Renal type I inositol 1,4,5-trisphosphate receptor is reduced in streptozotocin-induced diabetic rats and mice

KUMAR SHARMA,1 LEWEI WANG,1 YANQING ZHU,1 AURORA DEGUZMAN,1 GAO-YUAN CAO,2 RICHARD B. LYNN,2 AND SURESH K. JOSEPH3

1Nephrology Division and 2Gastroenterology and Hepatology Division of the Department of Medicine and 3Department of Anatomy, Pathology, and Cell Biology, Thomas Jefferson University School of Medicine, Philadelphia, Pennsylvania 19107

Sharma, Kumar, Lewei Wang, Yanqing Zhu, Aurora DeGuzman, Gao-Yuan Cao, Richard B. Lynn, and Suresh K. Joseph. Renal type I inositol 1,4,5-trisphosphate receptor is reduced in streptozotocin-induced diabetic rats and mice. Am. J. Physiol. 276 (Renal Physiol. 45): F54–F61, 1999.—The mechanisms underlying glomerular hypertrophy and hyperfiltration in diabetes remain unclear. We have previously demonstrated that the cytokine transforming growth factor-β1 (TGF-β1) is increased in early diabetic kidney disease and TGF-β1 inhibits the expression of the inositol 1,4,5-trisphosphate (IP₃)–gated calcium channel, the type I IP₃ receptor (IP₃R), in mesangial cells. To test the hypothesis that reduced type I IP₃R may be important in diabetic kidney disease, we evaluated type I IP₃R expression in the kidney of streptozotocin-induced diabetic rats and mice. Two-week-old diabetic rats have decreased renal type I IP₃R protein and mRNA levels. Immunostaining of normal rat kidney demonstrated presence of type I IP₃R in glomerular and vascular smooth muscle cells, whereas diabetic rats had reduced staining in both compartments. Reduction of type I IP₃R also occurred in parallel with renal hypertrophy, increased creatinine clearance, and increased renal TGF-β1 expression in the diabetic rats. Two-week-old diabetic mice also had reduced renal type I IP₃R protein and mRNA expression in association with renal hypertension and increased TGF-β1 mRNA expression. These findings demonstrate that there is reduced type I IP₃R in glomerular and vascular smooth muscle cells in the diabetic kidney, which may contribute to the altered renal vasoregulation and renal hypertrophy of diabetes.

Diabetes characteristically induces glomerular hyperfiltration in experimental animals and in patients. Hyperfiltration likely occurs as a result of enhanced glomerular blood flow due to afferent arteriolar vasodilatation, increased filtration surface area from mesangial cell relaxation, and enhanced glomerular capillary pressure due to relative efferent arteriolar vasoconstriction (10). Despite intensive investigation, it remains unclear how diabetes induces afferent arteriolar and mesangial cell relaxation (20). The glomerulus from diabetic animals exhibits decreased contraction to vasoconstrictors (12) that is likely due to reduced responsiveness of the mesangial cell to various vasoconstrictors, such as ANG II, endothelin, vasopressin, and norepinephrine (9, 16, 26). Although reduction of surface receptors may play a role (3), it remains possible that diabetes induces effects on common intracellular signaling pathways that may alter response to a variety of vasoconstricting agonists.

It is widely accepted that vasoconstrictors such as ANG II, endothelin, norepinephrine, and vasopressin all raise free intracellular calcium ([Ca²⁺]i) to promote contraction of the cell (4). The observation that mesangial cells from diabetic rat glomeruli, as well as human and rat mesangial cells cultured in high glucose, exhibit an impaired [Ca²⁺]i increase in response to these vasoconstrictors (9, 16) is consistent with the argument that an intracellular alteration may be responsible. It is interesting that apart from high glucose, exposure of several cell types to transforming growth factor-β (TGF-β) also impairs agonist-induced [Ca²⁺]i. Pretreatment of vascular smooth muscle cells with TGF-β1 (35) inhibits ANG II–induced [Ca²⁺]i release, and in our study with mesangial cells, pretreatment with TGF-β1 inhibited platelet-derived growth factor (PDGF)-induced [Ca²⁺]i (2). A common pathway of raising [Ca²⁺]i with PDGF and ANG II is the generation of inositol 1,4,5-trisphosphate (IP₃) from phosphatidylinositol 4,5-bisphosphate. The increased intracellular levels of IP₃ bind to IP₃ receptors (IP₃R) in the endoplasmic reticulum to release stored [Ca²⁺] into the cytoplasmic space (4). Of the various IP₃Rs identified (types I, II, and III), the types I and III IP₃Rs appear to be the predominant isoforms expressed in glomerular mesangial and vascular smooth muscle cells (17, 32). Recently, we found that TGF-β1 inhibits the expression of the type I IP₃R in rat and mouse mesangial cells (24). It is clear that diabetes leads to overexpression of TGF-β1 in the kidney of streptozotocin (STZ)-induced diabetes mellitus in the rat and mouse (18, 21, 23, 25, 31). Therefore, it is possible that, in these models of diabetic kidney disease that are associated with overexpression of TGF-β, there would be consequent downregulation of the type I IP₃R. Reduction of IP₃R expression may contribute to impaired [Ca²⁺]i mobilization to vasoconstrictors and diabetes-related alterations in mesangial cell contraction. In the present study, we demonstrate that expression of the type I IP₃R is downregulated in two models of diabetic kidney disease and associated with enhanced renal TGF-β1 expression.

Materials and methods

Animals. Sprague-Dawley rats weighing 220–260 g were made diabetic by a single intraperitoneal injection of STZ (65 mg/kg body wt; Sigma, St. Louis, MO) in 10 mmol/l sodium citrate, pH 5.5. Controls were injected with buffer alone. The levels of blood glucose were determined 2 days after injection, and rats with blood glucose >16 mmol/l were used as diabetic.
The diabetic rats were divided into two groups of six animals each. One group received daily subcutaneous insulin of 2.0 U of NPH insulin (Eli Lilly, Indianapolis, IN) to maintain hyperglycemia but avoid ketosis. The other diabetic group received daily high-dose insulin (7–10 U) to maintain relative euglycemia (~15 mmol/l). Diabetic and nondiabetic rats were given standard rat diet and water ad libitum. After 14 days of diabetes, rats were placed in metabolic cages for 24-h urine collection for creatinine clearance, as measured by a colorimetric assay (Sigma). At the end of the experiment, blood was collected, the right kidney was weighed, and the kidney cortex was excised and stored at –70°C. After removal of the right kidney, the left kidney was perfused with PBS and formaldehyde, removed, snap-frozen in liquid nitrogen, and stored at –70°C.

C57Bl mice were fed a standard pellet laboratory diet and provided with water ad libitum. Diabetes was induced in 7- to 8-wk-old mice (17–21 g) by two consecutive daily intraperitoneal injections of STZ (200 mg/kg). On the same day that glucosuria was noted, 0.5 U NPH insulin was administered to allow for hyperglycemia (~20 mmol/l) and to prevent ketonuria. Following 2 wk of diabetes, mice were killed, and kidneys were weighed and snap-frozen in liquid nitrogen and stored at –70°C for Western and Northern analysis (see below).

Immunohistochemistry. The left kidney of the rats in each group was perfused with PBS and formaldehyde in vivo prior to extraction and postfixed in PBS-formaldehyde for 1 h prior to storage at –20°C. Five-micron sections from kidney blocks were obtained by microtome, placed on poly-l-lysine-plated glass slides, and incubated in 0.3% H2O2 in methanol for 30 min to block endogenous peroxidase prior to immunostaining. The primary antibody against the type I IP3R that was used for immunostaining was obtained from Affinity Bioreagents (Golden, CO) and used at a dilution of 1:500. Sections were incubated with primary antibody in Tris-buffered saline (TBS) for 16 h at 4°C. The sections were washed in TBS and incubated with a goat biotin-conjugated anti-rabbit IgG serum (Vector Laboratories, Burlingame, CA). The avidin biotin-peroxidase complex technique using diaminobenzidine (Vectorstain Elite ABC kit, Vector) was then employed. Negative controls were performed with the absence of primary antibody and revealed no background staining. Slides were viewed on a Laborlux 5 microscope, and photographs were taken of representative sections. Semiquantitative assessment of immunostaining intensity was scored on a scale of 0–3 from 15 glomeruli, separately from their accompanying intertubular regions, and mRNA levels were calculated relative to those of β-actin.

Northern analysis. Total RNA was isolated from rat and mouse kidney using acid guanidinium thiocyanate-phenol-chloroform (7). Poly(A)+ mRNA was isolated from total RNA by oligo(dT) affinity column (Promega, Madison, WI). Three micrograms of poly(A)+ mRNA was loaded onto a 1.2% agarose gel containing 2.2 M formaldehyde, electrophoresed, and transferred onto nylon membrane by capillary blotting, then ultraviolet cross-linked. Prehybridization was performed for 1 h at 65°C with a buffer containing 10% dextran sulfate, 1 M NaCl, and 1% SDS. DNA probes were labeled with 10 µCi [32P]dCTP (3,000 Ci/mmol; Du Pont-New England Nuclear, Boston, MA) via the random primer method (DNA labeling kit; Boehringer-Mannheim, Indianapolis, IN) and added to prehybridization fluid. The hybridization was performed at 65°C overnight after the addition of labeled DNA probe. The filters were washed twice at 65°C for 30 min in a solution containing 1 mM EDTA, 40 mM Na2PO4, pH 7.2, and 5% SDS and then in a solution of 1 mM EDTA, 40 mM Na2PO4, pH 7.2, and 1% SDS. The filters were exposed at –80°C for 1–3 days to Kodak XAR film.

The probes for the type I IP3R and TGF-β1 were synthesized by the PCR using specific oligonucleotide primers, based on the published cDNA sequence and murine kidney cDNA as template as previously described (24, 25). A CDNA probe for rat kidney type I IP3R was similarly prepared by PCR using rat kidney cDNA as template. Nucleotide sequencing of the PCR products confirmed the identity of the probes. To standardize for loading, membranes were stripped and reprobed with a β-actin cDNA probe (kindly provided by Dr. Pamela A Norton). Densitometry was performed as described above, and mRNA levels were calculated relative to those of β-actin.

Statistical analyses. Results are expressed as means ± SE. One-way ANOVA was used to test for differences between two groups and analyzed by Student's unpaired t-test. Bonferroni's correction was applied for comparisons between three groups. The variability within the groups was random. P < 0.05 was considered significant.

RESULTS

Metabolic parameters and kidney hypertrophy in the STZ-induced diabetic rats. Table 1 shows the body weight, kidney weight, blood glucose, and creatinine clearance measured in normal rats and diabetic rats at the end of the experimental period. Diabetic rats treated with a low dose of insulin (2 U of NPH) had significant hyperglycemia (23.3 ± 1.4 mmol/l), whereas diabetic rats treated with high-dose insulin (7–10 U) had mild hyperglycemia (13.2 ± 1.9 mmol/l) that was not significantly different from control values (8.9 ± 0.7 mmol/l).

<table>
<thead>
<tr>
<th>Group</th>
<th>Body Wt. g</th>
<th>Kidney Wt. g</th>
<th>Blood Glucose, mmol/l</th>
<th>Creatinine Clearance, ml/min</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal rats</td>
<td>407 ± 26</td>
<td>1.30 ± 0.06</td>
<td>8.9 ± 0.7</td>
<td>0.93 ± 0.17</td>
</tr>
<tr>
<td>Hyperglycemic diabetic rats</td>
<td>331 ± 20*</td>
<td>1.67 ± 0.02*</td>
<td>23.3 ± 1.4*</td>
<td>1.70 ± 0.29*</td>
</tr>
<tr>
<td>Diabetic rats (high-dose insulin)</td>
<td>378 ± 34</td>
<td>1.43 ± 0.13</td>
<td>13.2 ± 1.9</td>
<td>1.23 ± 0.08</td>
</tr>
</tbody>
</table>

Values are means ± SE of all rats in each group. *P < 0.05 vs. normal rat group.
Body mass of hyperglycemic diabetic rats was significantly lower compared with control. However, absolute kidney weight was significantly increased by 28% and creatinine clearance was increased by 82% in hyperglycemic diabetic rats compared with normal rats. All of the above parameters were attenuated in diabetic rats treated with high-dose insulin and not significantly different compared with control rats.

Distribution of type I IP₃R in normal and diabetic kidney. The expression and distribution of type I IP₃R protein in the kidney was evaluated by immunoperoxidase staining using a specific rabbit anti-type I IP₃R antibody. Immunohistochemistry from the normal rat kidney demonstrates presence of type I IP₃R primarily in glomerular cells and in arteriolar smooth muscle cells (Fig. 1A). Type I IP₃R staining in cortical tubular cells was less prominent than in glomerular and vascular cells. In contrast to the normal rat kidney, the diabetic rat kidney demonstrates reduced immunostaining for the type I IP₃R in glomerular cells and vascular smooth muscle cells (Fig. 1B). The diabetic rats treated with high-dose insulin had a pattern of immunostaining of the type I IP₃R similar to that of the control group (Fig. 1C). The results of glomerular and arteriolar immunostaining intensity scores for type I IP₃R of all the animals in each of the three groups are shown in Fig. 2.

Type I IP₃R protein in kidney cortex of normal and diabetic rats. Figure 3 shows a representative immunoblot of rat cerebellum and rat kidney cortex protein with an antibody raised against the COOH-terminal end of the rat type I IP₃R (13). A prominent ~240-kDa band was observed in rat kidney cortex from normal and diabetic rats that corresponds to the known size of the type I IP₃R from rat cerebellum (Fig. 3A). Densitometric analysis of all samples in each of the three groups for type I IP₃R expression is shown in Fig. 2.

Fig. 1. Type I inositol 1,4,5-trisphosphate (IP₃) receptor (IP₃R) is reduced in diabetic glomeruli and vascular smooth muscle cells. A: normal rat kidney (control); note distinct staining for type I IP₃R in glomerulus and vascular arterioles (see arrow). B: diabetic rat kidney; much reduced staining for type I IP₃R is present in glomerular cells and vascular smooth muscle cells (see arrow). C: kidney from diabetic rat treated with high-dose insulin; note similar intensity of immunostaining in glomerular and arteriolar cells compared with control (A).
groups revealed that diabetic rats with hyperglycemia have significantly decreased type I IP₃R expression compared with control rats (29±6% of control values, P<0.04; Fig. 3B). Decreased expression of type I IP₃R in diabetic kidney cortex was largely prevented with high-dose insulin treatment and tight control of blood glucose (81±35% of control values, P not significant).

Northern analysis of type I IP₃R and TGF-β1 expression in diabetic rat kidneys. A representative hybridization of poly(A)⁺ mRNA of rat kidney cortex from the normal, diabetic, and diabetic group treated with high-dose insulin probed for the type I IP₃R is shown in Fig. 4. The diabetic rat kidney has decreased expression of type I IP₃R mRNA compared with the normal rat kidney. In addition, reduction of type I IP₃R mRNA in the diabetic kidneys is associated with enhanced expression of TGF-β1 mRNA. Densitometric analysis of pooled samples in the diabetic group demonstrates a reduction of type I IP₃R mRNA (standardized for β-actin) of 27±5% of control values (P=0.01) (Fig. 4B), whereas TGF-β1 mRNA is increased by 196±26% of control value (P=0.03) (Fig. 4C). Treatment of diabetic rats with high-dose insulin prevented changes in type I IP₃R and TGF-β1 mRNA expression compared with control.

Metabolic parameters and kidney hypertrophy in the STZ-induced diabetic mouse. Table 2 shows the body weight, kidney weight, and blood glucose from normal and STZ-induced diabetic mice after 2 wk of diabetes. Diabetic mice demonstrated significant hyperglycemia and kidney hypertrophy despite a tendency toward reduced body weight compared with the normal group.

Renal type I IP₃R protein and mRNA expression in diabetic mice. Type I IP₃R expression in the kidneys from normal and diabetic mice was analyzed by immunoblot and Northern analysis. A representative immunoblot analysis of kidney protein with antibody to the type I IP₃R demonstrated decreased type I IP₃R protein in the diabetic kidney compared with normal mouse kidney (Fig. 5). Pooled analysis of all samples revealed that renal expression of type I IP₃R protein in the diabetic mice was reduced to 26±6% of control values (P=0.01) (Fig. 5B). This is a reduction of type I IP₃R protein similar to that noted in the kidneys from hyperglycemic diabetic rats.

Hybridization of poly(A)⁺ mRNA from mouse kidneys with the type I IP₃R cDNA probe revealed a band for type I IP₃R mRNA of the same size as that noted from mouse cerebellum (Fig. 6). The diabetic mouse kidney has less expression of type I IP₃R mRNA compared with the normal mouse kidney. A representative hybridization of poly(A)⁺ mRNA from mouse kidneys from the normal and diabetic groups with the type I IP₃R, TGF-β1 and β-actin cDNA probes is shown in Fig. 7. Similar to the diabetic rats, the kidneys from diabetic mice demonstrate reduction of type I IP₃R mRNA in

![Fig. 2. Semiquantification of glomerular and arteriolar immunostaining for type I IP₃R. Intensity of immunostaining for type I IP₃R was scored on a scale of 0 (negative staining) to 3 (maximal staining) on kidney sections from all rats in each of the three groups (n=6 per group). N, normal rats; D, diabetic rats treated with low-dose insulin; D+I, diabetic rats treated with high-dose insulin; Glom, glomeruli; Vessels, glomerular arteriolar vessels. Data shown are means ± SE of staining scores from each of the 3 groups (n=6 per group). * P<0.05 vs. control.](http://ajprenal.physiology.org/)

![Fig. 3. Reduction of type I IP₃R protein expression in diabetic rat kidney. A: samples (50 µg protein/lane) of kidney cortical protein from 2 separate normal rats (lanes 2 and 3), hyperglycemic diabetic rats treated with low-dose insulin (lanes 4 and 5), and diabetic rats treated with high-dose insulin (lanes 6 and 7) were resolved on 7% SDS-PAGE, transferred to nitrocellulose, and probed with type I IP₃R antibody. The ~240-kDa band migrated to the same position on the gel as the type I IP₃R from rat cerebellum (lane 1). B: densitometric quantitation of immunoreactive protein expressed relative to control. Data are means ± SE of band intensities from each of the 3 groups (n=6 per group). * P<0.05 vs. control.](http://ajprenal.physiology.org/)
association with enhanced expression of TGF-β1 mRNA. Densitometric analysis of pooled samples demonstrates a reduction of type I IP₃R mRNA (standardized for β-actin) of 51.6 ± 8.6% of control values (P = 0.006) (Fig. 7B), whereas TGF-β1 mRNA is increased by 194 ± 12% of control value (P = 0.05) (Fig. 7C).

DISCUSSION

The present results indicate that renal expression of type I IP₃R is reduced in two animal models of early STZ-induced diabetes. Immunostaining of diabetic rat kidneys demonstrates that the reduced expression of type I IP₃R is prominent in arteriolar smooth muscle cells and glomerular cells. In STZ-induced diabetic rats, there is an association of reduced renal type I IP₃R expression with diabetic renal hypertrophy and diabetic renal hyperfiltration, as measured by creatinine clearance. In both models of STZ-induced diabetic rats and mice, reduction of type I IP₃R mRNA expression is associated with increased renal TGF-β1 mRNA expression suggesting a possible relationship between these processes.

Table 2. Renal hypertrophy in diabetic mice

<table>
<thead>
<tr>
<th></th>
<th>n</th>
<th>Body Wt, g</th>
<th>Kidney Wt, g</th>
<th>Blood Glucose, mmol/l</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>5</td>
<td>21.3 ± 1.6</td>
<td>140 ± 10</td>
<td>8.1 ± 0.6</td>
</tr>
<tr>
<td>Diabetic</td>
<td>5</td>
<td>19.8 ± 1.3</td>
<td>170 ± 21*</td>
<td>28.3 ± 0.9*</td>
</tr>
</tbody>
</table>

Values are means ± SE of all mice in each group. *P < 0.05 vs. normal mice group.

Fig. 4. Reduced expression of type I IP₃R mRNA occurs in association with enhanced transforming growth factor-β1 (TGF-β1) mRNA in diabetic rat kidney. A: top: representative autoradiograph of a Northern blot of poly(A)⁺ mRNA isolated from kidney cortex from normal (N), diabetic (D), and diabetic rat treated with high-dose insulin (D + I) probed with rat type I IP₃R. The same blot was probed with TGF-β1 cDNA (A, middle) and was finally probed with β-actin cDNA (A, bottom) as a control for loading. B: densitometric quantitation of renal type I IP₃R mRNA/β-actin mRNA from each of the 3 groups. C: densitometric quantitation of renal TGF-β1 mRNA/β-actin mRNA from each of the 3 groups. Data are means ± SE of band intensities of pooled data from all samples in each of the 3 groups (n = 6 per group). *P < 0.05 vs. normal group.

Fig. 5. Reduction of type I IP₃R protein expression in diabetic mouse kidney. A: representative immunoblot analysis of samples (50 μg protein/lane) from normal (NL) mouse kidney and diabetic mouse kidney were resolved on 7% SDS-PAGE, transferred to nitrocellulose, and probed with type I IP₃R antibody. B: quantitation of immunoreactive protein expressed in diabetic kidney (D) relative to the control (N). Data are means ± SE of band intensities from all samples in each of the groups (n = 5 per group). *P < 0.05 vs. normal group.
The IP$_3$R family has previously been evaluated in mouse and rat kidney. Northern analysis of various mouse tissues revealed that mouse kidney had the strongest expression of type I IP$_3$R mRNA outside the brain (8). In situ hybridization of mouse kidney demonstrated type I IP$_3$R expression primarily in vascular structures (8). Immunostaining of mouse kidney with an antibody raised against the IP$_3$R from cerebellum showed expression of IP$_3$R primarily in glomerular mesangial cells (19) and light immunostaining in tubular epithelial cells (6). Immunostaining of normal rat kidneys with monoclonal antibodies specific for each of the IP$_3$R isoforms revealed expression of only the type I and type III IP$_3$R isoforms in glomerular mesangial cells and glomerular arteriolar structures (17). Microdissection of rat nephron segments and subsequent RT-PCR with primers specific for the type I and II IP$_3$R demonstrated that glomeruli only express type I IP$_3$R, whereas distal tubular cells express both types I and II IP$_3$R (32). RT-PCR analysis of Madin-Darby canine kidney cells, a model of polarized tubular epithelial cells, revealed expression of all three types of IP$_3$R (6) with the type III isoform appearing to be the most predominant. On the basis of the above results, it appears that the type I IP$_3$R is present predominantly in glomerular mesangial cells and glomerular arterioles. Our recent study in murine and rat mesangial cells in culture demonstrated expression of type I IP$_3$R protein and mRNA (24). Our present demonstration of immunostaining for type I IP$_3$R in rat kidney glomerular cells and smooth muscle cells corresponds to similar findings recently reported in rat kidney (17). Expression of type I IP$_3$R mRNA in rodent kidneys confirms prior findings by Furuichi et al. (8).

Regulation of IP$_3$R expression is likely to have functional consequences. Reduction of type I IP$_3$R protein expression has previously been shown to result in decreased IP$_3$ sensitivity and [Ca$^{2+}$] release in re-

---

**Fig. 6.** Type I IP$_3$R mRNA expression in mouse cerebellum and normal and diabetic mouse kidney. Top: autoradiograph of a Northern blot of poly(A)$^+$ mRNA isolated from mouse cerebellum, normal mouse kidney, and a diabetic mouse kidney probed with cDNA encoding murine type I IP$_3$R. The single band noted in mouse kidney corresponds to an identically located band from mouse cerebellum. Bottom: same blot was probed with β-actin cDNA as a control for loading.

**Fig. 7.** Reduced expression of type I IP$_3$R mRNA occurs in association with enhanced TGF-β1 mRNA in diabetic mouse kidney. A, top: representative autoradiograph of a Northern blot of poly(A)$^+$ mRNA isolated from normal (NL) mouse kidneys and diabetic mouse kidneys probed with murine type I IP$_3$R. The same blot was probed with TGF-β1 cDNA (middle) and was finally probed with β-actin cDNA (A, bottom) as a control for loading. B: densitometric quantitation of type I IP$_3$R mRNA/β-actin mRNA expressed in diabetic mouse kidney (D) relative to the control (N). C: densitometric quantitation of TGF-β1 mRNA/β-actin mRNA expressed in diabetic mouse kidney (D) relative to control (N). Data are means ± SE of band intensities of pooled data from all mouse samples in both groups ($n = 5$ per group). *P < 0.05 vs. normal group.
response to IP₃ mobilizing agonists in neuroblastoma cells and rat liver epithelial cells (5, 29, 30). In a rat model of dehydration expression of type II IP₃R mRNA, but not type I IP₃R mRNA, in collecting ducts was found to be downregulated (32). The authors speculated that type II IP₃R regulation may be important in maintaining body fluid homeostasis (32). In the present study, we demonstrate that type I IP₃R protein is reduced in glomerular cells and vascular smooth muscle cells of the diabetic rat in association with the development of renal hypertrophy and increased creatinine clearance. It is possible that reduced type I IP₃R expression in vascular smooth muscle cells and glomerular mesangial cells may play an important role in mediating diabetic glomerular hyperfiltration. Reduced type I IP₃R would lead to decreased IP₃ sensitivity and consequently an impaired rise in [Ca²⁺]ᵢ in response to a variety of vasoconstrictors, such as ANG II, norepinephrine, vasopressin, and endothelin. Impaired rise in [Ca²⁺]ᵢ would prevent maximal vasoconstrictive response to the above agents, thus leading to vascular dilatation and enhanced glomerular blood flow. Enhanced glomerular blood flow, due to afferent arteriolar dilatation, is likely the most important determinant in increasing glomerular capillary pressure and promoting glomerular hyperfiltration (10).

Although downregulation of the type I IP₃R may play a crucial role in mediating the vasodilatory effect of diabetes at the glomerulus, other isoforms of the IP₃R may compensate, and overall calcium mobilization may not be altered. In this context, regulation of the type III IP₃R isoform and possibly other intracellular calcium channels, such as the ryanodine receptor, will need to be evaluated. Although this remains an important consideration, it is also possible that discrete intracellular calcium channels control discrete pools of calcium and thus may regulate different functions in response to intracellular calcium-mobilizing agonists. In this regard, during the process of apoptosis in lymphocytes, there is upregulation of the type III IP₃R in the membrane fraction, whereas type I IP₃R expression in the cytosolic compartment is not affected (15).

The role of [Ca²⁺]ᵢ mobilization in explaining the basis for reduced contraction of mesangial cells to vasoconstrictors remains controversial. Studies by Whiteside and colleagues (11, 26) have not demonstrated reduced [Ca²⁺]ᵢ mobilization in mesangial cells grown in high glucose, although several other studies have demonstrated this phenomenon (9, 16). The basis for these discrepant results may be due to technical issues in calcium measurements and the passage number of mesangial cells used. It has also been demonstrated that actin filaments may play a role in the altered contractile function of mesangial cells grown in high glucose (33). Additionally, the involvement of the aldose reductase pathway and the protein kinase C (PKC) pathway have been implicated in disturbing actin assembly (34). Interestingly, PKC may also be involved in the inhibition of calcium mobilization by high glucose in rat mesangial cells (16) and rat aortic vascular smooth muscle cells (27, 28). Receptor downregulation to angiotensin II, arginine vasopressin, and endothelin has also been described in experimental models of diabetes and may also play a role in decreased responsiveness to vasoconstrictors (3, 28). Thus several pathways may contribute to altered vascular responsiveness of mesangial and smooth muscle cells grown in high glucose or in the diabetic milieu.

How might the diabetic state lead to reduced type I IP₃R expression in the kidney? Two recent studies from our group have demonstrated that both ANG II and TGF-β are capable of reducing IP₃R expression. ANG II reduces both type I and III IP₃R expression in rat liver epithelial cells, possibly via enhanced ubiquitination and proteasomal degradation (5). TGF-β1 leads to reduced expression of both the mRNA and the protein expression of the type I IP₃R in mesangial cells (24).

Numerous studies have demonstrated that both the renin-ANG II system (1, 14, 22) and the TGF-b system are stimulated in the glomeruli of diabetic kidneys (18, 21, 23) at a very early stage of diabetes. Our present study demonstrates an association with reduced type I IP₃R and overexpression of mRNA for TGF-β1 in diabetic rat and mouse kidneys. Whether reduced expression of the type I IP₃R in the diabetic kidney is due to upregulation of the TGF-β system or the renin-ANG II system remains to be determined.

In summary, we have demonstrated that renal hyper trophy, renal hyperfiltration and enhanced renal expression of TGF-β1 is associated with reduced expression of the type I IP₃R in diabetic rodents. Reduced type I IP₃R expression may play an important role in altered responsiveness of vascular structures in the pathogenesis of diabetic renal hyperfiltration and subsequent diabetic nephropathy.

We are grateful to Dr. Peter McCue (Department of Anatomy, Pathology, and Cell Biology) for expert advice on the immunohistochemistry studies and to Stephen Dunn (Division of Nephrology, Department of Medicine) for expert advice on statistical analysis. This work was supported in part by National Institute of Diabetes and Digestive and Kidney Diseases Grant K08-DK-02308 (to K. Sharma), an American Diabetes Association Research Award (to K. Sharma), and National Institutes of Health Grant R01-AA-10971 (to S. K. Joseph).

Address for reprint requests: K. Sharma, Division of Nephrology, Dept. of Medicine, Thomas Jefferson Univ., 1020 Locust St., JAH suite 353, Philadelphia, PA 19107.

Received 19 November 1997; accepted in final form 17 September 1998.

REFERENCES


