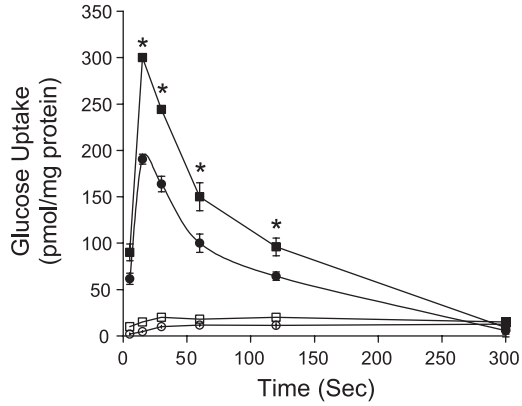

Fig. 1. Time course of glucose uptake in renal brush-border membrane vesicles (BBMV). Membrane vesicles were isolated from normotensive (○) or hypertensive (□) rats, and glucose uptake was measured at different time points in the presence (closed symbols) and absence (open symbols) of an Na^+ gradient. Each curve represents the mean ± SE of at least 5 different BBMV preparations. *$P < 0.05$ compared with normotensive rats.

glucose accumulation by 84, 93, 89, and 90% in BBMV from normotensive, hypertensive, ramipril-treated hypertensive, and losartan-treated hypertensive rats, respectively. Uptake of glucose at equilibrium (5 min) was identical in the presence and absence of a Na^+ gradient in all four experimental groups, indicating a similar size of membrane vesicles in all four groups of animals (14, 10, 13, and 12 pmol/mg protein) for BBMV from normotensive, hypertensive, ramipril-treated hypertensive, and losartan-treated hypertensive rats, respectively. Furthermore, in the absence of an Na^+ gradient (50 mM of Na^+ inside and outside the vesicle), the glucose uptake at 15 s was 0.5 ± 0.01 and 0.6 ± 0.03 pmol/mg protein for BBMV from normotensive and hypertensive rats, respectively.

To evaluate whether alterations in the glucose transport observed in the BBMV from hypertensive rats were related to changes in maximal transport capacity (V_{max}) and/or Na^+-glucose transporter affinity (K_m), glucose kinetics were determined. As shown in Fig. 3, Na^+-dependent glucose uptake was saturable and conformed to Michaelis-Menten kinetics in BBMV from normotensive and hypertensive rats. Furthermore,

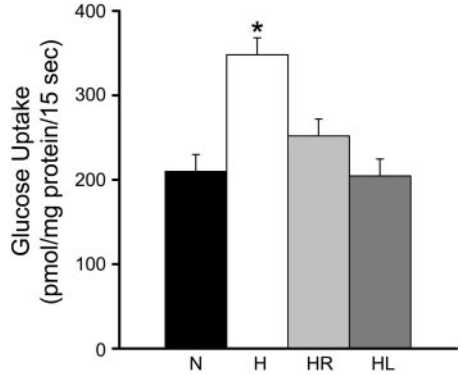

Fig. 2. Na^+-dependent glucose uptake by renal BBMV. Membrane vesicles were isolated from normotensive (N), hypertensive (H), ramipril-treated hypertensive (HR), or losartan-treated hypertensive (HL) rats. Na^+-dependent glucose uptake was measured at 15 s and calculated by subtracting the uptake in the absence of an Na^+ gradient from that in the presence of an Na^+ gradient. Values are means ± SE of at least 5 different BBMV preparations. *$P < 0.05$ when compared with normotensive rats.

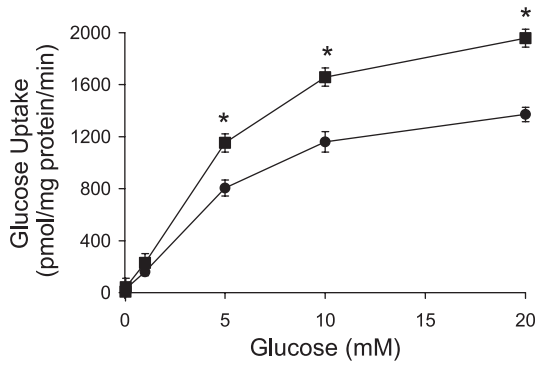

Fig. 3. Concentration-dependent glucose uptake by renal BBMV. Membrane vesicles were isolated from normotensive (●) and hypertensive (■) rats. Na^+-dependent glucose uptake was measured at 15 s in the presence of a Na^+ gradient. Values are means ± SE of at least 5 different preparations. $*P < 0.05$ compared with normotensive rats.

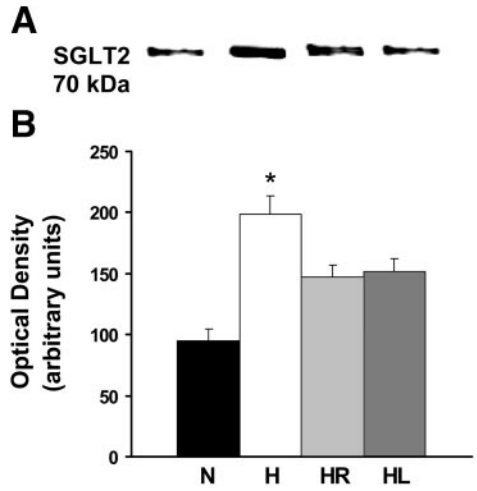

Fig. 4. Na^+-glucose cotransporter protein expression in BBMV. Protein from renal BBMV isolated from N, H, HR, or HL rats (see Fig. 2) were separated and identified by Western blot analysis. *A*: anti-SGL2 antibody recognized an immunoreactive protein of 70 kDa. *B*: relative density measured by optical densitometry. Values are means ± SE of 4 separate experiments. $*P < 0.01$ compared with N rats.

glucose uptake was higher in BBMV from hypertensive rats. Kinetic parameters for all four experimental groups are shown in Table 2. V_{max} values were higher in the BBMV from hypertensive rats compared with BBMV from normotensive rats. Moreover, treatment with either ramipril or losartan restored V_{max} values similar to that for the BBMV from normotensive rats. In contrast, similar K_m values for glucose were found in the BBMV from all experimental groups.

Na^+-glucose cotransporter immunoblotting. Western blot analysis performed to detect expression of the Na^+-glucose transporter (SGLT2) in the renal homogenate showed a small detection of the protein (16 ± 5 and 20 ± 9 arbitrary units for normotensive and hypertensive rats, respectively). However, when the immunoblotting was performed in the same batch of BBMV used for transport studies, the expression of the Na^+-glucose transporter in the BBMV isolated from the hypertensive rats was increased when compared with the normotensive rats, as shown in Fig. 4. In contrast in the BBMV isolated from the rats treated with either ramipril or losartan, Na^+-glucose cotransporter expression was not different to the levels found in the BBMV from normotensive rats.

Na^+-glucose cotransporter immunohistochemistry. Figure 5 shows paraffin-embedded sections with periodic acid-Schiff staining. Light microscopy revealed glomerullar hypoperfusion, glomerulatis, and mesangial expansion. However, the renal tissue from neither ramipril- nor losartan-treated hypertensive rats demonstrated any of these morphological abnormalities. Intensive, positive Na^+-glucose cotransporter was observed in the renal tissue of hypertensive rats (Fig. 5*B*). Na^+-glucose cotransporters were localized in the membrane of epithelial proximal tubular cells. Less immunostaining was observed in the renal tissue of normotensive and ramipril- or losartan-treated hypertensive rats (Fig. 5, *A*, *C*, and *D*). Moreover, expression of the transporter evaluated by computer-assisted analysis of 20 different fields for 5 rats for each group showed that the the Na^+-glucose cotransporter was expressed as 1,029 ± 71, 5,003 ± 292, 2,662 ± 136, 1,830 ± 125 arbitrary units in the normotensive, hypertensive, ramipril-, and losartan-treated hypertensive rats, respectively.

Table 2. *Kinetic constants for Na^+-glucose cotransporter*

	K_m, mM	V_{max}, nmol·mg protein^{-1}·min^{-1}
Normotensive	6.5±1	1.5±0.1
Hypertensive	6.8±2	3.0±0.2*
Hypertensive+ramipril	6.0±2	2.1±0.3
Hypertensive+losartan	6.4±3	1.8±0.3

Values are means ± SE of 5 independent determinations. $*P < 0.05$ vs. normotensive, ramipril-, or losartan-treated rats.

Na^+-glucose mRNA expression. To obtain information as to whether the increased protein levels of the Na^+-glucose cotransporter were associated with changes in transporter mRNA expression, we performed RT-PCR analysis. PCR amplification using primers specific for the Na^+-glucose cotransporter (SGLT2) was normalized with the data of PCR for GAPDH in the tissue from normotensive, hypertensive, ramipril-treated hypertensive, or losartan-treated hypertensive rats. Figure 6*A* shows how mRNA of Na^+-glucose transporter expression in renal tissue from hypertensive rats was increased fivefold compared with the expression of the mRNA of the transporter in renal tissue from normotensive rats. Furthermore, mRNA expression of the transporter in renal tissue from ramipril- or losartan-treated hypertensive rats was not different from that in normotensive rats. Moreover, to establish whether ANG II levels were responsible for induction of SGLT2 mRNA expression, we chronically (7 days) infused rats with a dose of 200 ng·kg^{-1}·min^{-1} of ANG II. Chronic ANG II infusion increased expression of renal SGLT2 mRNA to levels similar to those in rats with aortic coarctation (Fig. 6*B*). When we prevented the hypertensive effect of ANG II by treating ANG II-infused rats with the antihypertensive drug nifedipine (10 mg·kg^{-1}·day^{-1}), although an increase in blood pressure was prevented (from 160 ± 2 to 100 ± 3 mmHg for ANG II and ANG II-nifedipine-treated rats, respectively), the effect of ANG II on SGLT2 mRNA expression was not affected (Fig. 6*B*). Finally, to explore prostaglandins as the possible mediators of the effect of ANG II on SGLT2 mRNA expression, we treated the ANG II-infused rats with indomethacin (6 mg·kg^{-1}·day^{-1}). This did not affect blood pressure (153 ± 3

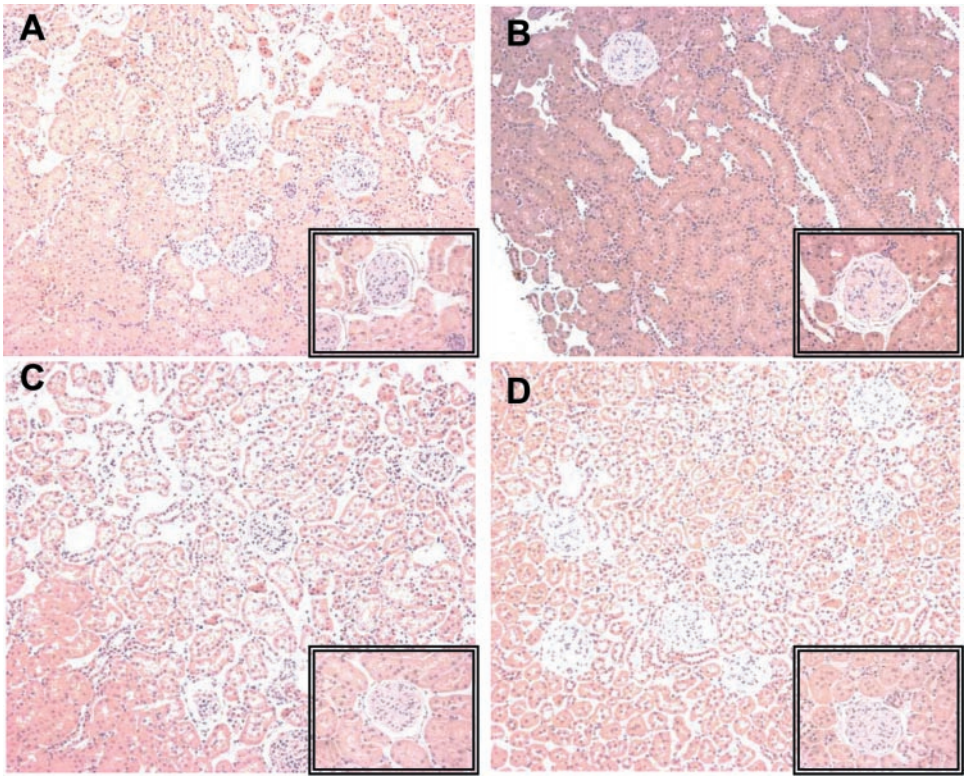

Fig. 5. Na^+-glucose cotransporter immunodetection in renal tissue. Microscopic slices from normotensive (*A*), hypertensive (*B*), ramipril-treated hypertensive (*C*), or losartan-treated hypertensive rats (*D*) are shown. Na^+-glucose cotransporter is detected in renal tissue by the brown staining. Each panel represents 1 of 6 separate experiments.

mmHg), and as can be seen in Fig. 6*B* inhibition of prostaglandin synthesis did not affect ANG II-dependent induction of SGLT2 mRNA expression.

DISCUSSION

This study demonstrates that Na^+-dependent glucose uptake is increased in BBMV prepared from renal cortex of hypertensive rats compared with normotensive rats. Increased activity of the Na^+-glucose cotransporter was associated with increased expression of Na^+-glucose cotransporter protein and mRNA. Furthermore, prevention of an increase in blood pressure by either inhibition of ANG II synthesis or blockade of the AT_1 receptor prevented the increased activity and expression of the Na^+-glucose cotransporter in hypertensive rats. Differences in glucose uptake were not related to variations in vesicle preparations, because purification and size of the BBMV, as measured from the enrichment of alkaline phosphatase, leucine aminopeptidase, and Na^+-dependent glucose uptake at equi-

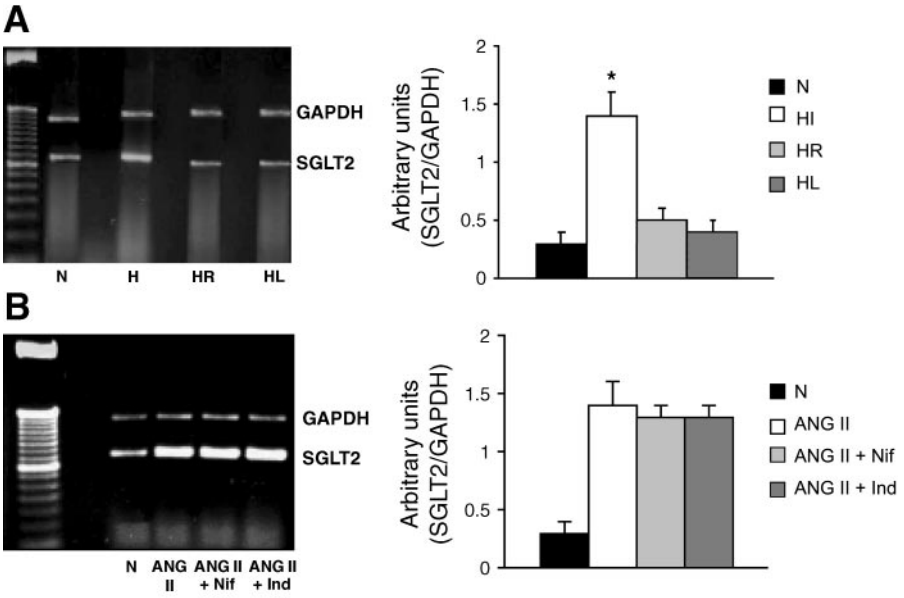

Fig. 6. Na^+-glucose cotransporter mRNA expression in renal tissue. RT-PCR was performed in kidneys from N, H, HR, or HL rats (*A*) or in kidneys from N, ANG II-infused rats (ANG II), ANG II-infused rats treated with either nifedipine (ANG II +Nif) or indomethacin (ANG II +Ind; *B*). *Left*: 1 of 4 cDNA gel electrophoresis of Na^+-glucose cotransporter. *Right*: relative density by densitometric analysis. Values are means ± SE of 4 separate experiments. $*P < 0.01$ compared with N rats.

librium (5 min), respectively, were similar among all four experimental groups (Table 1). Na^+ dependency was demonstrated by the data showing that in the absence of a Na^+ gradient there is no glucose uptake. Furthermore, this Na^+-dependent glucose uptake was through Na^+-glucose cotransporter activity, as evidenced by the inhibition of glucose uptake by phlorizin, a specific inhibitor of the Na^+-glucose cotransporter (29). Moreover, differences in glucose uptake were not present in the absence of the Na^+ gradient, further supporting Na^+ dependency.

Our data agree and disagree with previous reports. Morduchowicz et al. described a decrease in Na^+-dependent D-glucose uptake in renal BBMV prepared from SHR compared with control WKY rats; the reduction in glucose uptake was suggested to be related to a decrease in the density of the Na^+-glucose cotransporter (18). However, ANG II levels are also decreased in SHR (4). Thus differences between our data and these previous reports may be related to the presence of ANG II. Furthermore, Parenti et al. (21) reported that under specific experimental conditions, Na^+ uptake by BBMV from Milan hypertensive rats was faster than in the normotensive rats. Moreover, recently it has been reported that Na^+-coupled glucose transport through SGLT1 activity is increased in renal BBMV from high-salt-loaded DS rats (14). Kinetic studies of Na^+-dependent glucose uptake in jejunal BBMV from SHR and WKY suggested that enhancement in glucose transport in SHR could be due to an increase in the number of transport molecules (30).

Increased Na^+-dependent glucose transport has been suggested to be due to several cellular mechanisms, such as increased phosphorylation of the cotransporter (30) or induction of trafficking of the Na^+-glucose cotransporter from an intracellular pool into the BBMV (5). Thus to investigate the mechanism by which renovascular hypertension might regulate the increase in Na^+-dependent glucose uptake, kinetics studies were performed as well as mRNA and protein expression in BBMV from hypertensive and normotensive rats. As can be seen in Table 2, there were no differences in the K_m values in all four experimental groups. However, the V_{max} value was increased in the BBMV from hypertensive rats compared with normotensive or hypertensive rats treated with the angiotensin-converting inhibitor or the AT_1 receptor blocker. Thus kinetics of Na^+-dependent glucose uptake suggested that increased glucose transport in the BBMV from hypertensive rats could be related to an increase in the number of transporter molecules, because the apparent V_{max} of glucose transport was twofold higher in the BBMV from hypertensive rats than those from normotensive rats. In contrast, the affinity of the cotransporter was similar in BBMV from hypertensive and normotensive rats, because the apparent K_m was not different in both groups of rats. An increased number of Na^+-glucose cotransporters was further supported by our observations that either immunostaning or immunoblotting of the Na^+-glucose cotransporter in BBMV from hypertensive rats was higher compared with the BBMV from normotensive rats. We suggest that this increase in the number of cotransporters is related to the synthesis of the protein rather than a translocation from the intracellular organelle into the membrane, as suggested previously (5). We support this hypothesis based in our observation that increased expression of protein is observed in both homogenate or BBMV, that, in immnohistochemistry, increased expression of protein is observed in cytoplasm and membrane, and that increased expression of Na^+-glucose cotransporter mRNA was observed concomitantly with an increase in protein and activity. Therefore, we suggest that during development of renovascular hypertension, increased expression of Na^+-glucose transporter mRNA leads to induction of cotransporter protein that expresses a functional increase in Na^+-dependent glucose uptake. Thus the present results suggest the existence of an intrarenal or systemic mechanism responsible for induction of the Na^+-glucose cotransporter during development of renovascular hypertension. The aortic coarctaction model of renovascular hypertension is associated with increased activity of the renin-angiotensin system.

We (11) and others (15) have reported a direct effect of ANG II on mRNA expression in renal tissue. Therefore, we explored the role of ANG II in the regulation of the expression of Na^+-glucose cotransporter and increased Na^+-dependent glucose uptake in renovascular hypertension by testing the hypothesis that either inhibition of ANG II synthesis by inhibition of the angiotensin-converting enzyme or blockade of the AT_1 receptor could prevent increased expression of the Na^+-glucose cotransporter and, thereby, increased Na^+-dependent glucose uptake. Indeed, treatment of hypertensive rats with either ramipril or losartan prevented the induction of Na^+-glucose transporter protein, mRNA, and activity, suggesting that ANG II could be responsible for the regulation of the Na^+-glucose cotransporter. However, blockade of the ANG II system also prevents an increase in blood pressure in rats with aortic coarctation. Thus further experiments were performed to explore the role of altered tension on the regulation of Na^+-glucose cotransporter status. Therefore, we used a high-ANG II-concentration model through chronic infusion of ANG II and prevented hypertension with the vasodilator nifedipine. We demonstrated that chronic ANG II infusion was associated with an increase in SGLT2 mRNA and that prevention of hypertension did not affect this effect of ANG II, suggesting that hypertension was not participating in the regulation of SGLT2 mRNA expression. We also discarded prostaglandins as the mediators of the effect of ANG II by showing that ANG II induction of SGLT2 mRNA expression was not modified by prostaglandin inhibition. Thus our data suggest that ANG II was directly responsible for increased expression of the Na^+-glucose cotransporter and thereby increased epithelial Na^+ and glucose uptake. Therefore, induction of the Na^+-glucose cotransporter could represent a mechanism by which ANG II increases Na^+ reabsorption and fluid transport across proximal tubular epithelial cells (9).

In conclusion, we have shown that aortic coarctation-induced hypertension is associated with increased Na^+-glucose cotransporter activity through induction of SGLT2 protein and mRNA expression, probably by an ANG II-dependent mechanism. Increased Na^+ reabsorption through the Na^+-glucose cotransporter may be participating in the development of hypertension. Additionally, increased glucose epithelial uptake may play an important role in the adaptation of glucose transport to hypertension.

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