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TGF-β1 stimulates glucose uptake by enhancing GLUT1 expression in mesangial cells

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TGF-β1 stimulates glucose uptake by enhancing GLUT1 expression in mesangial cells.

Background. An increase in the expression of transforming growth factor-β1 (TGF-β1) has been proposed to play an important role in the excessive production of extracellular matrix (ECM) proteins seen in diabetes. Because the linkage between glucose metabolism and ECM protein production was found in mesangial cells overexpressed with the brain-type glucose transporter (GLUT1), we hypothesized that TGF-β1 could affect glucose metabolism.

Methods. To prove this hypothesis, we examined the effect of TGF-β1 on glucose uptake, the first step of glucose metabolism, in mesangial cells. 2-Deoxy-D-glucose (2DOG) uptake and the expression of GLUT1 were measured in mesangial cells exposed to various concentrations of TGF-β1. The kinetic constants were determined using 2DOG and 3-O-methyl-D-glucose (3OMG). The effect of anti–TGF-β neutralizing antibody on 2DOG uptake and GLUT1 mRNA was also examined in mesangial cells cultured under high-glucose (22.2 mM) conditions for 72 hours.

Results. TGF-β1 stimulated 2DOG uptake in mesangial cells by approximately 2.5-fold in a dose- (1.25 ng/ml maximum) and time-dependent manner, with a peak stimulation at nine hours. The increase in 2DOG uptake by TGF-β1 was completely abolished by the addition of 1 μg/ml cycloheximide, and kinetic analysis of 2DOG or 3OMG uptake revealed an increase in V_{max} by TGF-β1. Furthermore, TGF-β1 enhanced the expression of GLUT1 mRNA from one hour, followed by an enhancement of the expression of GLUT1 protein at nine hours. Finally, 2DOG uptake was significantly enhanced in cells cultured under high-glucose (22.2 mM) conditions as compared with that in cells under normal glucose (5.6 mM) conditions, and this increase in 2DOG uptake in cells under high-glucose conditions was inhibited by the addition of anti–TGF-β neutralizing antibody.

Conclusions. TGF-β1 stimulates glucose uptake by enhancing the expression of GLUT1 in mesangial cells, which leads to the acceleration of intracellular metabolic abnormalities in diabetes.

Key words: transforming growth factor-β, glucose transport, extracellular matrix, diabetic nephropathy, glomerular mesangial cells, brain type glucose transporter.

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Diabetic nephropathy is characterized histologically by an accumulation of extracellular matrix (ECM) proteins in glomerular mesangium [1–3]. Glomerular mesangial cells are considered to play a key role in the development of diabetic mesangial expansion, because mesangial cells were found to be capable of producing ECM proteins [4–7]. An enhancement of the production of type IV collagen and fibronectin was shown in mesangial cells cultured under high-glucose conditions [8, 9]. Metabolic abnormalities in mesangial cells specific to diabetes have been thought to contribute to the overproduction of ECM proteins in mesangial cells. An activation of polyol pathway and an increase in *de novo* synthesis of diacylglycerol followed by the activation of protein kinase C have been proposed as candidates of metabolic abnormalities in mesangial cells responsible for the overproduction of ECM proteins [9–13]. These metabolic abnormalities are caused by an excessive entry of glucose into mesangial cells through glucose transporters. Mesangial cells were found to express two types of glucose transporters, facilitative and sodium-coupled transporters (SGLTs) [14], and brain-type glucose transporter (GLUT1) was shown to be a predominant isoform (abstract; Kikkawa et al, *J Am Soc Nephrol* 3:830, 1992) [15]. The overexpression of GLUT1 in mesangial cells has been reported to result in an excessive production of ECM proteins even under normal glucose conditions [16]. Therefore, clarification of the regulation of GLUT1 in mesangial cells is considered to be essential to understand the mechanism of glucose-induced overproduction of ECM proteins.

Transforming growth factor-β1 (TGF-β1) is a multifunctional cytokine that regulates the proliferation and metabolism of ECM proteins in various types of the cells [17–19], including mesangial cells [7, 20]. Recently, an increase in the expression of TGF-β1 has been reported in glomeruli of both diabetic animals and diabetic patients with nephropathy [21–24], and treatment with anti–TGF-β neutralizing antibodies was shown to prevent the enhancement of an expression of ECM proteins in diabetic animals [25]. An increase in the production of TGF-β1 was also found in mesangial cells cultured

under high-glucose conditions, and treatment with anti–TGF-β neutralizing antibodies was again shown to prevent an enhancement of the expression of ECM proteins under high-glucose conditions [7, 25]. Thus, in addition to glucose-induced metabolic abnormalities in mesangial cells, the overexpression of TGF-β1 in mesangial cells under diabetic conditions is considered to play an important role in the overproduction of ECM proteins. However, a linkage between TGF-β and glucose metabolism has not yet been elucidated in mesangial cells.

Recently, TGF-β1 was reported to enhance glucose uptake and glycolysis in fibroblasts [26, 27] by inducing the expression of GLUT1 mRNA [27]. Furthermore, an enhancement of glucose uptake resulting from the up-regulation of GLUT1 mRNA was shown in mesangial cells cultured under high-glucose conditions [15]. From these observations, we hypothesized that TGF-β1 could enhance glucose uptake and thus modulate the production of ECM proteins not only by its direct effect, but by altering the glucose metabolism in glomerular mesangial cells. To evaluate this hypothesis, we examined the effect of TGF-β1 on glucose uptake, the first step of glucose metabolism, and the regulation of glucose transporters in cultured mesangial cells.

METHODS

Materials

Human platelet TGF-β1 and anti–TGF-β neutralizing antibody were obtained from R&D Systems (Minneapolis, MN, USA). Purified chicken IgG was purchased from ICN Pharmaceuticals, Inc. (Aurora, OH, USA). 2-Deoxy-D-glucose (2DOG), 3-O-methyl-D-glucose (3OMG), cycloheximide, and cytochalasin B were purchased from Sigma (St. Louis, MO, USA). 2-deoxy-D-[2,6-^{3}H]glucose (2-[^{3}H]DOG) was purchased from Amersham Life Science (Buckinghamshire, UK). 3-O-methyl-D-[^{3}H]glucose (3-[^{3}H]OMG) and [α-^{32}P] dCTP (3000 Ci/mmol) were purchased from NEN Research Products (Boston, MA, USA). Anti-GLUT1 antibody and GLUT1 control peptide were obtained from Santa Cruz Biotechnology (Santa Cruz, CA, USA). Rat GLUT1 cDNA was kindly provided by Dr. Yutaka Seino (University of Kyoto, Kyoto, Japan), and rat SGLT1 cDNA was prepared by reverse transcription-polymerase chain reaction (RT-PCR) from total RNA of rat duodenal epithelial cells using primers as follows: sense primer, 5′-GTGGTACCCGTCATGA TGGCTTCCCT-3′; antisense primer, 5′-TCTAGAAG GAGGATGATGCCGTTG-3′. All other reagents were of chemical grade and were purchased from standard suppliers.

Mesangial cell culture

Glomerular mesangial cells were obtained from a culture of glomeruli isolated from male Sprague-Dawley rats weighing 100 to 150 g by a sieving method as previously described [10]. Isolated glomeruli were cultured in RPMI 1640 medium containing 20% heat-inactivated fetal bovine serum (FBS), 100 U/ml penicillin, and 100 μg/ml streptomycin. Cultured cells were identified as mesangial cells by morphological and biochemical characters as previously described [28]. Cells at passages between 3 and 10 were used for the following experiments.

Measurement of 2DOG uptake and kinetic analysis of 2DOG uptake

Mesangial cells were grown in 35 mm culture dishes in 2 ml of RPMI 1640 medium containing 20% FBS. Confluent cells were made quiescent by reducing the concentrations of FBS to 0.4% for 24 hours. The quiescent mesangial cells were incubated in RPMI 1640 medium containing 0.4% FBS with the indicated concentrations of TGF-β1 at 37°C for the indicated time interval. The uptake of 2DOG was measured by incubating the cells in glucose-free KRP-Hepes buffer [131.2 mM NaCl, 4.71 mM KCl, 2.47 mM $CaCl_2$, 1.24 mM $MgSO_4$, 2.48 mM NaH_2PO_4, 10 mM HEPES, and 0.5% bovine serum albumin (BSA), pH 7.45] with 2-[^{3}H]DOG (1 μCi/well) and unlabeled 2DOG in a final concentration of 0.1 mM at 37°C for five minutes [29]. Incubation was terminated by rapid aspiration of the buffer, chilling on ice, and washing three times rapidly with ice-cold phosphate-buffered saline (PBS). The cells were then solubilized with 1 M NaOH, and the radioactivity remaining in the cells was determined by a liquid scintillation counter. For kinetic analysis of 2DOG uptake, the quiescent mesangial cells were incubated with TGF-β1 (2.5 ng/ml) for nine hours. The uptake of 2DOG was measured by incubating the cells in glucose-free KRP-Hepes buffer with 2-[^{3}H]DOG (1 μCi/well) and various concentrations of unlabeled 2DOG at 37°C for five minutes. The concentrations of cellular protein were determined using protein assay kit (Bio-Rad Laboratories, Hercules, CA, USA).

To examine the effect of high-glucose culture, mesangial cells were chronically adapted to the conditions of 5.6 mM glucose containing 20% FBS for a long period of time (at least for 30 days) and then cultured in RPMI 1640 medium with either 5.6 or 22.2 mM glucose for 72 hours. The concentrations of FBS were reduced to 5%, and the culture was continued for another 24 hours. Anti–TGF-β neutralizing antibody (30 μg/ml) or control chicken IgG were added during the last 24 hours of culture. Cells were rinsed extensively with PBS prior to the measurement of 2DOG uptake.

Kinetic analysis of 3OMG uptake

The uptake of 3OMG in mesangial cells was measured by the methods reported in fibroblasts or adipocytes [30–32]. In brief, the quiescent mesangial cells were incubated with TGF-β1 (2.5 ng/ml) for nine hours. The uptake

of 3OMG was measured by incubating the cells in glucose- and BSA-free KRP-Hepes buffer with 3-[^{3}H]OMG (1 μCi/well) and various concentrations of unlabeled 3OMG at 37°C for 10 seconds. After the incubation, the uptake was terminated by the addition of KRP-Hepes buffer containing 0.3 mM phloretin (stop solution), chilling on ice, and washing three times with ice-cold PBS. The radioactivity associated with the cells at zero time was determined by adding stop solution prior to the addition of the radioactive 3OMG. The intracellular radioactivity was determined by a liquid scintillation counter as described before. The total cell volume per dish was estimated from the equilibrium distribution of 3OMG for 15 minutes.

Northern blot analysis

Mesangial cells were grown in 100 mm culture dishes and treated with TGF-β1 as described previously in this article. Twelve micrograms of total RNA extracted using TRIzol® Reagent (GIBCO BRL, Grand Island, NY, USA) were electrophoresed through a 1% agarose formaldehyde gel and transferred on a nylon filter. The filter was prehybridized in Church buffer [500 mM $NaPO_4$, pH 7.0, 5% sodium dodecyl sulfate (SDS), 1% BSA, 1 mM ethylenediaminetetraacetic acid (EDTA)] at 65°C for two hours and hybridized with radiolabeled cDNAs at 65°C for 18 to 24 hours in a rotating hybridization oven. The filter was washed twice with wash buffer A (40 mM $NaPO_4$, pH 7.0, 5% SDS, 0.5% BSA, 1 mM EDTA) for 15 minutes at 65°C, twice with wash buffer B (40 mM $NaPO_4$, pH 7.0, 1% SDS, 1 mM EDTA) in the same conditions, and exposed to Kodak X-AR film with an intensifying screen at −80°C [33]. After stripping the radioactive probes from the filter, the filter was rehybridized with a radioactive probe of acidic ribosomal phosphoprotein PO (36 B4) cDNA as an internal standard [34].

Immunoblot analysis

Quiescent confluent mesangial cells were exposed to 2.5 ng/ml of TGF-β1 at 37°C for nine hours. The cells were harvested in 500 μl of cell lysis buffer (20 mM HEPES, pH 7.4, 1 mM EDTA, 250 mM sucrose, 1% Triton X-100, and 1 mM phenylmethylsulfonyl fluoride) and centrifuged at 14,000 × *g* for 10 minutes. The supernatants containing 50 μg of protein were subjected to 10% SDS-polyacrylamide gels and transferred onto Immobilon P membranes (Millipore, Bedford, MA, USA). The membranes were probed with anti-GLUT1 antibody (1:500). In the competition experiments, the membranes were treated with both GLUT1 control peptide (1:500) and anti-GLUT1 antibody. A horseradish peroxidase-conjugated secondary antibody (1:1000) was used to allow the detection of immunoreactive bands using enhanced chemiluminescence. The intensity of desired bands was quantitated by the densitometolic analysis.

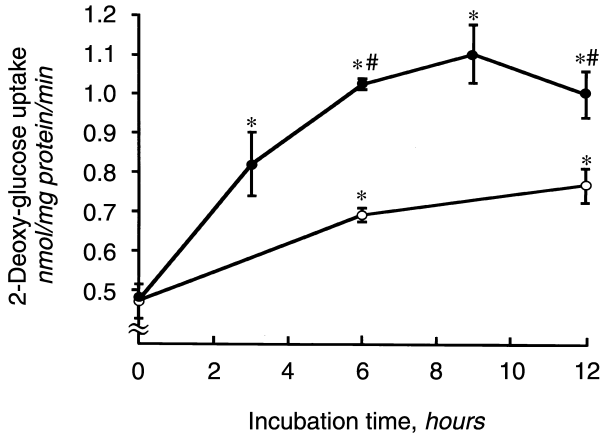

Fig. 1. Effect of transforming growth factor-β1 (TGF-β1) on 2-deoxy-D-glucose (2DOG) uptake in mesangial cells. 2DOG uptake in quiescent mesangial cells incubated in the presence (●) or absence (○) of 2.5 ng/ml TGF-β1 for the indicated time intervals was determined. The data shown are mean ± SD ($N = 4$). *$P < 0.01$ vs. basal 2DOG uptake (time 0); #$P < 0.01$ vs. 2DOG uptake in the cells incubated without TGF-β1 for the same period.

Statistical analysis

Analysis of variance followed by Scheffe's test was used to determine significant difference in multiple comparisons. Comparisons between two groups were analyzed by Student's unpaired *t*-test.

RESULTS

Effect of TGF-β1 on 2DOG uptake and kinetics of 2DOG and 3OMG uptake in mesangial cells

We first examined the time course of 2DOG uptake in mesangial cells treated with 2.5 ng/ml TGF-β1. As shown in Figure 1, TGF-β1 stimulated 2DOG uptake in mesangial cells by approximately 2.5-fold, with a maximal stimulation at nine hours. Although a gradual increase in 2DOG uptake was observed even in the untreated cells, the uptake of 2DOG in the cells treated with TGF-β1 was significantly higher than that in untreated cells throughout the incubation periods (Fig. 1). The dose-dependent stimulation of 2DOG uptake in mesangial cells by TGF-β1 at nine hours is shown in Figure 2. The stimulation of 2DOG uptake in mesangial cells by TGF-β1 was observed from 0.3125 ng/ml and reached a maximal stimulation at 1.25 ng/ml.

Kinetics of 2DOG and 3OMG uptake in mesangial cells were analyzed by Hanes plot analysis (Fig. 3). TGF-β1 significantly increased the V_{max} for 2DOG from 1.93 ± 0.025 to 5.36 ± 0.097 nmol/mg/min (mean ± SD, $N = 3$, $P < 0.01$) without affecting Km (from 0.57 ± 0.06 to 0.66 ± 0.07 mM, mean ± SD, $N = 3$, P = NS). Similar to 2DOG uptake, TGF-β1 significantly increased the V_{max} for 3OMG from 67.57 ± 1.01 to 116.50 ± 0.27 pmol/μl/second (mean ± SD, $N = 3$, $P < 0.01$) without affecting

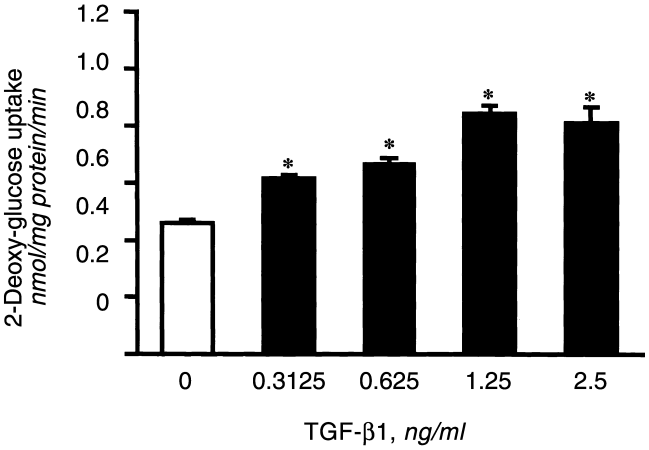

Fig. 2. Dose-dependent effect of TGF-β1 on 2DOG uptake in mesangial cells. 2DOG uptake into mesangial cells treated with various concentrations of TGF-β1 for nine hours was determined. The data shown are mean ± SD ($N = 4$). *$P < 0.01$ vs. basal (TGF-β1 0 ng/ml).

Km (from 8.38 ± 0.22 to 8.50 ± 0.24 mM, mean ± SD, $N = 3$, P = NS). These data suggest that TGF-β1 could stimulate glucose uptake by increasing the number of glucose transporters without affecting the affinity of transporters to glucose in mesangial cells. Although the values of Km for 3OMG were slightly different from those for 2DOG, which were concordant with the values reported previously in mesangial cells [15], the values of Km for 3OMG were similar to the values reported in human fibroblasts [31, 32, 35].

To know the requirement of protein synthesis in TGF-β1–induced stimulation of 2DOG uptake in mesangial cells, the effect of cycloheximide was examined. As shown in Figure 4, the TGF-β1–induced stimulation of 2DOG uptake was completely abolished by the coincubation with 1 μg/ml of cycloheximide, suggesting that the new protein synthesis was necessary for the TGF-β1–induced stimulation of glucose uptake in mesangial cells. From these observations, TGF-β1 is considered to stimulate glucose uptake by increasing the amount of glucose transporters in the mesangial cells.

To know the contribution of facilitative transporters to 2DOG uptake in mesangial cells, we next examined the effect of cytochalasin B on 2DOG uptake. As shown in Figure 5, 2DOG uptake in the cells treated with TGF-β1 was significantly inhibited by 1 μM cytochalasin B to the levels below 2DOG uptake in untreated cells. 2DOG uptake was almost completely abolished by 25 μM cytochalasin B in both untreated cells and the cells treated with TGF-β1. These results suggest that 2DOG could be taken up by mesangial cells mainly through facilitative transporters, and TGF-β1 is able to enhance the facilitative glucose transport.

Effect of TGF-β1 on the expression of GLUT1 mRNA and protein

Among the facilitative glucose transporters, GLUT1 was shown to be the predominant glucose transporter in cultured mesangial cells (abstract; Kikkawa et al, ibid) [15]. Although the study with cytochalasin B indicates that TGF-β1 could enhance the facilitative glucose transport, the previous report suggests that mesangial cells have two types of glucose transporters: facilitative and sodium-coupled transporters [14]. Thus, we next examined whether TGF-β1 could stimulate the expression of GLUT1 or SGLT-1. As shown in Figure 6 A and B, TGF-β1 was able to enhance the expression of mRNA of GLUT1 in a time- and dose-dependent manner, whereas mRNA of SGLT-1 could not be detected by Northern blot analysis (data not shown). An increase in the expression of GLUT1 mRNA was observed from one hour after treatment with TGF-β1 (Fig. 6A). The expression of GLUT1 protein in the mesangial cells is shown in Figure 7. Levels of the expression of 47 kDa protein, which selectively disappeared by competition with the GLUT1 control peptide (Fig. 7A), were increased by approximately threefold in the cells treated with TGF-β1 for nine hours, as compared with untreated cells (Fig. 7 B, C).

Effect of anti–TGF-β neutralizing antibody on 2DOG uptake and the expression of GLUT1 mRNA in mesangial cells cultured under high-glucose conditions

It has been recently reported that glucose uptake is enhanced in rat mesangial cell line cultured under high concentrations of glucose, indicating the up-regulation of glucose uptake by glucose [15]. To evaluate whether endogenous TGF-β1 induced by high concentrations of glucose could be involved in this glucose-induced up-regulation of glucose uptake, we examined the effect of anti–TGF-β neutralizing antibody on 2DOG uptake in mesangial cells cultured under high-glucose conditions. Cells were cultured in the conditions similar to the previous report [15]. As shown in Figure 8A, 2DOG uptake was significantly enhanced in mesangial cells cultured under high-glucose (22.2 mM) conditions as compared with that in cells under normal glucose (5.6 mM) conditions. The expression of TGF-β1 mRNA was also enhanced in cells cultured under high-glucose conditions (data not shown). This enhancement of 2DOG uptake in cells under high-glucose conditions was significantly inhibited by the addition of 30 μg/ml of anti–TGF-β neutralizing antibody (Fig. 8A). As shown in Figure 8B, an increase in the expression of GLUT1 mRNA under high-glucose conditions was also inhibited by the same concentrations of anti–TGF-β neutralizing antibody.

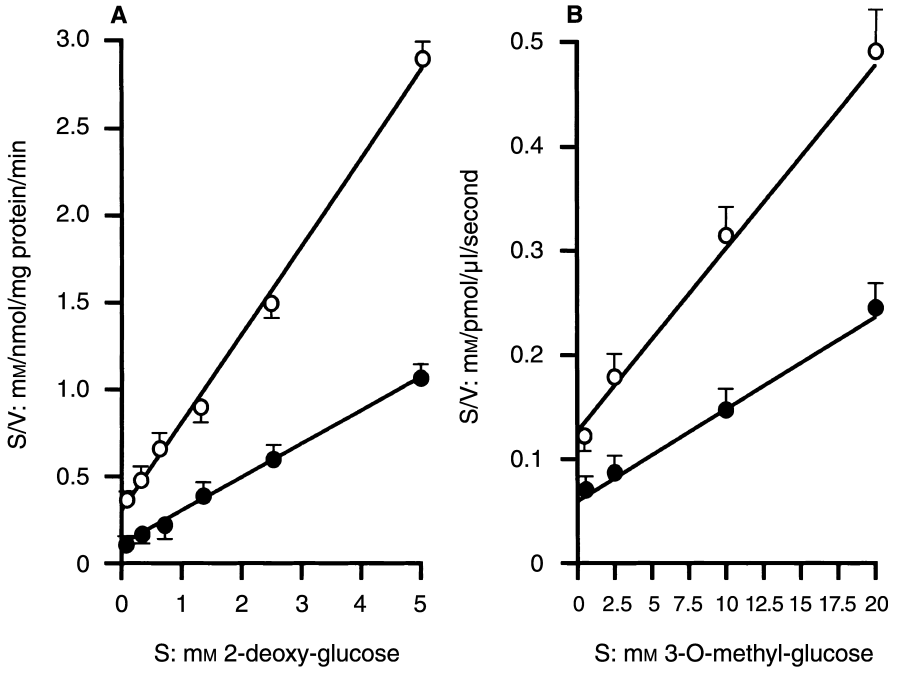

Fig. 3. Kinetic analysis of 2DOG and 3OMG uptake in mesangial cells. 2DOG and 3OMG uptake into mesangial cells incubated in the presence (●) or absence (○) of 2.5 ng/ml TGF-β1 for nine hours were determined. (*A*) 2DOG uptake was determined in the presence of various concentrations of unlabeled 2DOG ranging from 0.1 to 5.0 mM. (*B*) 3OMG uptake was determined in the presence of various concentrations of unlabeled 3OMG ranging from 0.1 to 20 mM. The data were analyzed by Hanes plots. The data shown are mean ± SD ($N = 3$).

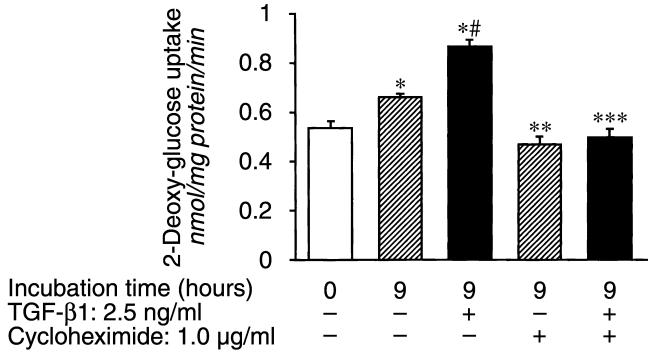

Fig. 4. Effect of cycloheximide on TGF-β1–induced stimulation of 2DOG uptake in mesangial cells. 2DOG uptake was determined in mesangial cells incubated in media with or without 2.5 ng/ml TGF-β1 in the presence or absence of 1 μg/ml cycloheximide for nine hours. The data shown are mean ± SD ($N = 4$). *$P < 0.01$ vs. basal 2DOG uptake (time 0); **$P < 0.01$ vs. 2DOG uptake in the cells incubated without TGF-β1 (time 9); ***$P < 0.01$ vs. 2DOG uptake in the cells incubated with TGF-β1; #$P < 0.01$ vs. 2DOG uptake in the cells incubated without TGF-β1 (time 9).

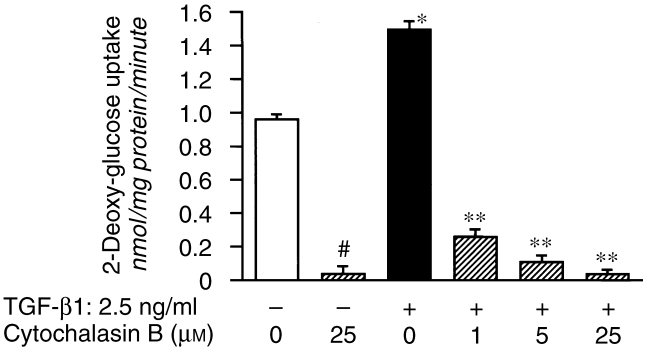

Fig. 5. Effect of cytochalasin B on 2DOG uptake in mesangial cells. Mesangial cells were incubated in media with or without 2.5 ng/ml TGF-β1 for nine hours. 2DOG uptake was determined in the presence or absence of indicated concentrations of cytochalasin B in glucose-free KRP-Hepes buffer at 37°C for five minutes. The data shown are mean ± SD ($N = 4$). *$P < 0.01$ vs. 2DOG uptake in the cells incubated without TGF-β1; **$P < 0.01$ vs. 2DOG uptake in the cells incubated with TGF-β1; #$P < 0.01$ vs. 2DOG uptake in the cells incubated without TGF-β1.

DISCUSSION

This study clearly indicates that TGF-β1 is able to stimulate glucose uptake, a first step of glucose metabolism, in glomerular mesangial cells by inducing the expression of mRNA and protein of GLUT1. The results also indicate that endogenous TGF-β1 produced by mesangial cells cultured under high-glucose conditions could stimulate glucose uptake and thus might accelerate glucose-induced metabolic abnormalities in mesangial cells.

Transforming growth factor-β1 is a multifunctional cytokine that regulates the proliferation and the metabolism of ECM proteins in various types of cells [17–19], including mesangial cells [7, 20]. However, the linkage between TGF-β1 and glucose metabolism has not been examined in mesangial cells. These results clearly indicate that TGF-β1 is able to stimulate 2DOG uptake in mesangial cells in a time- and dose-dependent manner. An increase in V_{max} by TGF-β1 in kinetic analysis of 2DOG and 3OMG uptake suggests an increase in numbers of glucose transporters by TGF-β1. The TGF-β1–induced

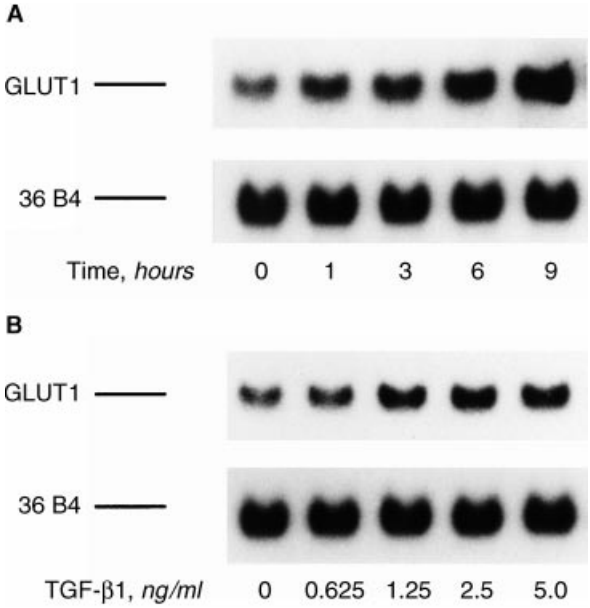

Fig. 6. Effect of TGF-β1 on GLUT1 mRNA expression in mesangial cells. (*A*) Cells were treated with 2.5 ng/ml TGF-β1 for indicated time intervals. Total RNA (12 μg) was subjected to Northern blot analysis. (*B*) Cells were treated with indicated concentrations of TGF-β1 for six hours. Total RNA (12 μg) was subjected to Northern blot analysis. *36 B4*; human acidic ribosomal phosphoprotein PO.

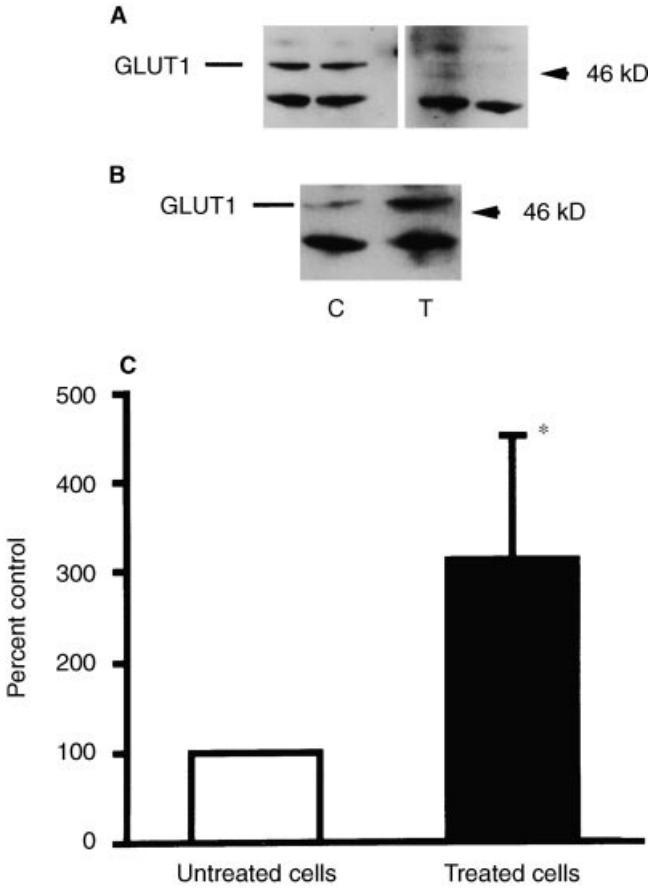

Fig. 7. Effect of TGF-β1 on GLUT1 protein expression in mesangial cells. Cells were incubated with or without 2.5 ng/ml TGF-β1 for nine hours, and immunoblot analysis for GLUT1 was performed. (*A*) A competition experiment using a GLUT1 control peptide. All lanes were loaded with 50 μg of total protein from TGF-β1–treated cells. The antisera for the right panel were preabsorbed with GLUT1 control peptide before immunoblot analysis. (*B*) An immunoblot analysis of a GLUT1. Lanes were loaded with 50 μg of total protein from TGF-β1–untreated (C) and treated (T) cells. (*C*) The results of a densitometric analysis of four independent experiments were shown. The data shown are mean ± SD, $N = 4$. $^*P < 0.05$ vs. (C).

increase in 2DOG uptake was abolished by the addition of cycloheximide, indicating the requirement of new protein synthesis for a TGF-β1–induced increase in 2DOG uptake. The results of the Northern blot analysis indicate that TGF-β1 is able to stimulate the expression of mRNA for GLUT1 in a time- and dose-dependent manner in mesangial cells. Furthermore, the expression of GLUT1 protein was also induced by TGF-β1. These results clearly indicate that TGF-β1 stimulates glucose uptake by inducing GLUT1 mRNA and protein expressions.

Mesangial cells have been reported to express two types of glucose transporters: facilitative and sodium-coupled transporters [14]. Although a small amount of the expression of GLUT4 was shown in mesangial cells [36], we and others have reported that GLUT1 is the predominant glucose transporter in mesangial cells (abstract; Kikkawa et al, ibid) [15]. In this study, we examined the expression of SGLT1 mRNA using cDNA prepared by RT-PCR from the RNA of rat duodenal epithelial cells. However, we were unable to detect SGLT1 in this study. In addition, 2DOG uptake was almost completely inhibited by cytochalasin B in both untreated and TGF-β1–treated cells, suggesting that 2DOG was taken up by mesangial cells mainly through facilitative transporters, and that TGF-β1 could enhance the facilitative glucose transport. The importance of GLUT1 in mesangial cells has been suggested from a recent report which found that the overexpression of GLUT1 in mesangial cells leads to an excessive production of ECM proteins even under normal glucose conditions [16]. Therefore, TGF-β–induced up-regulation of GLUT1 followed by an increase in glucose uptake, as shown in this study, is considered to be an important factor in order to understand the mechanisms of not only the action of TGF-β, but the regulation of glucose uptake in mesangial cells.

Various growth factors, cytokines, oncogene products and serum were found to induce the expression of glucose transporter at a transcriptional level [29, 37–40]. Two distinct enhancer elements have been identified in the 5′ region and second intron of GLUT1 gene [40]. One contains the homologous sequences with two 12-O-tetradecanoylphorbol-13-acetate-responsive elements (TREs), one serum responsive element, one cAMP-responsive element (CRE), and three GC boxes, and the other con-

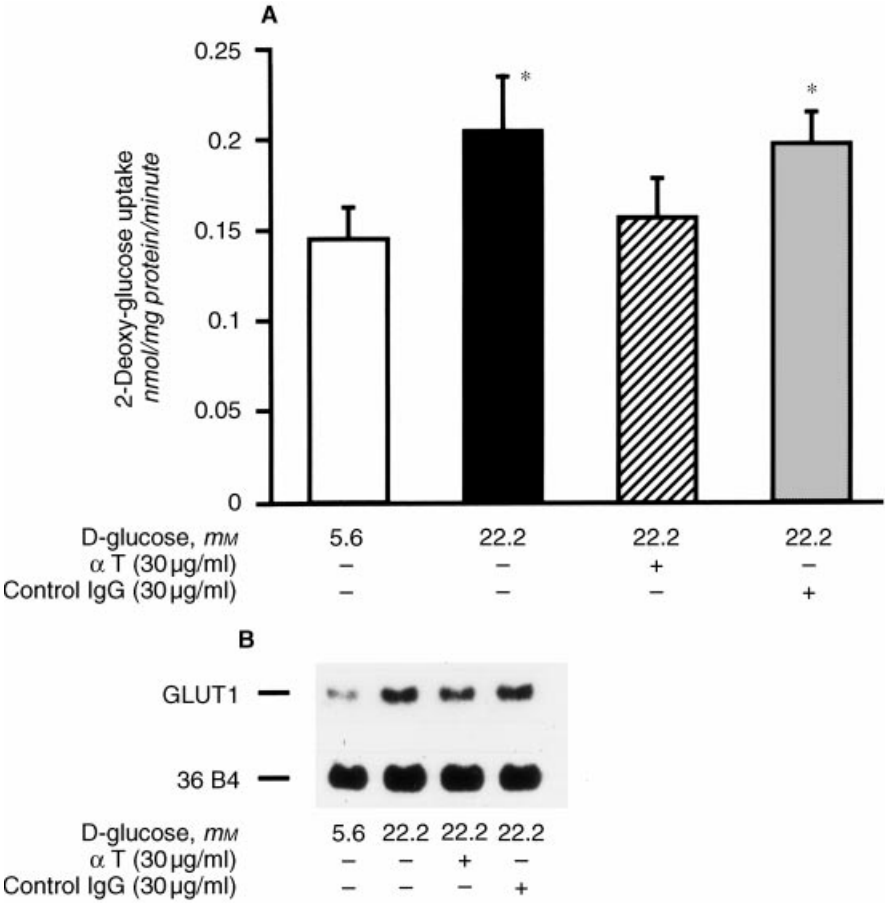

Fig. 8. Effect of anti–TGF-β neutralizing antibody on 2DOG uptake and the expression of GLUT1 mRNA in mesangial cells cultured under high glucose conditions. (*A*) Mesangial cells were first maintained in RPMI 1640 medium with 5.6 mM glucose containing 20% FBS for at least 30 days and then cultured in RPMI 1640 medium with either 5.6 or 22.2 mM glucose for 72 hours. The concentrations of FBS were reduced to 5%, and the culture was continued for another 24 hours. Anti–TGF-β neutralizing antibody (30 μg/ml; αT) or control chicken IgG (30 μg/ml; control IgG) were added during the last 24 hours of culture. The data shown are mean ± SD ($N = 8$). *$P <$ 0.01 vs. 5.6 mM glucose. (*B*) Total RNA (12 μg) isolated from the cells under the identical conditions of the 2DOG uptake experiment under high-glucose condition was subjected to Northern blot analysis. *36 B4*; human acidic ribosomal phosphoprotein PO.

tains the homologous sequences with two TREs and one CRE [40]. Because TGF-β1 has been reported to induce its own gene expression through a TRE-like sequence, which exists within the TGF-β1 promoter [41] and the expression of fibronectin gene through CRE sequence [42], TGF-β1 may regulate the GLUT1 gene expression through these enhancer elements present in GLUT1 gene in mesangial cells. These possibilities need to be examined further.

Various metabolic abnormalities secondary to hyperglycemia, such as an activation of the polyol pathway and an increase in *de novo* synthesis of diacylglycerol followed by the activation of protein kinase C, have been proposed to play an important role in the development of diabetic nephropathy [10, 43–46]. Excessive influx of glucose into mesangial cells is the first step of these metabolic abnormalities. Recently, an increase in glucose uptake and in the expression of GLUT1 mRNA and protein has been reported in mesangial cells cultured under normal glucose conditions for long periods of time and then exposed to high concentrations of glucose [15]. We hypothesized that TGF-β1 produced from mesangial cells cultured under high-glucose conditions might be responsible for this glucose-induced increase in glucose uptake. In our study, 2DOG uptake and the expression of GLUT1 mRNA were enhanced in cells cultured under high-glucose conditions for similar durations as reported earlier [15]. This increase in both 2DOG uptake and the expression of GLUT1 mRNA was prevented by the addition of anti–TGF-β neutralizing antibody. Thus, in mesangial cells under high-glucose conditions, various metabolic alterations, such as the activation of protein kinase C, might induce the production of endogenous TGF-β1, and TGF-β1, in turn, could stimulate glucose uptake and thus accelerate the metabolic alterations.

Recent evidence suggests that an increase in the expression of TGF-β1 in the glomeruli of both diabetic animals and diabetic patients, as well as in mesangial cells cultured under high-glucose conditions, plays an important role in the excessive production of ECM proteins seen in diabetic nephropathy [7, 21–24]. The interaction between TGF-β1 and GLUT1, shown in our study, might provide the evidence for the action of TGF-β1 that is different from its direct effect on the production of ECM proteins, and thus might be an important factor towards understanding the mechanisms of mesangial cell abnormalities that lead to the development of diabetic nephropathy.

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REFERENCES

1. Mauer SM, Steffes MW, Ellis EN, Sutherland DER, Brown DM, Goetz FC: Structural-functional relationships in diabetic nephropathy. *J Clin Invest* 74:1143–1155, 1984
2. Steffes MW, Østerby R, Chavers B, Mauer SM: Mesangial expansion as a central mechanism for loss of kidney function in diabetic patients. *Diabetes* 38:1077–1081, 1989
3. Nerlich A, Schleicher E: Immunohistochemical localization of extracellular matrix components in human diabetic glomerular lesions. *Am J Pathol* 139:889–899, 1991
4. Steffes MW, Bilous RW, Sutherland DER, Mauer SM: Cell and matrix components of the glomerular mesangium in type I diabetes. *Diabetes* 41:679–684, 1992
5. Kreisberg JI, Ayo SH: The glomerular mesangium in diabetes mellitus. *Kidney Int* 43:109–113, 1993
6. Ayo SH, Radnik RA, Garoni JA, Glass WF II, Kreisberg JI: High glucose causes an increase in extracellular matrix proteins in cultured mesangial cells. *Am J Pathol* 136:1339–1348, 1990
7. Ziyadeh FN, Sharma K, Ericksen M, Wolf G: Stimulation of collagen gene expression and protein synthesis in murine mesangial cells by high glucose is mediated by autocrine activation of transforming growth factor-β. *J Clin Invest* 93:536–542, 1994
8. Haneda M, Kikkawa R, Horide N, Togawa M, Koya D, Kajiwara N, Ooshima A, Shigeta Y: Glucose enhances type IV collagen production in cultured rat glomerular mesangial cells. *Diabetologia* 34:198–200, 1991
9. Kreisberg JI, Kleisberg SH: High glucose activates protein kinase C and stimulates fibronectin gene expression by enhancing a cAMP response element. *Kidney Int* 48(Suppl 51):S3–S11, 1995
10. Kikkawa R, Umemura K, Haneda M, Arimura T, Ebata K, Shigeta Y: Evidence for existence of polyol pathway in cultured rat mesangial cells. *Diabetes* 36:240–243, 1987
11. Bleyer AJ, Fumo P, Snipes ER, Goldfarb S, Simmons DA, Ziyadeh FN: Polyol pathway mediates high glucose-induced collagen synthesis in proximal tubule. *Kidney Int* 45:659–666, 1994
12. Keogh RJ, Dunlop ME, Larkins RG: Effect of inhibition of aldose reductase on glucose flux, diacylglycerol formation, protein kinase C, and phospholipase A_2 activation. *Metabolism* 46:41–47, 1997
13. Pugliese G, Pricci F, Pugliese F, Mene P, Lenti L, Andreani D, Galli G, Casini A, Bianchi S, Rotella CM, Mario UD: Mechanisms of glucose-enhanced extracellular matrix accumulation in rat glomerular mesangial cells. *Diabetes* 43:478–490, 1994
14. Wakisaka M, He Q, Spiro MJ, Spiro RG: Glucose entry into rat mesangial cells is mediated by both Na^+-coupled and facilitative transporters. *Diabetoloia* 38:291–297, 1995
15. Heilig CW, Liu Y, England RL, Freytag SO, Gilbert JD, Heilig KO, Zhu M, Concepcion LA, Brosius FC III: D-glucose stimulates mesangial cell GLUT1 expression and basal and IGF-I-sensitive glucose uptake in rat mesangial cells: Implications for diabetic nephropathy. *Diabetes* 46:1030–1039, 1997
16. Heilig CW, Concepcion LA, Riser BL, Freytag SO, Zhu M, Cortes P: Overexpression of glucose transporters in rat mesangial cells cultured in a normal glucose milieu mimics the diabetic phenotype. *J Clin Invest* 96:1802–1814, 1995
17. Ewen ME, Sluss HK, Whitehouse LL, Livingston DM: TGFβ inhibition of cdk4 synthesis is linked to cell cycle arrest. *Cell* 74:1009–1020, 1993
18. Ignotz RA, Endo T, Massagué J: Regulation of fibronectin and type I collagen mRNA levels by transforming growth factor-β. *J Biol Chem* 262:6443–6446, 1987
19. Sharma K, Ziyadeh FN: Hyperglycemia and diabetic kidney disease: The case for transforming growth factor-β as a key mediator. *Diabetes* 44:1139–1146, 1995
20. Schoecklmann HO, Rupprecht HD, Zauner I, Sterzel RB: TGF-β1-induced cell cycle arrest in renal mesangial cells involves inhibition of cyclin E-cdk 2 activation and retinoblastoma protein phosphorylation. *Kidney Int* 51:1228–1236, 1997
21. Yamamoto T, Nakamura T, Noble NA, Ruoslahti E, Border WA: Expression of transforming growth factor β is elevated in human and experimental diabetic nephropathy. *Proc Natl Acad Sci USA* 90:1814–1818, 1993
22. Nakamura T, Fukui M, Ebihara I, Osada S, Nagaoka I, Tomino Y, Koide H: mRNA expression of growth factors in glomeruli from diabetic rats. *Diabetes* 42:450–456, 1993
23. MacKay K, Striker LJ, Stauffer JW, Doi T, Agodoa LY, Striker GE: Transforming growth factor-β: Murine glomerular receptors and responses of isolated glomerular cells. *J Clin Invest* 83:1160–1167, 1989
24. Sharma K, Ziyadeh FN, Alzahabi B, McGowan TA, Kapoor S, Kurnik BRC, Kurnik PB, Weisberg LS: Increased renal production of transforming growth factor-β1 in patients with type II diabetes. *Diabetes* 46:854–859, 1997
25. Sharma K, Jin Y, Guo J, Ziyadeh FN: Neutralization of TGF-β by anti-TGF-β antibody attenuates kidney hypertrophy and the enhanced extracellular matrix gene expression in STZ-induced diabetic mice. *Diabetes* 45:522–530, 1996
26. Masumi A, Akamatsu Y, Kitagawa T: Modulation of the synthesis and glycosylation of the glucose transporter protein by transforming growth factor-β1 in Swiss 3T3 fibroblasts. *Biochim Biophys Acta* 1145:227–234, 1993
27. Kitagawa T, Masumi A, Akamatsu Y: Transforming growth factor-β1 stimulates glucose uptake and the expression of glucose transporter mRNA in quiescent Swiss mouse 3T3 cells. *J Biol Chem* 266:18066–18071, 1991
28. Haneda M, Kikkawa R, Maeda S, Togawa M, Koya D, Horide N, Kajiwara N, Shigeta Y: Dual mechanism of angiotensin II inhibits ANP-induced mesangial cGMP accumulation. *Kidney Int* 40:188–194, 1991
29. Haneda M, Kikkawa R, Togawa M, Koya D, Kajiwara N, Shigeta Y: Metabolic actions of insulin-like growth factor I in cultured glomerular mesangial cells. *Metabolism* 40:1311–1316, 1991
30. Whitesell RR, Gliemann J: Kinetic parameters of transport of 3-O-methylglucose and glucose in adipocytes. *J Biol Chem* 254: 5276–5283, 1979
31. Palfreyman RW, Clark AE, Denton RM, Holman GD, Kozka IJ: Kinetic resolution of the separate GLUT1 and GLUT4 glucose transport activities in 3T3-L1 cells. *Biochem J* 284:275–281, 1992
32. Longo N, Griffin LD, Langley SD, Elsas LJ: Glucose transport by cultured human fibroblasts: Regulation by phorbol esters and insulin. *Biochim Biophys Acta* 1104:24–30, 1992
33. Sugimoto T, Haneda M, Togawa M, Isono M, Shikano T, Araki S, Nakagawa T, Kashiwagi A, Guan KL, Kikkawa R: Atrial natriuretic peptide induces the expression of MKP-1, a mitogen-activated protein kinase phosphatase, in glomerular mesangial cells. *J Biol Chem* 271:544–547, 1996
34. Laborda J: 36B4 cDNA used as an estradiol-independent mRNA control is the cDNA for human acidic ribosomal phosphoprotein PO. *Nucleic Acids Res* 19:3998, 1991
35. Longo N, Griffin LD, Elsas LJ: Influx and efflux of 3-O-methyl-D-glucose by cultured human fibroblasts. *Am J Physiol* 254:C628–C633, 1988
36. Brosius FC III, Briggs JP, Marcus RG, Barac-Nieto M, Charron MJ: Insulin-responsive glucose transporter expression in renal microvessels and glomeruli. *Kidney Int* 42:1086–1092, 1992
37. Rollins BJ, Morrison ED, Usher P, Flier JS: Platelet-derived growth factor regulates glucose transporter expression. *J Biol Chem* 263:16523–16526, 1988
38. Hiraki Y, Rosen OM, Birnbaum MJ: Growth factors rapidly induce expression of the glucose transporter gene. *J Biol Chem* 263:13655–13662, 1988
39. Bilan PJ, Mitsumoto Y, Ramlal T, Klip A: Acute and long-term effects of insulin-like growth factor I on glucose transporters in

muscle cells: Translocation and biosynthesis. *FEBS Lett* 298:285–290, 1992
40. Murakami T, Nishiyama T, Shirotani T, Shinohara Y, Kan M, Ishii K, Kanai F, Nakazuru S, Ebina Y: Identification of two enhancer elements in the gene encoding the type 1 glucose transporter from the mouse which are responsive to serum, growth factor, and oncogenes. *J Biol Chem* 267:9300–9306, 1992
41. Kim SJ, Denhez F, Kim KY, Holt JT, Sporn MB, Roberts AB: Activation of the second promoter of the transforming growth factor-β1 gene by transforming growth factor-β1 and phorbol ester occurs through the same target sequences. *J Biol Chem* 264:19373–19378, 1989
42. Kreisberg JI, Garoni JA, Radnik R, Ayo SH: High glucose and TGFβ1 stimulate fibronectin gene expression through a cAMP response element. *Kidney Int* 46:1019–1024, 1994
43. Ayo SH, Radnik R, Garoni JA, Troyer DA, Kreisberg JI: High glucose increases diacylglycerol mass and activates protein kinase C in mesangial cell cultures. *Am J Physiol* 261:F571–F577, 1991
44. Haneda M, Araki S, Togawa M, Sugimoto T, Isono M, Kikkawa R: Mitogen-activated protein kinase cascade is activated in glomeruli of diabetic rats and glomerular mesangial cells cultured under high glucose conditions. *Diabetes* 46:847–853, 1997
45. Koya D, Jirousek MR, Lin YW, Ishii H, Kuboki K, King GL: Characterization of protein kinase Cβ isoform activation on the gene expression of transforming growth factor-β, extracellular matrix components, and prostanoids in the glomeruli of diabetic rats. *J Clin Invest* 100:115–126, 1997
46. Ishii H, Jirousek MR, Koya D, Takagi C, Xia P, Clermont A, Bursell SE, Kern TS, Ballas LM, Heath WF, Stramm LE, Feener EP, King GL: Amelioration of vascular dysfunctions in diabetic rats by an oral PKCβ inhibitor. *Science* 272:728–731, 1996