Expression and functional characteristics of tubular transporters: P-glycoprotein, PEPT1, and PEPT2 in renal mass reduction and diabetes

Gianfranco Tramonti, Ping Xie, Elisabeth I. Wallner, Farhad R. Danesh, and Yashpal S. Kanwar

1Department of Nephrology, University of Pisa, Pisa, Italy; 2Pathology and 3Medicine, Northwestern University Medical School, Chicago, Illinois

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The kidney plays a critical role in the clearance of various molecules including drugs, toxins, and endogenous metabolites. This process, traditionally known as renal clearance, involves glomerular filtration, tubular secretion, and reabsorption. In renal tubules, a number of membrane transport proteins are involved during excretion and reabsorption. Tubular secretion of drugs and other substances is handled by several classes of transporters that are located at the basolateral membrane which mediates cellular uptake, whereas those expressed at the brush-border membrane promote the exit and secretion into tubular lumen (6, 12, 16). There are certain other membrane transport proteins which reabsorb filtered molecules, including amino acids or peptides/proteins and sugars, perhaps to economize and prevent their urinary loss (3, 15, 28, 35).

One of the transporters involved in tubular secretion of xenobiotics is P-glycoprotein (Pgp). Pgp is a plasma membrane glycoprotein with a Mr of 170,000, and it belongs to the ATP-binding cassette family. It is composed of two similar segments, each containing six putative transmembrane domains and an intracellular tail. Pgp acts as a membrane-bound, ATP-consuming drug efflux pump that actively extrudes a variety of structurally unrelated compounds across the plasma membrane (6, 11, 16, 31). It is located on the apical side of cells of many secretory organs such as the liver, intestine, and kidney. In the kidney, Pgp is expressed mainly in the proximal tubule and to a lesser extent in the thick limb of Henle’s loop, collecting ducts, and glomerular mesangium. Many substances are actively transported by Pgp, e.g., calcium channel blockers, immunosuppressants, cardioactive glycosides, antibiotics, antineoplastic and several other peptides and steroids (7, 26). Thus Pgp functions as an efflux pump which represents a protective mechanism to exclude endogenous and exogenous toxins from normal cells and to ultimately excrete them into the bodily secretions. In humans, two genes [multidrug resistance (MDR1 and MDR2)] encode this protein, whereas in rodents there are three homologs of this protein, i.e., mdr1 (mdr1b), mdr2, and mdr3 (mdr1a) (6, 12, 16).

Renal tubular reabsorption of peptides at the brush border of cells is handled by two transporters designated as PEPT1 and PEPT2. They are similar and exhibit 50% amino acid homology and mediate an electrogenic H+-coupled cotransport of dipeptides and tripeptides (10). PEPT1 is found in the S1 segment of the proximal tubule, whereas PEPT2 is located in S2 and S3 segments (27). PEPT1 is a low-affinity/high-capacity and PEPT2 a high-affinity/low-capacity transporter, and they perform a number of functions, as ascertained by various expression cloning and target gene disruption experiments (8, 25). Of interest are peptide-like drugs such as ß-lactam antibiotics, angiotensin-converting enzyme inhibitors, and some of the anticancer drugs that are also handled by them. Previous studies demonstrated an increased expression of PEPT1 in experimental diabetes (11) and upregulation of PEPT2 in the setting of reduced renal mass (30). Furthermore, high-glucose ambience modifies the expression of a large number of proteins under basal conditions and in the setting of reduced renal mass (33). However, no comprehensive data regarding the influence of concomitant reduction of renal mass and diabetes on Pgp, PEPT1, and PEPT2 have been reported.
This study was initiated to ascertain whether the reduction in renal mass influences expression of these tubular transporters, i.e., Pgp, PEPT1, and PEPT2, in diabetic state and under basal conditions. Protein and gene expression of transporters was evaluated in normal, diabetic rats and in rats who had undergone uninephrectomy. In addition, uptake of peptide transporters, PEPT1 and PEPT2, was evaluated by studying the transport of [H]glycylsarcosine across the renal tubular brush-border membrane vesicles (BBMV).

MATERIALS AND METHODS

Animals. A total of 40 Sprague-Dawley female rats weighing ∼120 g was used. The female rats are used to avoid variability in the compensatory hypertrophy of the kidney following nephrectomy in the hyperglycemic state which may be related to certain circulating growth factors in males. Moreover, the degree of streptozotocin-induced hyperglycemia is at times quite variable in males. Twenty female rats were sham-operated. Two weeks after surgery, 10 of them were made diabetic by administering a single intraperitoneal injection of streptozotocin (60 mg/kg). Twenty other rats were subjected to left uninephrectomy, and 10 of them were made diabetic 2 wk after surgery. Rats were kept on standard laboratory diet and had free access to tap water for another 2 wk, and then they were killed and blood was collected for various serologic parameters. The kidneys were removed, immersed in liquid nitrogen, stored at −80°C, and utilized for various expression and transport experiments. Except for one diabetic rat that had blood glucose >450 mg/dl, the others had ranging from 250 to 350 mg/dl. In controls, blood glucose levels were removed, immersed in liquid nitrogen, stored at −80°C, and utilized for various expression and transport experiments. Except for one diabetic rat that had blood glucose >450 mg/dl, the others had ranging from 250 to 350 mg/dl. In controls, blood glucose levels

Gene expression studies. Total RNA was isolated with the use of Promega RNAsen Total RNA Isolation System (Promega, Madison, WI). RNA isolated was treated with DNase to remove genomic DNA. Two micrograms of total RNA were reverse transcribed using M-MLV reverse transcriptase (Promega). To quantify cDNA, competitive PCR was performed as previously described (17, 34). The primers employed for the wild-type cDNA are listed in Table 1. They were custom synthesized by Integrated DNA Technologies. The PCR conditions are summarized in Table 2.

For quantitative PCR, a competitive minigene plasmid (mutant) containing sequences for various matrix proteins previously synthesized was used (25). The competitive (mutant) DNA for mdr1b was obtained by PCR using the minigene construct and the following primers: sense: 5′-AGT GAC ACT GGT GCC TCT TAC CCT GGT CGG CAA CCC CAA GAC CAT CCC CCC GCC CGT GTG TCA CGG AGG CC-3′, which is a combination of the sense primer of fibronectin, a linker, and the sense primer of mdr1b; and antisense: 5′-GAC ACA CTG GTT GTA TGC ACG GTA GAC CAT CCT GCA GAG CC-3′, which is a combination of antisense primer of β-actin, the linker, and the antisense primer of mdr1b. The competitive DNA for PEPT2 was obtained by PCR of the minigene and the following primers: sense: 5′-GAG TAT CTC CAG CAT GCT GGT CAA GAG CCC CCC GCC CTG GTG TCA CGG-3′, a combination of the sense primer of fibronectin and the sense primer of PEPT2; and antisense: 5′-CAG AAG GGT GGA GGA GGA GGA CAG GGC CC-3′, a combination of antisense primers of β-actin and PEPT2. PEPT1 competitive was made by using the minigene and primers consisting of combination of PEPT1 and PEPT2 primers used for wild gene. The mdr1a competitive was made by PCR using the minigene and the following primers: sense: 5′-GGG CCA CAT GAT CAA GAG GCC GGA GTA TCT CCA GCA TGC TGG TCA AGG-3′, which represents combination of mdr1a and PEPT2 primers; and antisense: 5′-GAG CAG CTT CAT TGG CAA GCC TGG GAG GGC GCA GTG GCC AT-3′, which is the combination of mdr1a and β-actin primers. PCR products of the competitive containing primers for PEPT1 and PEPT2 and for mdr1a and mdr1b were ligated into pCR II Vector (Invitrogen) and amplified for competitive PCR analyses. The expected size of PCR products (bp) for wild and competitive (mutant) DNAs are included in Table 3.

Finally, for competitive PCR analyses, first the amount of wild and competitive DNAs to be used was standardized by performing competitive PCR using β-actin primers. Then fixed amounts of cDNAs and logarithmic dilutions of plasmids containing competitive DNAs were amplified using the same primers and amplification conditions as for the standard PCR. The density of the individual bands was calculated by scanning the PCR bands with optical densitometer and then the bar graphs were prepared. Because the PCR band at the highest range of the PCR were normalized against this value. Then the average band density and standard deviations were calculated from data of five different animals.

Protein expression studies. Protein expression was evaluated by Western blot analyses. Membrane-enriched fractions were isolated as follows. The kidneys were homogenized in a Dounce homogenizer at 4°C. The homogenate was centrifuged at 400 g for 10 min to remove the nuclei. The supernatant was centrifuged at 30,000 g for 30 min. The resulting pellet was resuspended by sonicating for 1 min in 1 ml of the buffer. After the protein concentration was measured by Bradford assay, the membrane-enriched fractions were stored at −80°C until further use. All procedures were performed at 4°C.

The membranes, containing 500 mg of protein, were suspended in the loading buffer and sonicated on ice for 30 s. Samples were treated in a boiling bath for 5 min, cooled on ice, and loaded onto 7.5% SDS-PAGE. The proteins resolved in the gel were electroblotted onto nitrocellulose membrane (Hybond ECL, Amersham Pharmacia Biotech) at 4°C, at a constant current of 36 mA overnight. Nonspecific sites were blocked with 5% nonfat milk. For Pgp detection, the blots were incubated with C219 monoclonal antibody (Signet Laboratories) for 2 h. The signal for Pgp was detected by incubating with secondary anti-rabbit antibody conjugated with horseradish peroxidase (ECL system, Amersham, and Brosius), and the signal was detected by incubating with anti-rabbit antibody conjugated with horseradish peroxidase (ECL

Table 1. Primers employed for wild-type cDNA

<table>
<thead>
<tr>
<th>Sense</th>
<th>Antisense</th>
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<tbody>
<tr>
<td>β-Actin</td>
<td>GAC GAC ATG GAG AAG ATG TGG</td>
</tr>
<tr>
<td>mdr1a</td>
<td>GGG CCA CAT GAT CAA GAG GGGG</td>
</tr>
<tr>
<td>mdr1b</td>
<td>AGT GAC ACT GGT GCC TCT GA</td>
</tr>
<tr>
<td>PEPT1</td>
<td>GCA CCC TTA ACG AGA TGA TCA CC</td>
</tr>
<tr>
<td>PEPT2</td>
<td>GAG TAT CTC CAG CAT GCT GTG CAA GG</td>
</tr>
</tbody>
</table>
tissues were embedded in paraffin and 4-
overnight at 4°C. After dehydration in graded series of ethanol, the
aldehyde (PLP) solution (0.01 M NaIO₄, 0.075 M lysine HCl,
followed by perfusion with freshly prepared periodate-lysine-parafor-
kidneys were briefly perfused via abdominal aorta with warm saline,
anti-rabbit IgG (1:100 dilution) conjugated with FITC. The sections
were washed with PBS three times and then incubated with anti-mouse or
either of the following antibodies: anti-Pgp, -PEPT1, and
sections were prepared, air-dried, washed with PBS buffer, and incubated
with sister of P-glycoprotein (sPgP) exclusively expressed in the liver
Smith et al. (27). The C219 Pgp monoclonal antibody cross-reacts
standard deviations about the mean were calculated from data of five
determinations. It was designated as 1 and the measurements of others
intensity of individual bands. Because the band of uninephrectomy
systems were scanned by an optical densitometer to determine the
expression levels of MDR1a, MDR1b, PEPT1, PEPT2, and
MgCl₂ and rehomogenized. The homogenate was re-centrifuged at
30,000 g for 30 min. The final pellet consisting of BBMV was
washed in buffers containing 100 mM mannitol, 100 mM KCl, 10 mM Mes, pH 6.0, for transport studies and the protein concentra-
tions were adjusted to 10 mg/ml. A fixed amount of BBMV preparation
(250 μl, 2.5 mg) was incubated at 37°C for 10 min with 15 μl of
valinomycin (1 mg/ml in ethanol) to generate an inwardly directed
negative membrane potential. After incubation, 25 μl [H]l-glucosecarosine with specific activity of 60 Ci/mmol (American Radiolabeled Chemical) were added. Fixed amounts (50 μl, 431 μg) of the solution, containing BBMV and the tracer, were placed on a precoated Millipore HAWP filter and washed with TCA
undertaken at vacuum for 10, 20, 30, 60, and 120, and 600 s. After being washed, the filters were immersed in the scintillation cocktail, and radioactivity was measured using a spectrometer and expressed as 2 × 10⁻³
cpm/mg of the protein.

RESULTS

Gene expression. Competitive PCR analyses of various
genes are included in Fig. 1. The top lanes represent the PCR
product of wild-type cDNA with expected size of PCR products
of 450, 340, 480, and 492 bp for MDR1a, MDR1b, PEPT1, PEPT2, and β-actin genes, respectively, whereas the bottom lanes represent the PCR products utilizing competitive (mutant) plasmid DNA with expected size of 320, 245, 349, 366, and 288 bp for MDR1a, MDR1b, PEPT1, PEPT2, and β-actin genes, respectively. In each group, i.e., SN, UN, SD, and UD, the four lanes represent 10⁻⁶-10⁻⁹ log dilutions of the competitive (mutant) DNA. At such a dilution range, one could visualize bands of PCR products of both the wild-type cDNA and competitive (mutant) DNA. The densitometric readings of the bands of PCR products were made. Finally, the density of PCR bands of cDNA was expressed as a relative score of 1–50, and the extent of expression was determined. Relative average density of bands of wild-type DNA (Fig. 1, top band, *) from five different animals was bar graphed and included in Fig. 2

Table 3. Expected size of PCR products for wild and competitive (mutant) DNAs

<table>
<thead>
<tr>
<th>Gene</th>
<th>Wild-type cDNA, bp</th>
<th>Competitive (Mutant) DNA, bp</th>
</tr>
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<tbody>
<tr>
<td>β-Actin</td>
<td>492</td>
<td>288</td>
</tr>
<tr>
<td>mdr1a</td>
<td>450</td>
<td>320</td>
</tr>
<tr>
<td>mdr1b</td>
<td>340</td>
<td>245</td>
</tr>
<tr>
<td>PEPT1</td>
<td>489</td>
<td>349</td>
</tr>
<tr>
<td>PEPT2</td>
<td>480</td>
<td>366</td>
</tr>
</tbody>
</table>
For MDR1a (Fig. 1A), the bands for the wild-type cDNA of uninephrectomy (UN) group had a relatively high expression score compared with the sham-operated (SN) group, suggesting 10-fold upregulated gene expression (5 → 50, *) in the former UN group of rats. Similarly, in diabetic rats, the expression scores were higher in the uninephrectomized (UD) group compared with the sham-operated ones (SD) with about twofold upregulation. Interestingly, although hyperglycemia led to about fivefold increase in MDR1 expression (5 → 25, *) in SD vs. SN group, however, no further added effect on the gene expression was observed as a result of hyperglycemia (UN vs. UD). In fact, uninephrectomy by itself in the UN group yielded the highest relative upregulated MDR1 expression. For MDR1b (Fig. 1B), although there was a certain degree of gene upregulation on uninephrectomy or in diabetic state, however, the degree of change was not as much as that seen for the MDR1a gene. About 50% increase in the MDR1b expression was observed after uninephrectomy (UN vs. SN). Similarly, a 30% increase was observed in the diabetic rats that had undergone uninephrectomy (UD vs. SD). The changes in MDR1b expression were marginally different between control sham-operated (SN) and diabetic group (SD), suggesting a mild contribution of hyperglycemia.

For PEPT1 (Fig. 1C), the changes in the expression were comparable to those observed for MDR1a gene. The wild-type cDNA at a 10^{-9} log dilution of competitive mutant DNA had an approximately fourfold upregulated gene expression (10 → 40), with 50 being the highest and others normalized against this value.

For MDR1a (Fig. 1A), the bands for the wild-type cDNA of uninephrectomy (UN) group had a relatively high expression score compared with the sham-operated (SN) group, suggesting 10-fold upregulated gene expression (5 → 50, *) in the former UN group of rats. Similarly, in diabetic rats, the expression scores were higher in the uninephrectomized (UD) group compared with the sham-operated ones (SD) with about twofold upregulation. Interestingly, although hyperglycemia led to about fivefold increase in MDR1 expression (5 → 25, *) in SD vs. SN group, however, no further added effect on the gene expression was observed as a result of hyperglycemia (UN vs. UD). In fact, uninephrectomy by itself in the UN group yielded the highest relative upregulated MDR1 expression. For MDR1b (Fig. 1B), although there was a certain degree of gene upregulation on uninephrectomy or in diabetic state, however, the degree of change was not as much as that seen for the MDR1a gene. About 50% increase in the MDR1b expression was observed after uninephrectomy (UN vs. SN). Similarly, a 30% increase was observed in the diabetic rats that had undergone uninephrectomy (UD vs. SD). The changes in MDR1b expression were marginally different between control sham-operated (SN) and diabetic group (SD), suggesting a mild contribution of hyperglycemia.

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Fig. 1. Competitive RT-PCR analyses of MDR1a, MDR1b, PEPT1, PEPT2, and β-actin. The band intensity of various PCR products of wild-type DNA (top bands) is given as a relative expression score with a range of 1 to 50. An increased expression of MDR1a, MDR1b, PEPT1, and PEPT2 is observed in animals that underwent uninephrectomy (UN) compared with sham-operated (SN) rats, the highest being for MDR1a like the protein expression of Pgp (Fig. 3). This is quite evident at the highest dilution (10^{-9}) of the wild-type DNA (*) in each of the groups. Similarly, a mild-to-moderate increase in the expression of various cotransporters is observed in diabetic animals that had undergone uninephrectomy (UD) compared with control diabetic (SD) rats. The expression is slightly increased in the SD group compared with SN. Interestingly, there are no significant differences in the expression between UN and UD groups, rather it seems to be somewhat decreased. The β-actin expression is similar in all the groups.

Fig. 2. Bar graphs representing the average relative density of the wild-type PCR bands of P-glycoprotein (Pgp), PEPT1, PEPT2, and β-actin included in Fig. 1. The bar graphs reflect the average density data and standard deviation of wild-type PCR bands at the highest dilution (10^{-9}) derived from 5 animals from each group. Because the band of uninephrectomy group of sham-operated rats had the highest intensity by densitometric measurements, it was designated as 50 and the measurements of other variables were normalized against this value. P values reflect comparison between SN vs. UN or SD vs. UD groups.

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40, *) in the UN group compared with the sham-operated (SN) group. Similarly, in diabetic rats, the expression scores were notably higher in the uninephrectomized (UD) group compared with the sham-operated ones (SD) with approximately twofold upregulation. Interestingly, hyperglycemia led to a mild (~30%) increase (SN vs. SD) in PEPT1 expression; and a further added effect (~100%) on the gene expression was observed as a result of hyperglycemia (SD vs. UD). However, like for MDR1a, uninephrectomy by itself in the UN group yielded the highest upregulated relative gene expression. For PEPT2, the expression paralleled that seen in the MDR1b gene (Fig. 1D). About threefold upregulated expression (10 → 30, *) was seen in the uninephrectomy group compared with sham-operated group (UN vs. SN). In contrast, a mild (~20%) relative increase in the expression was observed in the diabetic rats that had undergone uninephrectomy (UD vs. SD). The changes in PEPT2 expression were marginally significant between control sham-operated (SN) and diabetic group (SD), suggesting a mild contribution of hyperglycemia. Finally, no significant differences for the β-actin expression were observed among all the groups (Fig. 1E).

Protein expression studies. Western blot analyses of various proteins are included in Fig. 3A, and the corresponding relative intensity of the bands is given in Fig. 4. For Pgp (Fig. 3A), a band of ~170 kDa was detected in the kidneys of nondiabetic sham-operated (SN) rats. Following uninephrectomy (UN), the renal Pgp expression was upregulated, as reflected by an approximately threefold increase in the band intensity compared with that of the SN (Fig. 4). The intensity of the band in kidneys of sham-operated diabetic rat (SD) was similar to that of the SN group. After uninephrectomy in diabetic rat (UD), the renal Pgp expression was also upregulated (~2-fold) compared with SD group, and the increase was somewhat less than in uninephrectomized nondiabetic rats with respect to SN.

For PEPT1, a band of ~90 kDa was seen by immunoblot in SN group (Fig. 3B). The band intensity increased by about fourfold in UN group, suggesting a notable upregulation of PEPT1 after uninephrectomy (Fig. 4). Also, an approximately twofold increase was noted in diabetic SD rats compared with the sham (SN) group. In contrast, to Pgp, a ~50% increase in the expression of PEPT1 was observed in diabetic rats that had undergone uninephrectomy (UD) compared with SD group. For PEPT2, a band ~85 kDa was detected by Western blotting (Fig. 3C), and changes in the expression were similar to that of PEPT1 with certain exceptions. The increase in the expression following uninephrectomy was about twofold in both nondiabetic (UN) and diabetic (UD) groups compared with their respective controls, i.e., SN and SD. However, no significant differences were observed between the rats that had not undergone uninephrectomy, i.e., SN vs. SD. The expression of β-actin that served as a control for various group of rats, i.e., SN, UN, SD, and UD, did not change significantly, as reflected by similar intensities of the ~43-kDa band (Figs. 3D and 4).

IF and IH studies. Both IF and IH studies were performed to evaluate their localization in various compartments of the kidney and to delineate the “qualitative” changes in their expression following uninephrectomy or hyperglycemia. By IF microscopy, Pgp was seen in the brush borders of tubular epithelia confined to the middle cortex in control sham-operated animals (Fig. 5A). In addition, a mild expression in the glomerular mesangium was seen (arrowheads). The expression increased markedly in kidneys of uninephrectomized rats, as reflected by the accentuated IF staining of the brush borders of tubular epithelia (Fig. 5B). The staining was intense to such an extent that the IF blended with the cytoplasm of the tubular epithelia. Along with this, IF also increased in the glomerular mesangium (arrowheads in Fig. 5, B and C). The PEPT1 staining was seen confined to the brush border of the outer cortical proximal tubular epithelia in the control animals (Fig. 5D), and minimal expression in the glomerular mesangium was seen (arrowhead). In kidneys of uninephrectomized animals, a notable increase in the intensity of IF staining was observed in tubular epithelia (Fig. 5E). Interestingly, the expression of PEPT1 extended into the tubules of the deeper cortex. The expression in the glomerular mesangium increased as well (arrowhead, Fig. 5F), but it was readily
noticedable in the S1 segment of the proximal tubule (arrow, Fig. 5F). The PEPT2 expression was seen in the middle cortex, and it seemed to be relatively higher than PEPT1 in the proximal tubules, corresponding to their S2 and S3 segments in control animals (Fig. 5G). In the uninephrectomized rats, intense IF staining in the tubules was seen with extension of PEPT2 expression into the deeper cortex and outer medulla (Fig. 5H). Similar to Pgp, the brush-border expression was quite intense and blended with cytoplasm of the tubular epithelia (Fig. 5I). However, unlike Pgp and PEPT1, the PEPT2 expression in the glomeruli did not increase significantly (arrowheads). The level of renal expression of Pgp, PEPT1, and PEPT2 in diabetic animals with or without nephrectomy increased slightly from the controls but was not appreciably different from that seen in uninephrectomized rats.

For better elucidation of protein expression of the transporters PLP perfusion-fixed kidneys were utilized and IH was performed. As noted previously (27), precise localization of Pgp, PEPT1, and PEPT2 to S2 and S3 segments (Fig. 5H, asterisks) of proximal tubules was readily delineated. However, it was difficult to evaluate the differences of brownish peroxidase staining among different variables. Also, by PLP perfusion fixation, the anti-Pgp reactivity of the antibody was completely lost, suggesting one’s dependency on IF staining when evaluating the expression of these transporters in different experimental states.

Transport studies. The results of [H\textsuperscript{3}]glycylsarcosine transport across BBMV are given in Fig. 6 in the presence (▲, ●, ○) or absence (△, □, ○) of valinomycin. The values of various variables were normalized to 1 at 10 s from the start of the experiment. At subsequent time intervals, i.e., 20 to 600 s, no significant increase in the [H\textsuperscript{3}]glycylsarcosine uptake in the sham-operated (○), uninephrectomized (△), and diabetic + uninephrectomized (□) groups in the absence of valinomycin was observed. Similarly, in the uninephrectomized (●) group the transport of the tracer did not increase at subsequent time points in presence of valinomycin. However, an upward trend was observed in the diabetic + uninephrectomized (■) group, and ~100% increase in the uptake of the tracer was observed at 30 s. Intriguingly, a remarkable (>4-fold) uptake of the [H\textsuperscript{3}]glycylsarcosine was observed in the uninephrectomized (▲) group in the presence of valinomycin. The values included in Fig. 6 are the average of five experiments on BBMV preparations isolated from different rat kidneys. No appreciable further increase in the uptake was observed at 60, 120, and 600 s (10 min) after the start of the experiment. A single
measurement for each of the variable was also made at 20 min, and no appreciable increase was observed.

**DISCUSSION**

This investigation was intended to conduct comparative analyses and to delineate expression of some of the well-characterized transporters under conditions of unilateral reduction of renal mass while the other kidney undergoes hypertrophy due to compensatory mechanisms by themselves or in combination with hyperglycemia. The data suggest that a reduction in renal mass influences the expression of Pgp, PEPT1, and PEPT2, the transporters located at the luminal surface of the tubular cells that handle small molecules, including xenobiotics, di- or triptides. Their expression increases with the reduction of renal mass although to a varying degree. Besides upregulation of protein and gene expression, their capacity for the uptake of compounds prototypic of dipeptides, i.e., [3H]glycylsarcosine, also increases. Interestingly, the effect of hyperglycemia in the event of unilateral reduction in the renal mass seems to be somewhat marginal. These observations may likely have certain bearings on the management of patients with altered renal pathophysiology.

From a clinical standpoint, it is pertinent to address the state of tubular transport of drugs, including antibiotics or xenobiotics, in renal diseases where one encounters reduced renal mass and hyperglycemia, as one finds such situations quite often. A prototypical example would be patients suffering from diabetes mellitus having undergone renal transplantation. Currently, reduction in the renal mass and having diabetes mellitus represent the leading cause in patients requiring dialysis worldwide. The present study was carried out in uninephrectomized rats because this model mimics renal transplantation and patients that have undergone uninephrectomy for other causes, e.g., kidney neoplasm. In the 5/6 nephrectomy model, it is likely that with surgical ablation of kidney poles there is a local stimulation of cytokine production, thus making a scenario far from that encountered in clinical practice. Thus the expression of various transporters could very well vary depending on the model employed in a given study. Nevertheless, there are remarkable physiological changes, in terms of excretory activity, in the nephrons of a remnant kidney model (9). The changes that occur initially may be attributable to the compensatory mechanisms after the immediate reduction of the renal mass, whereas later alterations may be due to the uremic toxicity. Certainly, in the initial compensatory phase the remnant kidney goes into a hypermetabolic state with increased oxygen consumption and gluconeogenesis (13). Besides increased oxygen consumption, there is an increased production and excretion of malondialdehyde (MDA), the latter change perhaps reflecting a protective mechanism to shield the nephron from the anticipated oxidative stress or lipid peroxidation (23). Interestingly, the alterations are also reflected in various transport mechanisms; for instance, loss of renal mass results in a selective augmentation of 22Na flux (lumen-to-bath) in the cortical collecting tubules (32). These pioneering studies carried out more than a decade ago set the stage for the investigation of recently discovered transporters, e.g., for glucose, amino acids, small peptides, antibiotics, and xenobiotics.

The importance of Pgp has been well emphasized in several recent reviews, and it had been shown to act as an ATP-dependent “efflux pump” operative during multidrug resistance, drug-drug interactions, and drug bioavailability under various pathological states (1). The bioavailability of drugs to various tissues conceivably depends on the expression of the transporters/countertransporters. Our study demonstrated a higher Pgp expression in uninephrectomized rats both at the transcription as well as translation levels, which as suggested in previous metabolic studies (13, 23) may indicate that the reduction of renal mass represents a stimulus to tubular cells to increase Pgp production. Of course, this compensatory response may occur to improve tubular excretory capacity. However, in the model of unilateral nephrectomy with concomitant subtotal removal of the other kidney, the Pgp levels remain unchanged or rather they were decreased (19). This may again be due to the fact that there would be an expected rise in cytokine production, e.g., TGF-β, in the subtotal nephrectomy model. Alternatively, this may be due to the fact that Pgp levels were measured at 6 wk, the period at which chronic changes in the form of fibrosis, conceivably induced by TGF-β, have already set in, and thus they do not reflect the events of the compensatory phase following nephrectomy. The role of TGF-β has been well established in glomerulosclerosis, and at times its levels are parallel to the increased expression of glucose transporters in the mesangium during diabetic state (14). Although other transporters like Pgp and PEPT1, as observed in this investigation, are increased in the mesangium, their relationship to glomerulosclerosis with the associated increase in fibrogenic cytokines, such as TGF-β or CTGF, remains to be investigated. In any instance, the question that needs to be addressed is, what are the potential changes in the tubular cells with such an increased Pgp expression? It has been reported that the increased Pgp expression makes the cells more sensitive to drugs, nonionic detergents, and physical shear, the hypothesis being that increased expression of Pgp may disrupt membrane integrity and thus sensitizes the cells to injury induced by agents that bind to the plasmalemma.
other possibility could be that Pgp activity requires a large amount of ATP and its consequent intracellular depletion would be expected to lead to an overall generalized compromise in the functions of a given cell. Along these lines, literature data indicate that Pgp overexpression in transplant patients confers a relatively high resistance to steroid or cyclosporine therapy for acute rejection crises, which partly may be due to a compromise in cellular functions and, in part, to the increased efflux pump activity of the Pgp (18). In regard to the effect of glucose on Pgp, the literature data are somewhat sparse. However, depletion of glucose leading to an increased expression of Pgp in hepatoma cells in vitro has been reported (20). This observation is in contrast to the findings of our study where hyperglycemia induced an increase rather than decrease in Pgp expression, although there was no synergistic effect between uninephrectomy and hyperglycemia. These differences may be attributable to the fact that the previous literature studies were carried out in vitro and with the cells that utilize anaerobic rather than aerobic metabolism, as operative in tubular cells in vivo were used (20). Besides this isolated literature report, there are, however, a few studies which include the information regarding the status of other transporters, e.g., PEPT1, in hyperglycemic states (11).

An increased expression of PEPT1 has been described in the hyperglycemic state 96 h after the induction of diabetes with streptozotocin in rats (11). At the same time, \(^{3}H\)glycylsarcosine uptake was also increased under conditions when an inwardly directed negative membrane potential was generated with valinomycin. Although PEPT2 has similar functions but with differing affinity and capacity, its status in diabetes has not been reported. Both PEPT1 and PEPT2 are expressed in kidney; however, the former has a more restricted distribution, whereas PEPT2 has a broad range of distribution including in the nonexcretory organs such as the brain, spleen, and lung (5). In the current study utilizing a model that is a combination of uninephrectomy and hyperglycemia, an increased expression of both PEPT1 and PEPT2 was observed; although, PEPT1 expression was notably upregulated, that of the PEPT2 was somewhat marginal. Nevertheless, we observed a remarkable increase in the expression of both peptide transporters 4 wk after uninephrectomy. A similar increase for PEPT2 has been described in an acute remnant kidney model while that of PEPT1 was unaffected 2 wk after 5/6 nephrectomy (30). Similarly, like in our study the H\(^{+}\)-dependent glycylsarcosine uptake by the BBMV was increased. However, the mRNA levels of both PEPT1 and PEPT2 along with the SGLT2, a glucose cotransporter, were found to be depressed in the chronic remnant model, i.e., 16 wk after 5/6 nephrectomy (22). Also, uptake of both the Na\(^{+}\) gradient-dependent \(\alpha\)-glucose and H\(^{+}\) gradient-dependent glycylsarcosine by BBMV was reduced. This would suggest that in the chronic model of a remnant kidney fibrosis may have set in, which globally reduced the expression and activity of both the glucose as well as of the peptide transporters. Another interesting corollary of the study by these groups of investigators (22) may mean that the expression and activity of \(\alpha\)-glucose and peptide transporters parallel one another as PEPT1 and SGLT2 are low-affinity/high-capacity transporters expressed in the S1 segment while the ones with high affinity/low capacity, i.e., PEPT2 and SGLT1, are expressed in the S3 segment of the nephron. Further studies are needed to assess the correlative aspects of their activities in a setting of reduced renal mass in a diabetic state. In any instance, a firm conclusion can be drawn from our study and of others that the expression of peptide transporters is remarkably affected during the compensatory phase following unilateral or 5/6 nephrectomy. The influence of hyperglycemia seems to be intriguing in that it somehow dampens the expression in a setting of unilateral nephrectomy. Furthermore, the fact that there was a somewhat lower degree of \(^{3}H\)glycylsarcosine uptake by the BBMV prepared from diabetic animals that had undergone uninephrectomy, as observed in our study, affirms such an assertion. The question of reduced uptake of \(^{3}H\)glycylsarcosine in the diabetic state, whether related to increased or decreased expression of peptide transporters, needs to be addressed with respect to its clinical significance and ultimate delivery of the filtered xenobiotics to the target organs. Of interest are the ACE inhibitors that are widely employed because of their well-established ameliorative effect on progression of kidney damage in various renal diseases including diabetic nephropathy. The expression and activity of these transporters may be equally relevant in circumstances when other xenobiotics, e.g., cyclosporine or FK-506, are being administered in patients with diabetes that had undergone renal transplantation.

In conclusion, reduction of renal mass induces an increase in the expression of peptide transporters (influx) and Pgp (efflux) located at the brush border of renal tubular epithelial cells. In diabetic rats, the increase in the expression of these transporters is less evident; rather, it decreases to a certain extent, suggesting that in the setting of reduced renal mass the presence of hyperglycemia hinders the compensatory response of the tubular cells. At present, the mechanism(s) leading to the increased expression of Pgp, PEPT1, or PEPT2 in vivo systems is unknown. To address this issue, use of transgenic animal models with overexpression or deletion of these genes would be required, and then various mechanism(s) can be delineated following induction of the diabetic state with or without reduced renal mass. Conversely, it would be interesting to investigate the status of these transporters in states of increased renal growth, such as during embryonic and neonatal periods as a number of other organic anion or cation transporters are developmentally regulated (24, 29). Hopefully, this discussion and the data of the present study yield an impetus to such lines of investigation in the near future.

GRANTS

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REFERENCES

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