Renal expression and localization of the facilitative glucose transporters GLUT1 and GLUT12 in animal models of hypertension and diabetic nephropathy

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Renal expression and localization of the facilitative glucose transporters GLUT1 and GLUT12 in animal models of hypertension and diabetic nephropathy. Am J Physiol Renal Physiol 290: F205–F213, 2006. First published August 9, 2005; doi:10.1152/ajprenal.00237.2004.—Renal tubular glucose reabsorption is mediated by facilitative glucose transporter (GLUT) proteins and energy-dependent sodium glucose luminal transporters. Glucose transport in the diabetic kidney is upregulated and has been implicated in the pathogenesis of progressive diabetic nephropathy. Hyperglycemia, hypertension, and activation of the renin-angiotensin system are believed important in the development of the disease. The present study examines the renal expression of the facilitative glucose transporters GLUT1 and GLUT12 in rat models of diabetic nephropathy. Sprague-Dawley and transgenic (mRen-2)27 rats received either streptozotocin-induced diabetes or vehicle. GLUT12 expression and localization were determined by immunohistochemistry, immunoblotting, in situ hybridization, and confocal immunofluorescence. GLUT1 immunolabeling was detected on the basolateral membrane throughout the nephron. GLUT12 was localized to the distal tubules and collecting ducts. A significant increase in GLUT12 immunolabeling was measured in Ren-2 controls and Ren-2 diabetic animals compared with Sprague-Dawley controls. GLUT12 expression was higher in Ren-2 diabetic compared with Sprague-Dawley diabetic rats. Long-term diabetes resulted in significant increases in GLUT1 levels in the renal proximal tubules and expression was higher in Ren-2 diabetic than Sprague-Dawley diabetic rats. GLUT12 protein was localized to the cytoplasm and to the apical membrane of human and rat distal tubules and collecting ducts. The apical localization of GLUT12 in the distal tubules and collecting ducts suggests that it could contribute to additional glucose reabsorption in the late nephron. Levels of both GLUT1 and GLUT12 are elevated in animal models of hypertension and diabetic nephropathy.

(mRen-2)27 rat; hyperglycemia; hypertension; glucose reabsorption

DIABETIC NEPHROPATHY IS A microvascular disease affecting approximately one-third of patients with type 1 and a significant number of patients with type 2 diabetes (1, 35). Hyperglycemia, hypertension, and activation of the renin-angiotensin system (RAS) are factors believed important in the development of the disease (3). Glucose transport in the diabetic kidney is upregulated and appears to be part of the response to hyperglycemia (12).

The proposed model of renal glucose reabsorption involves sodium glucose luminal transporter (SGLT)-mediated luminal influx of glucose across the brush-border membrane (BBM) of the proximal tubule. Glucose is then transported down its concentration gradient by the facilitative glucose transporter isoforms, GLUT1 and GLUT2 (8). Glucose transporters (GLUT) are transmembrane proteins that exhibit distinct tissue distributions, biochemical properties, and regulatory mechanisms, mediating controlled glucose uptake under different physiological conditions (6). The GLUT family is divided into three subclasses, according to the sequence similarities between its members (14).

In diabetic, hyperglycemic rats, modulation of GLUT1, GLUT2, and the fructose-specific transporter, GLUT5, has been reported in response to the intracellular concentration of glucose (10, 33). Common strains of the laboratory rat develop only minimal diabetic renal disease. The transgenic (mRen-2)27 rat with the mouse Ren-2 gene inserted into the genome of the Sprague-Dawley (SD) rat is hypertensive and exhibits elevated levels of local ANG II (25). The streptozotocin (STZ)-induced diabetic Ren-2 rat develops advanced glomerulosclerosis and tubulointerstitial disease and has been used extensively as a model of progressive diabetic nephropathy (17).

Using the Xenopus laevis oocyte expression system, the glucose transport properties have recently been described for GLUT12, a newly identified class III glucose transporter (27). In normal adult human tissues, GLUT12 protein is expressed in the insulin-responsive tissues, fat, and skeletal and cardiac muscle (29). In the fetal rat, GLUT12 protein expression has been demonstrated in a wider range of tissues, including the distal tubules and collecting ducts of the kidney (21). GLUT12 is also expressed in prostate and breast cancer and this expression is thought to reflect activation of a gene in cancer cells that is also expressed in the fetus (7, 28). Precedents for recapitulation of developmental programs in renal disease have also previously been described (20).

To investigate possible mechanisms for upregulation of tubular glucose transport, the present study examines the renal expression of the facilitative glucose transporters, GLUT1 and GLUT12, in rat models of hypertension and diabetic nephropathy.

MATERIALS AND METHODS

Animals

Rats were housed in a stable environment (maintained at 19–23°C with a 12:12-h light-dark cycle) and allowed free access to tap water and standard rat chow containing 0.25% Na+ and 0.76% K+ (GR2; Clark-King, Gladstoneville, Australia). Six-week-old female SD rats and female heterozygous Ren-2 rats were starved overnight and randomized to receive either 55 mg/kg STZ (Sigma, St. Louis, MO) diluted in 0.1 mol/l citrate buffer, pH 4.5, to induce experimental type I
diabetes or citrate buffer alone (nondiabetic) by tail-vein injection. Each week rats were weighed and blood glucose was measured using a glucose meter (Advantage Blood Glucose Monitor, Roche Diagnostics). Diabetic rats received subcutaneous insulin injections (2–4 U, Human NPH Isophane, Eli Lilly, Indianapolis, IN) to maintain blood glucose at 27 mmol/l and to reduce mortality and encourage weight gain. Systolic blood pressure was recorded every 4 wk by tail-cuff plethysmography on preheated, conscious rats. Experimental procedures adhered to the guidelines of the National Health and Medical Research Council of Australia’s Code for the Care and Use of Animals for Scientific Purposes and were approved by the Bioethics Committee of St. Vincent’s Hospital, Melbourne, Australia.

Tissue Preparation

At 36 wk of age (30 wk post-STZ or vehicle), rats (n = 6 per group) were euthanized (Lethobarb, 60 mg/kg body wt ip, Virbac, Peakhurst, Australia) and kidneys were surgically removed, weighed, decapsulated, and sliced in transverse sections. The left kidney was diced, snap-frozen in liquid nitrogen, and stored at −80°C for immunoblotting and for immunofluorescence as detailed below. The right kidney was postfixed in formalin overnight, routinely processed, and embedded in paraffin for histological examination. Tissue integrity was assessed from serial hematoxylin-and eosin-stained sections. Human nephrectomy tissue was obtained from the Department of Pathology, St. Vincent’s Hospital Melbourne under approval from the St. Vincent’s Hospital Human Ethics Committee.

Antibodies

Rabbit polyclonal GLUT12 antibody, R1396, raised to the 16 COOH-terminal amino acids of human GLUT12 (29), was used for all immunohistochemical (1:500), immunoblotting (1:500), and immunofluorescence (1:200) experiments. Rabbit polyclonal anti-GLUT1 (Chemicon International), raised to the 12 terminal amino acids of the COOH terminus of GLUT1, was used for immunohistochemistry (1:500). A polyclonal epidermal growth factor antibody (Chemicon) was used for immunohistochemistry (1:50). Nonimmune rabbit serum from preimmunization bleeds was used for negative controls.

Immunohistochemistry

Sections were dewaxed and incubated in 5% H2O2/methanol for 30 min. Sections were blocked with 10% normal swine serum (NSS; Institute of Medical and Veterinary Science, Gilles Plain, Australia) and 5% PBS (CSL Biosciences, Parkville, Australia) in PBS for 30 min and then incubated overnight at 4°C with primary antibodies diluted in 5% PBS/PBS. Sections were rinsed with 0.1% Tween/PBS (PBST) followed by incubation with biotinylated swine anti-rabbit IgG (DAKO). Sections were finally incubated for 20 min with the avidin-biotin peroxidase complex VECTASTAIN (Vector Laboratories, Burlingame, CA), and a signal was detected with 3,3'-diaminobenzidine (Sigma) diluted in distilled water and counterstained with hematoxylin. At least two sections per kidney for each antibody were stained with n = 6 animals per group. For control slides, R1396 serum (1:500) was incubated on ice overnight with 50 or 100 µg/ml of either competitive peptide (the peptide used to generate the R1396 antibody) or an unrelated noncompetitive peptide.

Immunohistochemical Quantitation

Positive GLUT12 and GLUT1 staining was quantitated using image analysis software, the Analytical Imaging Station (AIS, Version 6, Imaging Research, St. Catharines, Canada). Images were captured using a light microscope (Olympus BX-50, Olympus Optical, Tokyo, Japan), equipped with a high-resolution camera (Fujix HC-2000, Fujifilm, Japan), connected to an IBM Pentium III computer. Tubulointerstitial staining was highlighted using a selective color tool. The scan area, which correlated to the number of pixels in the outlined area, determined the proportional area of staining in each of five representative ×400 images (n = 6 animals per group).

Protein Isolation and Immunoprecipitation

Frozen tissue (untreated SD heart and kidney) was homogenized (Polytron homogenizer; Kinematica, Lucerne, Switzerland) in sucrose homogenization buffer, pH 7 (250 mM sucrose, 5 mM NaCl, 2 mM EGTA, 10 mM NaHCO3, 1 mM PMSF). The crude extract was then subjected to ultracentrifugation (65,000 rpm at 4°C for 60 min), and the supernatant was discarded and the resulting pellet containing membrane proteins was dissolved in 1% SDS overnight at 4°C on a shaker. The protein concentrations of kidney extracts were determined by a Bradford protein assay (Bio-Rad, Hercules, CA). For the immunoprecipitation, 2 mg of either kidney or heart membrane protein were incubated with 25 µl of washed Agarose A beads (Upstate, Lake Placid, NY) for 60 min at 4°C to eliminate nonspecific binding. The beads were then pelleted by centrifugation and the kidney protein extract was incubated with 2 µg affinity-purified GLUT12 polyclonal antibody R1396 and 25 µl of washed Agarose A beads. The heart protein extract was incubated with either 2 µg of affinity-purified GLUT12 antibody as a positive control or 2 µg of alphal (338–358) AMP-activated protein kinase (AMPK) affinity-purified antibody (24) as a negative control (a kind gift from Dr. B. Michell, St. Vincent’s Institute of Medical Research, Melbourne, Australia) and 25 µl of washed Agarose A beads were added. The samples were placed on a rotating wheel overnight at 4°C. The resulting immunocomplex was then washed twice with cold PBS/1% Triton X-100/0.5 M NaCl and then once with cold PBS. The beads were resuspended in 20 µl of reducing loading buffer (62.5 mM Tris, pH 6.8, 10% glycerol, 2% SDS, 0.05% bromophenol blue, 5% β-mercaptoethanol) and incubated at 100°C for 5 min. Samples were then centrifuged to pellet the beads and the supernatant was used for Western analysis.

Western Blot Analysis

The immunoprecipitated proteins were separated on 10% SDS-PAGE gels and transferred to polyvinylidene difluoride (PVDF) transfer membrane (Roche Diagnostics, Mannheim, Germany). Membranes were blocked in a 5% wt/vol nonfat dry milk block solution for 60 min at room temperature and immunoblotted with GLUT12 polyclonal antibody R1396 (1:500 in 2% wt/vol nonfat dry milk) overnight at 4°C. Following incubation in horseradish peroxidase-conjugated anti-Protein G (Bio-Rad, in 0.1% TTBS) for 60 min at room temperature, immunolabeled proteins were detected using Lumi-Light chemiluminescent detection (Roche Molecular Biochemicals). As a control, R1396 serum (1:500 in 2% skim milk powder/TBS) was incubated with 100 µg/ml of competitive peptide, the peptide used to generate R1396, or an unrelated noncompetitive peptide for 1 h at room temperature, before immunoblotting of the membrane as described above.

Table 1. Body weight, kidney weight, systolic blood pressure, and plasma glucose in control and diabetic SD and Ren-2 rats (30 wk post-STZ or vehicle control)

<table>
<thead>
<tr>
<th></th>
<th>Body Weight, g</th>
<th>Kidney Weight, g</th>
<th>Blood Pressure, mmHg</th>
<th>Plasma Glucose, mmol/l</th>
</tr>
</thead>
<tbody>
<tr>
<td>SD control</td>
<td>488.4±36.0</td>
<td>1.5±0.08</td>
<td>150.4±4.0</td>
<td>7.0±0.8</td>
</tr>
<tr>
<td>SD diabetic</td>
<td>446.0±6.6*</td>
<td>2.3±0.2</td>
<td>154.0±3.8</td>
<td>23.9±1.3*</td>
</tr>
<tr>
<td>Ren-2 control</td>
<td>403.7±21.1</td>
<td>1.5±0.1</td>
<td>189.6±1.7†</td>
<td>75.5±0.5</td>
</tr>
<tr>
<td>Ren-2 diabetic</td>
<td>288.7±18.1*</td>
<td>1.5±0.04</td>
<td>201.4±6.8†</td>
<td>28.4±2.3*</td>
</tr>
</tbody>
</table>

Data are expressed as means ± SE; n = 6 per group. SD, Sprague-Dawley; STZ, streptozotocin. *P < 0.05 when compared with respective nondiabetic rats. †P < 0.05 when compared with SD rats.

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In Situ Hybridization

A 350-bp partial human GLUT12 cDNA was produced by PCR for use as a probe for in situ hybridization experiments. For PCR reactions, the full-length GLUT12 cDNA (29) was used as a template and forward (5'-CTT CTA CGT GAC CAT GGT ACC TG-3') and reverse (5'-TAT CTG TCT ATC AGG ACC CCT CGG-3') primers designed based on the GLUT12 nucleotide sequence. PCR conditions were 95°C for 30 s, 53°C for 30 s, and 72°C for 1 min for 40 cycles on an ABI thermocycler. The PCR product was cloned into pGEM-T Easy (Promega, Madison, WI), transformed into Escherichia coli strain SURE-2 (Stratagene, La Jolla, CA), and sequenced by Micromon Sequencing Facility (Melbourne, Australia). In situ hybridization was performed as previously described (16). A 32P-labeled anti-sense GLUT12 riboprobe was generated from an AatII linearized...
template using T7 RNA polymerase (Promega, [³⁵S]pUTP obtained from Genereworks, Adelaide, Australia). Sections were dewaxed, rehydrated, and microwaved in 0.01 M citrate buffer, pH 6.0, for 5 min. After being fixed in 4% paraformaldehyde in PBS for 10 min at room temperature, sections were washed in 0.1 M phosphate buffer, pH 7.2, and then digested with Pronase E (125 μg/ml in 50 mM Tris·HCl, pH 7.2, 5 mM EDTA, pH 8.0) at 37°C for 10 min. Tissue sections were washed in 0.1 M phosphate buffer, fixed in 4% paraformaldehyde for 10 min at room temperature, and rinsed again. They were then dehydrated through graded ethanol and air dried. Sections were hybridized under coverslips with the ³⁵S-labeled anti-sense GLUT12 probe (2–4 × 10⁶ cpm/μl) in hybridization buffer (300 mM NaCl, 10 mM Tris·HCl, pH 7.5, 10 mM Na₂HPO₄, pH 6.8, 5 mM EDTA, pH 8.0, 1× Denhardt’s solution, 50% formamide, 17 mg/ml yeast tRNA, and 10% wt/vol dextran sulphate) overnight at 58°C in humidified chambers (50% formamide/2× SSC). Control sections were hybridized with a GLUT12 sense probe. After hybridization, coverslips were removed in standard saline citrate containing 50% formamide at 50°C.

Slides were washed for a further 1 h in the above solution at 55°C, rinsed three times in RNase buffer (10 mM Tris·HCl, pH 7.5, 1 mM EDTA, pH 8.0, 0.5 M NaCl) at 37°C, and incubated in RNase A (150 μg/ml) for 1 h at 37°C. Sections were washed in 2× SSC at 55°C for 45 min, dehydrated in graded ethanol, air dried, and exposed to Kodak BioMax MR Autoradiography film for 5 days at room temperature. After development of the X-ray film, sections were dipped in LM-1 nuclear emulsion (1:1 with 2% glycerol, Amersham, UK), stored in a dark, airtight container with dessicant at 4°C for 21 days, developed in Phenisol (Ilford, Cheshire, UK), fixed in Ilford Hypam, and then stained with hematoyxin and eosin (H&E).

Confocal Immunofluorescence Microscopy

Fresh kidney tissue was rapidly frozen in cryomoulds submerged in liquid nitrogen-cooled isopentane and sectioned at 6 μm on a cryotome (Shandon Scientific, Runcorn, UK) at −20°C. Frozen sections were fixed with acetone, incubated in 100 mM glycine for 15 min, and permeabilized with 0.1% Triton X-100 (Ajax Chemicals, Sydney, Australia) for 15 min. Cryosections were then rinsed in distilled water, washed in PBS (3 × 5 min), and blocked for 30 min in 10% NSS, 5% FBS/PBS. Sections were incubated overnight at 4°C in primary antibody diluted in 5% FBS/PBS (GLUT12 R1396 serum or nonimmune rabbit serum). Following a 60-min incubation in the dark with swine anti-rabbit FITC-conjugated secondary antibody (1:100, DAKO), nuclei were stained with propidium iodide [20 μl of 250 ng/ml propidium iodide (Sigma) in 1% Triton X-100, 2 μl of 500 μg/ml RNase, 660 μl PBST] in the dark for 10 min and slides were mounted with fluorescent aqueous mountant (DAKO). A Bio-Rad MCR1024 inverted laser-scanning confocal microscope with Laser-Sharp 2000 software (Bio-Rad) was used for analysis. FITC (GLUT12) staining was detected at 488 nm as green, whereas propidium iodide nuclear staining was detected at 522 nm as red.

Statistical Analysis

Statistical package for the social sciences (SPSS) version 10 was used for statistical analysis of results. All values are expressed as means ± SE. Two-way ANOVA, with a Fisher post hoc comparison, was used to determine statistical significance between group means. P < 0.05 was regarded as statistically significant.

RESULTS

Physiological Parameters

Rats that received STZ for 30 wk all exhibited a diabetic phenotype (Table 1). Blood glucose levels were significantly greater in diabetic animals compared with their respective controls and no significant difference in blood glucose levels was observed between Ren-2 and SD rats. Thirty-week post-STZ diabetic animals gained significantly less weight than control groups. Long-term diabetes was associated with increased kidney weight in SD diabetic rats and decreased kidney weight in Ren-2 rats. Ren-2 rats were hypertensive (Table 1) and systolic blood pressure (SBP) was significantly higher when compared with SD rats.

Expression and Localization of GLUT12 in Adult Rat Kidney

Immunohistochemical analysis. GLUT12 expression in kidneys of SD and Ren-2 control and diabetic rats was determined by immunohistochemistry. Comparisons were made to GLUT1, as the expression and localization of this facilitative glucose transporter in the kidney are well understood. GLUT1 staining was present at the basolateral membrane in the tubular epithelium of all groups examined (Fig. 1, A–D). GLUT12 immunolabeling was also observed in all rat kidney sections but in contrast to GLUT1 was restricted to the epithelial cells of the distal tubules and collecting ducts (Fig. 1, E–H). In addition, and also in contrast to GLUT1, GLUT12 staining was present in the cytoplasm and at the plasma membrane of these cells. GLUT12 localization patterns were similar in all four animal groups. No immunolabeling was observed in sections incubated with nonimmune rabbit serum (Fig. 1, I–L).

Immunoprecipitation and immunoblotting. Immunoprecipitated rat heart and kidney membrane proteins were subjected to Western immunoblot analysis using the R1396 GLUT12 polyclonal antibody. Immunoblots demonstrated the presence of a single protein species with a molecular mass of 60 kDa following immunoprecipitation with the R1396 GLUT12 polyclonal antibody (Fig. 2). No GLUT12-immunoreactive proteins were detected following immunoprecipitation of rat heart membrane proteins with an unrelated control antibody (Fig. 2). The specificity of GLUT12 immunoblotting in the rat kidney was further demonstrated by preabsorption experiments. Significantly reduced band intensities of the immunoreactive species was observed in control immunoblots of immunoprecipitated rat heart and kidney (SD control) membrane proteins after

![Fig. 2](http://ajprenal.physiology.org/)} by 10.20.33.5 on July 8, 2017 http://ajprenal.physiology.org/ Downloaded from
preincubation of the serum with 100 μg/ml of competitive peptide, but not the noncompetitive peptide (Fig. 2).

Localization of the distal tubules and collecting ducts by immunohistochemical detection of epidermal growth factor expression. Epidermal growth factor (EGF) expression has previously been shown to be localized to the distal tubules and collecting ducts (15). Therefore, to confirm the specific pattern of expression of GLUT12, serial sections were immunolabeled with GLUT12 and EGF polyclonal antibodies. Results of representative immunohistochemistry experiments are shown in Fig. 3. Immunolabeling with GLUT12 and EGF antibodies demonstrate a similar staining pattern, indicating GLUT12 expression is localized to the distal tubules and collecting ducts. GLUT12 immunolabeling was also observed after preincubation of R1396 GLUT12 polyclonal antibody with 100 μg/ml of noncompetitive peptide (Fig. 3B). However, reduced intensity was evident in tissue immunolabeled with serum preincubated with 100 μg/ml of competitive (to which the antibody was raised) peptide (Fig. 3C).

In situ hybridization. GLUT12 mRNA expression in the rat kidney was examined using in situ hybridization. Figure 4A is an in situ hybridization photomicrograph illustrating GLUT12 mRNA expression in the renal cortex. The results correlate well to the demonstration by immunohistochemistry of GLUT12 expression specifically in the distal tubules and collecting ducts. A similar pattern of labeling was observed in SD and Ren-2 control and diabetic kidneys.

Renal Expression of GLUT12 Is Elevated in the Ren-2 Rat

Immunohistochemical assessment of GLUT12 expression indicated that the number of positively stained tubules present was higher in Ren-2 animals. Positive tubulointerstitial GLUT12 immunolabeling was therefore quantitated using the AIS system to determine the mean proportional area of GLUT12 staining, as described in MATERIALS AND METHODS. A significant increase in GLUT12 immunolabeling was observed in Ren-2 animals compared with SD controls (*P < 0.05; Fig. 5A). GLUT12 immunolabeling was also significantly greater in Ren-2 diabetic compared with SD diabetic animals (#P < 0.05; Fig. 5A). In comparison, GLUT1 immunolabeling in the proximal tubules was significantly elevated in both SD and Ren-2 diabetic animals compared with the respective control groups (*P < 0.05; Fig. 5B). GLUT1 proximal tubule staining was also significantly higher in Ren-2 diabetic compared with SD diabetic animals (#P < 0.05; Fig. 5B). No differences in GLUT1 immunostaining levels were found between SD and Ren-2 control animals (Fig. 5B). In addition, no significant differences in intensity of GLUT1 staining in the distal tubules and collecting ducts were found between the groups (data not shown).

Immunofluorescence Subcellular Localization of GLUT12

As described above, immunolabeling experiments demonstrated cytoplasmic and plasma membrane staining for GLUT12

Fig. 3. Epidermal growth factor (EGF) immunolabeling. Representative photomicrographs of Ren-2 diabetic kidney sections immunolabeled with: EGF (A) GLUT12 after preincubation of R1396 polyclonal antibody with 100 μg/ml of noncompetitive peptide (B) GLUT12 after preincubation with 100 μg/ml of competitive (to which the antibody was raised) peptide (C). Original magnification ×400.

Fig. 4. In situ hybridization of renal GLUT12 mRNA (A) and sense control (B). GLUT12 mRNA was localized to the renal cortex.
in the epithelial cells of the distal tubules and collecting ducts. The subcellular localization of GLUT12 protein was further studied by immunofluorescence laser confocal microscopy. GLUT12 labeling was observed in the epithelial cytoplasm, and plasma membrane staining was predominantly localized to the apical membrane of rat as well as human tubular epithelial cells (Fig. 6, A and C). Similar localization of GLUT12 was recorded for SD and Ren-2, nondiabetic and diabetic kidneys (data not shown). Control sections, incubated with nonimmune rabbit serum, were negative (Fig. 6, B and D). GLUT12 was predominantly localized to the tubular basolateral membranes (Fig. 6E).

**DISCUSSION**

The Ren-2 rat displays an amplified tissue RAS and develops severe hypertension from 4 wk of age (25). Hypertension and an elevated RAS have been closely associated with the development of nephropathy in diabetic animals (2, 17). The present study demonstrated that the novel facilitative glucose transporter GLUT12 is expressed in the adult rat kidney and is localized to the apical membrane of the distal tubules and collecting ducts. The study also indicates that both GLUT1 and GLUT12 protein expression is elevated in animal models of hypertension and diabetic nephropathy.

As previously described, GLUT1 expression was detected basolaterally throughout the nephron (11). In comparison, GLUT12 immunohistochemical staining was specific for the distal tubules and collecting ducts in SD and Ren-2 control and diabetic animals. GLUT12 has previously been shown to exhibit a specific pattern of expression in the distal tubules and collecting ducts of the fetal rat kidney and it has been postulated that GLUT12 may be important in glucose delivery to developing tissues (21). Serial kidney sections immunolabeled with GLUT12 and EGF, a protein specifically expressed in the distal tubules and collecting ducts (15), exhibit a similar staining pattern, confirming the specific localization of GLUT12 (Fig. 3). This pattern of localization is in accordance with the results obtained by in situ hybridization, demonstrating GLUT12 mRNA expression in the renal cortex (Fig. 4). Immunoblotting detected a 60-kDa protein in the kidney homogenate of SD and Ren-2 control and diabetic rats (Fig. 2). Previous studies reported GLUT12 mRNA at low levels in adult human kidney, although no detectable immunolabeling was observed by Western blot analysis (29). As the distal tubules and collecting ducts comprise only ~10% of the renal cortex, GLUT12 may not have been detectable in homogenates of small tissue samples available from human kidney.

Confocal immunofluorescence microscopy revealed both cytoplasmic and apical membrane labeling of GLUT12 in renal tubular epithelial cells of rat as well as human kidney (Fig. 6). Cytoplasmic and plasma membrane localization of GLUT12 has been described in rat lactating mammary cells, in addition to human breast and prostate tumors (7, 22, 28, 29). In cultured breast and prostate cancer cell lines, GLUT12 is present in a perinuclear location and at the plasma membrane (7, 28). Like GLUT4, it is thought that glucose transport into cells via GLUT12 is regulated by trafficking of the transporter protein from intracellular pools to the plasma membrane. GLUT4 is sequestered intracellularly in muscle and adipose cells and translocates to the plasma membrane in response to insulin (13). There are a number of conserved targeting motifs that are thought to control the intracellular localization of GLUT4 and GLUT12. Di-leucine (LL) motifs are present in the NH2 and COOH termini of GLUT12, at similar positions as the FQQL and LL targeting motifs of GLUT4.

Previously, only two facilitative hexose transporters had been described on the apical membrane of the nephron. GLUT2, a low-affinity, high-capacity transporter, has been described on the apical BBM of the proximal tubule in hyper-
glycemic conditions (10) and GLUT5 is thought to transport fructose at the BBM (31). STZ-induced diabetes has been reported to stimulate the insertion of GLUT2 into the BBM of the proximal tubule and to increase facilitative glucose uptake (23), although the signals that direct apical targeting are not well understood (4). GLUT12 protein has also been described at the apical plasma membrane of rat lactating mammary epithelial cells (22) and future studies may reveal the specific targeting domain(s) and signals that control GLUT12 subcellular localization and trafficking in the kidney.

The bulk of renal glucose reabsorption normally occurs in the proximal convoluted tubules. This is thought to be a two-step process involving reabsorption at the BBM by SGLT active transporters, followed by facilitative transport at the basolateral membrane via GLUT2 and GLUT1 (32). Early studies suggested that under certain conditions glucose is additionally reabsorbed in the later parts of the nephron. Experiments performed in the dog demonstrated glucose reabsorption in the distal tubules following combined glucose load and extracellular volume expansion (34). In the rat, carrier-mediated reabsorption of 2-deoxy-d-glucose in the distal nephron has also been reported (19). The apical localization and expression of GLUT12 in the distal tubules of the nephron suggest that GLUT12 may play a role in glucose reabsorption when the glucose reabsorption capacity of the proximal region is exceeded. Recently, expression of another class III transporter, GLUT8, has been demonstrated in glomerular podocytes and in the basolateral region of distal tubular epithelial cells of the murine kidney. Although Western analysis demonstrated increased GLUT8 levels in the diabetic kidney, it is not known whether this involved elevated GLUT8 levels in the distal tubule region (30).

GLUT1 protein levels were significantly elevated in the proximal tubules of diabetic animals. Following Western blot analyses, D’Agord Schaan et al. (9) reported that GLUT1 levels are increased in the rat renal cortex after 45 days STZ-induced diabetes. Other studies reported that untreated STZ-induced diabetes for 2 to 4 wk resulted in a reduction of GLUT1 protein levels in the proximal tubules. Treatment with insulin, 4 U/day resulted in a return to normal GLUT1 levels (10). In the current study, diabetic rats received subcutaneous insulin injections (2–4 U) to maintain blood glucose at 27 mmol/l and to reduce mortality and encourage weight gain. Our study was performed 30 wk post-STZ treatment, a time point that represents advanced nephropathy. At this stage, the kidney develops significant pathological changes such as glomerulosclerosis and tubulointerstitial fibrosis (18). Our results implicate the upregulation of GLUT1 in the pathogenesis of progressive diabetic nephropathy.

Expression of both GLUT1 and GLUT12 is increased in the kidneys of Ren-2 animals compared with SD controls (Fig. 5). As levels of GLUT12 were not significantly elevated in STZ-
induced diabetes (Fig. 5A), it is unlikely that hyperglycemia alone is responsible for increased GLUT12 levels. Elevated levels of a local kidney RAS that occur in the tubules of the Ren-2 rat may directly increase GLUT expression. There are precedents for regulation of glucose transporter expression by the RAS. For example, recent studies demonstrated increased glucose reabsorption in proximal tubule membrane vesicles and increased expression of SGLT2 in response to renovascular hypertension in rats. Increased SGLT2 levels were also associated with chronic ANG II infusion (5). A study of GLUT1 expression in cultured mesangial cells showed that ANG II upregulated GLUT1 mRNA in a time- and dose-dependent manner (26).

The current study demonstrates that GLUT12 is expressed in the rat kidney and its distinct expression indicates that it is likely to play a role in glucose transport in the distal tubules and collecting ducts. The apical localization of GLUT12 in the rat and human kidney suggests that it may be involved in an additional glucose reabsorptive mechanism in the late nephron. In addition, GLUT1 and GLUT12 expression are upregulated in the rat kidney in animal models of hypertension and diabetic nephropathy. Thus both GLUT1- and GLUT12-facilitated glucose reabsorption may contribute to the processes occurring in the progression of diabetic nephropathy.

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GRANTS

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