Reduced expression of organic cation transporters rOCT1 and rOCT2
in experimental diabetes

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Non-standard abbreviations: BLM, basolateral membrane; γ–GT, gamma-glutamyltranspeptidase; OCT, organic cation transporter; STZ, streptozotocin; TEA, tetraethylammonium
Abstract
Recent reports have documented a functional deficit of organic cation transport in diabetic rats by an unknown mechanism. This study was designed to test the hypothesis that experimental diabetes decreases expression of organic cation transporters at the basolateral membrane (BLM). Streptozotocin (STZ)-induced diabetic rats were maintained for varying durations after induction of diabetes. A second group of age-matched control rats were maintained in a parallel manner. Kinetic analysis of tetraethylammonium accumulation in freshly isolated proximal tubular cells indicated a significantly lower Vmax value for the diabetics versus controls with no statistical difference in Km values between the two groups. Cortex sections were processed by standard procedures for Northern and immunoblot analysis. Protein expression of the cation transporters rOCT1 and rOCT2 progressively decreased with increasing duration of diabetes. After 21 days of diabetes, rOCT1 and rOCT2 were maximally reduced by 50% and 70% respectively. Quantification of mRNA expression revealed that the roct1 transcript remained unchanged while the roct2 transcript was decreased by 50% after 14 days of diabetes. Treatment with insulin prevented the reductions in transporter levels. These results support the hypothesis by demonstrating that experimental diabetes decreased expression of both rOCT1 and rOCT2 protein and also of roct2 mRNA accumulation. On the other hand, roct1 mRNA levels were unaffected by the diabetic state. This suggests that differences in rOCT2 protein may result from transcriptional and/or translational changes while rOCT1 deficits may be due to post-transcriptional alterations.
While nephropathy is a common outcome of clinical diabetes mellitus, only recently has attention focused on the effect of the disease on organic substrate transport systems of the proximal tubule. Our laboratory has demonstrated a progressive impairment of the ability of the model organic cation tetraethylammonium to accumulate in freshly isolated proximal tubular cells (Rathi and Cacini, 1996) and renal slices prepared from STZ-induced diabetic rats (Grover et al., 2002). The renal organic cation transport system of the proximal tubule plays an important role in mediating the removal of a wide variety of toxicants and drugs from the body (see reviews, Pritchard and Miller, 1996; Inui et al., 2000). This system moves positively charged molecules from the peritubular blood to the tubular lumen for elimination in urine. The overall process is energy dependent, highly efficient and dependent upon discrete transport proteins located in the basolateral and brush border membranes of the proximal tubule.

Grundemann et al. (1994) were the first to identify a polyspecific organic cation transporter, rOCT1, in rat kidney via expressional cloning. Subsequently, numerous homologous cation transporters were identified (see reviews, Koepsell and Gorboulev, 1999; Burckhardt and Wolf, 2000). Currently, rOCT2, rOCT3, and two subtypes of rOCT1 have been cloned using hybridization techniques (Okuda et al., 1996; Kekuda et al., 1998). Comparisons of the primary sequence of the proteins reveal that rOCT2 contains 67% identity to rOCT1, whereas rOCT3 has less than 50% identity to rOCT1. While rOCT1 is found in the kidney, liver, and intestine, rOCT2 is only found in the kidney. High levels of rOCT3 are...
abundantly found in the placenta, intestine, heart, brain, but only very little in the kidney. It is now generally accepted that rOCT1 and rOCT2 mediate organic cation transport at the BLM of renal proximal tubule cells (Urakami et al., 1998; Sweet et al., 2000; Sugawara-Yokoo et al., 2000). These transporters are not uniformly distributed along the length of the proximal tubule such that rOCT1 is primarily located in the S1 and S2 segments and rOCT2 is mainly expressed in S2 and S3 segments (Karbach et al., 2000).

The current study was undertaken to test the hypothesis that the diabetes-associated functional deficits in organic cation transport are due to a decrease in the expression of rOCT1 and/or rOCT2. The results support the hypothesis. Protein expression for both transporters was significantly lower in renal cortex from diabetic rats compared to levels observed in non-diabetic control rats. While the mRNA level for roct2 was decreased in the diabetics, roct1 mRNA expression was not different from the control levels. Insulin treatment prevented the decline in the transporters.
Materials and Methods

**Induction of Diabetes.** On day 1, diabetes was induced in male Sprague-Dawley rats (225-250 g initial body weight) by injection of streptozotocin (STZ, Sigma Chemical Co., St. Louis, MO) as previously described (Sarangarajan and Cacini, 1996). Briefly, freshly dissolved STZ in a 0.05 M citrate buffer (pH 4.5) was injected into a single lateral tail vein at a dose of 65 mg/kg body wt. Nondiabetic control rats were injected with buffer alone. On day 3, diabetes was confirmed if the blood glucose level exceeded 300 mg/dl (One Touch glucometer, LifeScan Inc., Milipitas, CA) and glucosuria was evident (TesTape, Eli Lilly Co., Indianapolis, IN). All rats were maintained for varying intervals for up to 42 days with free access to food and tap water in temperature and humidity controlled quarters. Some rats were treated with NPH insulin (Novolin N, 100 U/ml, Novo Nordisk, Bagsvaerd, Denmark) after confirmation of diabetes. Insulin was administered subcutaneously beginning on day 3 of diabetes with an initial dose of 40 U/kg. Blood glucose concentrations were monitored twice daily and subsequent doses were provided as necessary. The individual doses of supplemental insulin were calculated by the method of Moglia and Phelps (1996). Animals with a plasma glucose level below 150 mg/dl were considered to be controlled diabetics.

**Isolation of proximal tubule cells.** The isolation method was originally described by Boogaard et al. (1989). Briefly, rats were anesthetized with pentobarbital (60 mg/kg body weight, ip). The abdomen was opened and the
Kidneys were initially flushed in situ via the aorta with a modified Hank-HEPES buffer of the following composition (mM): NaCl 137, KCl 5, MgSO4 0.8, Na2HPO4 0.33, KH2PO4 0.44, NaHCO3 26, HEPES 25, glucose 11.1. The kidneys were then excised as a pair, mounted in an organ bath, and subjected to collagenase (type 2, Worthington Biochemical Corporation, Freehold, NJ) perfusion followed by Accudenz gradient centrifugation. Proximal tubule cells appeared as a well-defined band at the interface between two layers of the gradient. The band was transferred to a test tube on ice using a Pasteur pipet and suspended in ice-cold Hank-HEPES buffer to an initial estimated concentration of 8-12 million cells/ml.

**Cell incubations.** Isolated cells were incubated essentially as described by Boogaard et al (1989). Typically, a 0.9 ml aliquot of the cell suspension, diluted in H-H BSA buffer to approximately 2.3 million cells/ml, was pipetted into a 25 ml Erlenmeyer flask and pre-incubated for 15 minutes. Following this, 0.1 ml of the appropriate stock solution of 14C-labeled TEA (Perkin Elmer Life Sciences, Boston, MA) was added and the cells were incubated for 2 minutes (final volume = 1 ml) at 37 degrees. The incubation was stopped by addition of 10 ml of ice-cold buffer. After rapid centrifugation, the cells were washed with an additional 10-ml of buffer, centrifuged and the supernatant was discarded. The cells were lysed with 0.5 ml of distilled water, deproteinized by addition of 0.5 ml of 10% (w/v) trichloroacetic acid and centrifuged. Radioactivity in the supernatant was quantified and the results were expressed as nmoles/mg protein.
Determination of cell number. Cell yield was determined by visual counting of an aliquot of cell suspension under light microscopy using a standard Neubauer hemacytometer (Hausser Scientific Company, Horsham, PA). The hemacytometer was charged by addition of a 100 µl aliquot, and cell counts were completed by counting a square millimeter of two different samples. The yield was expressed as the total number of cells (i.e. number of cells/ml X ml of stock).

Cell viability. An aliquot of isolated cells were suspended in HH-BSA buffer which was added to an equal volume of 0.04% Trypan blue solution. The cells were examined microscopically and considered to be viable if the dye was excluded. The viability was expressed in terms of percent of total cells (i.e., viable cells/total cells X 100 = viability). Cell preparations with viability of 85% or more were used in experiments.

Gamma-glutamyltranspeptidase (γGT) Located in the brush border, γGT has been identified as a marker enzyme for the proximal tubule cell (Neiss and Klehn, 1981). γGT activity of the freshly isolated cells was determined using a commercially available diagnostic kit (Sigma, St. Louis, MO). Activity was expressed in nmoles of product/min.
Northern blotting. At the conclusion of the experiment, the rats were sacrificed using carbon dioxide in a gas chamber. The kidneys were rapidly excised and decapsulated. A thin longitudinal slice of cortex was cut (Stadie-Riggs microtome, 0.5 mm thick) from the surface of both sides of each kidney (i.e. 4 total slices) which were flash-frozen in liquid nitrogen. Total RNA was isolated from cortical tissue using RNAzol-B (Tel-Test, Friendswood, TX). Samples (10 µg), quantified spectrophotometrically, were denatured in formaldehyde, fractionated on 1% agarose gels, and then transferred to a nitrocellulose membrane (GeneScreen Plus, DuPont, Wilmington, DE). Equality of RNA loading per lane was verified by densitometric analysis of ethidium bromide stained 18S and 28S ribosomal RNA. Plasmids containing rOCT1 and rOCT2 cDNAs were generously provided by Dr. Hermann Koepsell (Universität Würzburg, Germany) and Dr. John Pritchard (NIEHS, North Carolina, USA) respectively. Isolated rOCT1 and rOCT2 cDNAs were randomly labeled with [32P]deoxy-CTP (NEN Research, Boston, MA) using the random primer method of Feinberg and Vogelstein (1983). After transfer, stringent hybridization and wash procedures were completed according to previously published methods (Church and Gilbert, 1984). Finally, x-ray film was exposed to the membrane and developed for visualization of roct1 or roct2 mRNA.

Immunoblotting. Renal slices (described above) were homogenized in an ice-cold buffer containing 25 mM Tris-HCl (pH 7.4), 3 mM MgCl2, 0.32 M sucrose, 2 mM EGTA, 0.1 mM spermine, and 50 µg/ml each of leupeptin and aprotinin.
Following microcentrifugation (5 min) at 4\(^\circ\) C to remove insoluble cellular material, total protein was determined spectrophotometrically by a commercially available kit using bovine serum albumin as standard (Biorad Laboratories, Melville, NY). Individual protein samples (20 µg) were fractionated by 10% SDS-PAGE and electrophoretically transferred onto a polyvinylidene difluoride blotting membrane (Immobilion-P, Millipore). The blotting membrane was blocked overnight at 4\(^\circ\) C with 5% non-fat dry milk or 5% bovine serum albumin in a phosphate buffered saline. The blots were washed and then incubated for 2 h at 25\(^\circ\) C with a polyclonal primary antibody (1:1,000) of anti-rOCT1 or anti-rOCT2 (Alpha Diagnostic Int., San Antonio, TX). The bound antibody was visualized on X-ray film by chemiluminescence detection using a secondary antibody coupled to horseradish peroxidase (Pierce, Rockford, IL).

**Data Analysis.** Densitometric quantification was completed using a NucleoVision imaging workstation camera (NucleoTech Corp., San Carlos, CA) attached to an IBM compatible computer running the image analysis software, GelExpert (NucleoTech Corp., San Carlos, CA). Values were corrected for background and are expressed as either % compared to non-diabetic values for protein expression studies or relative fraction of mRNA transcript to the 18S rRNA for Northern studies. Statistical differences (p<0.05) were determined by one way analysis of variance coupled to the Student/Newman/Keuls post test multiple comparisons.
Results

**Characteristics of freshly isolated PT cells.** A typical isolation of PT cells from a pair of kidneys yielded about 3.2 X 10⁶ cells of which 90% were viable as determined by trypan blue exclusion. The characteristics of PT cells isolated from 21 day diabetic and age-matched non-diabetic rats are shown in Table 1. No statistical differences in yield, viability, γGT activity, or protein content were found between the two groups. Therefore, the uptake data for the kinetic experiments are normalized to mg protein.

**Kinetic analysis.** Concentration-dependent uptake of TEA in isolated cells from non-diabetic and 21-day diabetic animals was compared by incubating PT cells with TEA at various concentrations for 2 minutes (Figure 1). As expected, TEA uptake (nmoles/mg protein/min) in non-diabetic cells was concentration-dependent and saturable. Uptake in diabetic derived cells was also saturable, but TEA accumulation was significantly lower for all concentrations as compared to the non-diabetic derived cells. At the highest concentration (1 mM) the diabetic derived cells accumulated 57% less TEA compared to the non-diabetic cells. Saturable TEA uptake could be described with the Michaelis-Menten equation; the results are shown in Table 2. The Vmax generated by the diabetic PT cells was a significant 46% lower than that from non-diabetic cells. There was no significant difference in the apparent Km between the two cell populations.
Effect of duration of diabetes on rOCT1 and rOCT2 protein. Immunoblot analysis of OCT1 and OCT2 was conducted to determine the effect of diabetes on organic cation transporter protein levels. The results are summarized in Figure 2A. An approximate 66 kDa and 76 kDa protein was detected for rOCT1 and rOCT2, respectively, which is consistent with previous reports (Urakami et al., 1998, 1999, 2000). A decrease in both proteins was observed in samples obtained from rats after 14, 21 and 42 days of diabetes compared to the non-diabetics. Densitometric analysis indicated that rOCT1 protein was decreased by 45% after 14 days and rOCT2 protein was decreased by a 53% after 7 days of diabetes (Figure 2B). The maximal decrease for both proteins was observed at 21 days where rOCT1 and rOCT2 were approximately 50% and 70%, respectively, lower than that observed in the non-diabetics.

Effect of duration of diabetes on roct1 and roct2 expression. To determine whether changes in transport protein were due to diabetes-related alterations of gene expression, roct1 and roct2 expression was examined in rats with increasing duration of diabetes. Transcripts of 1.9 kb and 2.2 kb roct1 and roct2 respectively were detected (Figure 3A). Expression of roct1 was not statistical different in diabetics as compared to rats in the non-diabetic group (Figure 3A and B). On the other hand, roct2 was significantly decreased within 14 days of diabetes and maximally decreased by about 50% after 21 days.
Effect of Insulin treatment. Based on these results, an experiment was conducted to test whether early insulin treatment could prevent the observed changes in transporter expression. Rats were assigned to the following groups: 1) non-diabetic, 2) untreated diabetic, and 3) diabetic + insulin. Figure 4 shows blood glucose concentrations determined over the 21-day experimental period. Values for the untreated diabetic rats remained above 300 mg/dl while those in the insulin-treated diabetics were significantly lower and, in fact, fluctuated around the non-diabetic group values. The effect of insulin treatment on the expression of the transport proteins on day 21 is shown in Figure 5. As demonstrated in Figure 2A, a decrease in both proteins occurred after 21 days of diabetes as compared to the non-diabetics. Densitometric results shown in Figure 5B, revealed that rOCT1 protein in the insulin-treated rats was significantly greater (69% of non-diabetic rat value) compared to that determined in the untreated diabetic rats (41% of non-diabetic rats). The rOCT2 protein level was not significantly different from non-diabetic rats. The effect of insulin treatment on roct1 and oct2 mRNA expression is shown in Figure 6. There were no significant differences in expression of roct1 mRNA among the 3 groups. On the other hand, insulin treatment significantly attenuated the diabetes-related decrease in roct2 mRNA expression.
Discussion

The importance of a functional cation transport system for efficient renal excretion of therapeutic and toxic substances is well documented. In this study, we demonstrated that STZ-induced diabetes decreased basolateral organic cation transporter expression (Figure 2). The decline was rapid with rOCT2 expression decreased by more than 50% within 7 days following induction of diabetes and that for rOCT1 by 14 days. These results coincide with the functional decline pattern for TEA uptake in cortex slices derived from STZ diabetic rat kidneys that were recently reported by Grover et al. (2002). In this study, decreased TEA accumulation in rat renal cortex slices was evident after 7 days of STZ-induced diabetes and was attenuated by 14 days with the maximal decline detected by 21 days. These results clearly suggest a mechanistic link between function and rOCT expression. Our kinetic data also support this conclusion. However some comments on the limitations of the kinetic data are in order. In this initial report of the effect of diabetes on rOCT and roct levels we felt that our kinetic experiments should be compared to reports from investigators who used a kinetic approach in normal rats that included the isolation and incubation techniques that we used. Under these conditions, Boom et al. (1992) reported that TEA uptake into PT cells from normal rats was linear during the first 4-5 minutes of incubation. An earlier report by Boogaard et al. (1989) had shown the same linearity limit for N1-methylnicotinamide. Accordingly, we applied their 2-minute time for comparison reasons. The relatively long period may have allowed an unknown level of outflux of the TEA. While this may have been a
source of error, nevertheless, the Vmax and k values for TEA in our non-diabetic rats are similar to those reported by Boom et al. (1992).

Northern blot analysis showed that roct2 mRNA levels declined in a manner corresponding to the protein expression results (Figure 3). This suggests that diabetes alters transcription of rOCT2. In contrast, roct1 mRNA levels were unchanged regardless of the duration of diabetes, an unexpected result that did not correspond with the apparent decline of rOCT1 protein. This suggests post-transcriptional alteration of rOCT1 may occur in diabetes. While the mechanism of this is unclear, a logical progression would implicate non-enzymatic glycation, of proteins, a leading mechanistic hypothesis for development of diabetic end-organ damage (Brownlee, 1995).

Hyperglycemia promotes excessive attachment of glucose to amino acids (primarily lysine) to extracellular and intracellular proteins resulting in an initial reversible Schiff base adduct which can ultimately become an irreversible advanced glycation end product (AGEP). The resulting residues presumably change the structure and/or functionality of the affected proteins. The monitoring of degree of glycation of hemoglobin (in this case, a model intracellular protein) is commonly measured in diabetic patients on the assumption that this provides a value that predicts diabetic complications. The glycation process can be reasonably fast. We have documented a near doubling of glycation of hemoglobin within 7 days after STZ injection in rats (Grover et al., 2002). While rOCT1 protein may actually be decreased despite an unchanged mRNA level, at this point, we cannot eliminate the possibility that the lowered protein expression
may have been due to an inability of the anti-OCT1 antibody to recognize the excessively glycated protein.

While this is the first demonstration of down regulation of organic cation transporters in a diabetes model, adaptations of the facilitative glucose transporters (GLUTs) have been demonstrated in STZ-induced diabetes in both functional and molecular studies (Dominguez et al., 1994; Chin et al., 1997; Noonan et al., 2001). Goralski et al. (2001) reported enhanced transport of the antiviral drug amantadine after 4 days of STZ-induced diabetes via a bicarbonate-dependent transporter the nature of which is yet to be defined. These, together with the present study, suggest that experimental diabetes is associated with significant alterations of organic substrate transporter levels. At present, little is known about regulation of this transport system (for review see Berkhin and Humphreys, 2001). The primary structure of the OCT transport proteins includes potential phosphorylation sites for protein kinase C (PKC) and protein kinase A (PKA). Mehrens et al. (2000) demonstrated enhanced transport subsequent to activation of tyrosine kinases. They further provided evidence that PKC phosphorylates rOCT1 and suggested that this may be responsible for altering the substrate affinity of the transport protein. Other mechanisms which regulate transcription and/or translation may be involved in experimental diabetes. Working within the diabetic model, insulin is an obvious potential candidate for altering transcription or translation. This hormone is well known to affect both processes in multiple cell types (Kimball et al., 1994; O’Brien and Granner, 1996). Indeed, when exogenous insulin was administered in this study,
normal levels of rOCT protein and mRNA were maintained (Figures 5 and 6). This result argues against the likelihood that the decrease of transporter expression was due an acute toxic action of STZ or an artifact of the experimental design. Therefore, it is reasonable to conclude that the observed changes documented in this study can be traced to the diabetic state itself.

More than thirty years ago Bowman and Hook (1972) observed that testosterone affected the functional transport of TEA in renal cortical slices. The mechanism remains unresolved. However, recent molecular studies by Urakami, et al. (1999; 2000) indicate that testosterone increased expression of rOCT2 protein and mRNA in normal rats. Slitt et al. (2001) have demonstrated that male rats express four times the amount of roct2 mRNA in kidney compared to that from females and showed that gonadectomy in males resulted in mRNA expression approximating the significantly lower level found in females. All rats in the current study were males. These observations suggest a regulatory role for androgens in the maintenance of rOCT transporters. Studies utilizing an experimental diabetes model may prove useful in light of the fact that male diabetic rats are deficient not only insulin but also of testosterone (Ikeda et al, 2000; Tanaka et al, 2001). Furthermore, the activities of these two hormones may be intertwined. Morimoto et al. (2001) demonstrated that testosterone has a direct positive effect on insulin gene expression in pancreatic beta cells. Clearly, more research will be needed to define the roles of hormones in the regulation of organic cation transporters.

While the primary structural characteristics of the typical rOCT substrate
includes a hydrophobic backbone and a positive charge, cisplatin, an inorganic nephrotoxic anticancer drug, was recently identified as an atypical substrate that accumulates in the S3 segment of rat proximal tubule (Pan et al., 1999; Endo et al., 2000). This is of interest because the significant decrease in rOCT2 expression at the protein level demonstrated in the present study provides a novel mechanism for the remarkable “protection” afforded to STZ-diabetic rat kidneys against cisplatin-induced nephrotoxicity. (Scott et al., 1989). It is clear that this protection is related to less accumulation of the toxicant versus levels seen in non-diabetics (Sarangarajan and Cacini, 1996,1997). A deficit of rOCT2 expression in the basolateral membrane could, at least in part, explain these observations.

In conclusion, this study has provided evidence that previously reported impaired organic cation transport at the BLM in experimental diabetes is associated with decreased rOCT1 and rOCT2 protein. The mechanism mediating the protein insufficiency appears to reside post-transcriptionally for rOCT1, whereas differences in rOCT2 protein may result from transcriptional and/or translational changes.
References


with special reference to the chemodifferentiation of the proximal tubule.


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Figure Legends

**Figure 1** Concentration-dependent TEA uptake in proximal tubule cells isolated from 21-day STZ-diabetic and age-matched non-diabetic rats. Concentration-dependent TEA uptake (0.025-1.0 mM) in PT cells was completed in the presence and absence of 5 mM quinine to determine nonspecific uptake. Cell uptake values represent the mean ± SE of five different isolations where cell incubation time was 2 minutes. TEA uptake is expressed as nmoles/mg protein/min. The solid lines represent the Michaelis-Menten fit in combination with linear diffusion and the dashed lines represent uptake by diffusion.

**Figure 2** Effect of duration of diabetes on the BLM organic cation transport proteins, rOCT1 and rOCT2. (A) Representative individual immunoblots of rOCT1 and rOCT2 are shown. Each lane represents a single animal and was loaded with 20 µg of total protein from a single slice. (B) Quantitative analysis of immunoblots was completed by densitometry of two separate experiments. Each value represents the mean ± SE of 4 animals where non-diabetic values were set to 100% for comparison of diabetic values. An asterisk indicates a significant difference (p<0.05) from the non-diabetic value.

**Figure 3** Effect of duration of diabetes on roct1 and roct2 mRNA. (A) Individual Northern Blots of rOCT1 and rOCT2 are shown. Each lane represents a single animal and was loaded with 10 µg of total RNA from a single slice. The ribosomal 28S and 18S units are shown for comparison of actual total RNA
loaded per well. (B) Densitometric analysis of oct1 and roct2. Each value represents the relative fraction of roct1 or roct2 to 18S and is reported as the mean ± SE. * indicates significant difference (p<0.05) from the non-diabetic value.

**Figure 4** Blood glucose concentrations for the insulin study. Three groups were monitored for 21 days: 1) non-diabetic (squares), 2) diabetic (circles) and 3) diabetic + insulin (triangles). Each value represents the mean ± SE of 4 animals.

**Figure 5** Effect of insulin treated diabetic animals on the transport proteins, rOCT1 and rOCT2. (A) Immunoblots of rOCT1 and rOCT2 are shown. Each lane represents a single animal and was loaded with 20 µg of total protein from a single slice. (B) Quantitative analysis of immunoblots. Each value represents the mean ± SE. Non-diabetic values were set to 100% for comparison of diabetic values. An asterisk indicates significant difference (p<0.05) from the non-diabetic value. † indicates significant difference (p<0.05) from the diabetic value.

**Figure 6** Effect of insulin treated diabetic animals on rOCT1 and rOCT2 mRNA. (A) Individual Northern blots of rOCT1 and rOCT2 are shown. Each lane represents a single animal and was loaded with 10 µg of total RNA from a single slice. The 28S and 18S ribosomal units are shown for comparison of actual total RNA loaded per well. (B) Densitometric analysis of rOCT1 and rOCT2. Each value represents the relative fraction of rOCT1 or rOCT2 to 18S and is reported
as the mean ± SE. An asterisk indicates significant difference (p<0.05) from the non-diabetic value.
Table 1.
Comparison of freshly isolated proximal tubule cells from 21-day STZ-diabetic and age-matched non-diabetic rats

<table>
<thead>
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<th></th>
<th>Yield</th>
<th>% Viability</th>
<th>GGT activity</th>
<th>Protein</th>
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<tr>
<td></td>
<td>(10^6 cells)</td>
<td>(dye exclusion)</td>
<td>(nmoles/min)</td>
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<td>Diabetic</td>
<td>32.7±3.9</td>
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<td>Non-diabetic</td>
<td>32.6±3.7</td>
<td>90.4±1.1</td>
<td>251±5</td>
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</table>

Values are the mean ± SE of 15 cell isolation experiments.

GGT = γ glutamyltranspeptidase, a proximal tubular cell marker enzyme. There were no significant differences between diabetic and non-diabetic rat cells for any of the 4 values.
Table 2

Kinetic analysis of TEA uptake in freshly isolated proximal tubule cells from 21-day diabetic and non-diabetic rats

<table>
<thead>
<tr>
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<th>$V_{\text{max}}$ (nmoles/mg protein/min)</th>
<th>$K_m$ (µM)</th>
<th>$k$ (ml/mg protein/min)</th>
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<tr>
<td>Diabetic</td>
<td>0.64 ± 0.15*</td>
<td>422 ± 61</td>
<td>0.20 ± 0.03</td>
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<tr>
<td>Non-diabetic</td>
<td>1.17 ± 0.04</td>
<td>288 ± 39</td>
<td>0.33 ± 0.08</td>
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</table>

Each value represents the mean ± from five separate cell isolations. Statistical significance(*) was assessed by a Student’s test ($p< 0.05$). $k = \text{slope of the individual diffusion lines}$
FIGURE 2

(A) Duration of Diabetes (Days)

<table>
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<tr>
<td>rOCT1 66 kDa</td>
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<tr>
<td>rOCT2 77 kDa</td>
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(B) rOCT1 Protein

Duration of Diabetes (Days)

% Compared to Non-diabetic

Non-diabetic | 7 | 14 | 21 | 42 |
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rOCT2 Protein

Duration of Diabetes (Days)

% Compared to Non-diabetic

Non-diabetic | 7 | 14 | 21 | 42 |
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FIGURE 4

Blood Glucose (mg%) vs. Time (Days)

-1  3  7  11  15  19  23
Figure 5

(A) Western blots showing the expression of rOCT1 and rOCT2 proteins in non-diabetic, diabetic (21 days), and insulin-treated conditions. 

(B) Bar graphs showing the percentage of rOCT1 and rOCT2 proteins compared to non-diabetic conditions. The graphs indicate significant differences (*p < 0.05) and trends (†p < 0.1).
### Figure 6a

<table>
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<tr>
<td><strong>rOCT1</strong></td>
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<td>1.9 kb</td>
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<td>18S</td>
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(B)  

**rOCT1 mRNA**

- Non-diabetic
- Diabetic
- Insulin

**rOCT2 mRNA**

- Non-diabetic
- Diabetic
- Insulin

Figure 6B