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High Glucose Induced VEGF Expression via PKC and ERK in Glomerular Podocytes

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Podocytes are the major site of vascular endothelial growth factor (VEGF) production in the kidney, and up-regulation of VEGF plays a critical role in the progression of diabetic nephropathy. Using a differentiated mouse podocyte cell line, we investigated the roles of protein kinase C (PKC) and extracellular signal-regulated kinase (ERK) on the expression of VEGF under high glucose conditions. High glucose induced up-regulation of VEGF mRNA and protein expression in podocytes via activation of PKC (PKC-α and -βII isoforms) and ERK. High glucose stimulated [^{3}H]leucine incorporation in the podocytes. High glucose and the PKC stimulator, phorbol 12-myristate 13-acetate (PMA) induced activator protein-1 (AP-1)-dependent transcriptional activity and expression of VEGF. In addition, these phenomena were blocked by specific inhibitors of PKC (GF10902X) and ERK kinase (PD98059). These observations suggested that high glucose-induced VEGF expression in podocytes was largely mediated through PKC and ERK pathways that may be involved in diabetic nephropathy.

Key Words: **diabetic nephropathy; podocyte; vascular endothelial growth factor; protein kinase C; extracellular signal-regulated kinase; activator protein-1; high glucose.**

Nephropathy is one of the most serious complications in diabetes and is the major basis of chronic renal failure worldwide (1). Several prospective controlled studies have shown that hyperglycemia and subsequent biochemical events were correlated with the development of diabetic complications (2, 3). Vascular endothelial growth factor (VEGF) is one of the major factors promoting diabetic complications (4). VEGF is an endothelial mitogen and potent vasopermeability factor, the effects of which are mediated by endothelial cell-specific receptors (5). A recent study indicated that an antibody against VEGF treatment decreased hyperfiltration, albuminuria and glomerular hypertrophy in diabetic rats (6). These findings suggested that VEGF plays important roles in the pathogenesis of diabetic nephropathy and that VEGF may be a good therapeutic target molecule for diabetic nephropathy. However, the function of VEGF on diabetic nephropathy is not well established.

The VEGF family is comprised of 34- to 42-kDa heparin-binding, dimeric, disulfide-bound proteins. Molecular cloning of cDNAs for the VEGF family has revealed the existence of at least five isoforms in human tissues, formed by alternative splicing of mRNA to yield VEGF transcripts encoding polypeptides of 206, 189, 165, 145, and 121 (7). Although the physiological roles of each VEGF splicing variant are not well known, VEGF165 is the most abundant isoform expressed in the majority of normal human tissues and is detectable in human serum (7).

It is generally accepted that levels of VEGF and its receptor are increased in the kidneys of diabetic rats with glomerular lesions (8, 9). Since podocytes are the major producers of VEGF within the glomeruli (8, 10–13) and podocyte injury underlies progressive glomerulosclerosis in diabetes both in humans (14, 15) and experimental models (17–19), regulation of VEGF expression in the podocytes may provide novel insight into the pathogenesis of diabetic nephropathy. Unfortunately, the signaling cascade of VEGF regulation in the podocytes remains largely unknown due to lack of appropriate differentiated podocyte cell lines. A recently established immortalized mouse podocyte cell line is known to express several podocyte-specific characteristics (19) and is considered to be the most appropriate cell line mimicking podocytes *in vivo*. Using this podocyte cell line, the present study investigated the effects of high glucose on VEGF production in podo-

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cytes. In addition, we report here that high glucose induces VEGF expression through PKC and ERK pathways in podocytes.

MATERIALS AND METHODS

Cell culture and experimental design. The immortalized mouse podocyte clone was a generous gift from Dr. Mundel (19) and was maintained in Dulbecco's modified Eagle medium (DMEM: Gibco BRL, Grand Island, NY, USA) supplemented with 10% fetal bovine serum (FBS) and 10 U/ml recombinant mouse interferon-γ (IFN-γ, Sigma Chemical Corporation, St. Louis, MO) at 33°C (permissive conditions). To induce differentiation, podocytes were seeded sparsely (5×10^4 cells) on type I-collagen coated dishes (Iwaki glass, Tokyo, Japan) at 37.5°C without IFN-γ (nonpermissive conditions). Under nonpermissive conditions, the majority of cells had an arborized shape and expressed podocyte specific synaptopodin (19). After 4 days under non-permissive conditions, they were starved in DMEM containing 0.2% BSA and 0.4% FBS for 48 h. Then, the cells were divided into three groups: (1) normal glucose group (NG) as controls incubated in DMEM containing 5.5 mM glucose, (2) high glucose group (HG) incubated in DMEM containing 25 mM glucose, (3) the mannitol osmotic control group (MN) incubated in NG medium supplemented with mannitol (5.5 mM glucose + mannitol 19.5 mM) as an osmotic control for HG.

To determine the effects of activated PKC, cells were treated with 100 nM phorbol 12-myristate 13-acetate (PMA, Sigma) incubated in NG medium. Cells were harvested after 3 days for extraction of membrane and cytosolic fractions for analysis of PKC activities or protein for ERK1/2 activity. To evaluate VEGF production, cells were harvested for extraction of total RNA and culture media were collected for VEGF peptide ELISA. To determine the effects of the PKC inhibitor, GF10209X (Calbiochem, CA) (20) and MAP/ERK kinase (MEK) inhibitor, PD98059 (Calbiochem) (21), the cells were pretreated with these agents for 2 h before changing to HG or PMA medium. To evaluate ^{3}H-thymidine or [^{3}H]leucine incorporation, starved podocytes were incubated with NG, HG or MN for 18 h, and then added to either [^{3}H]thymidine or [^{3}H]leucine (1 μCi/ml, Amersham Pharmacia Biotech) for 6 h. The incorporation was determined by liquid scintillation counting (22).

RNA preparation and reverse transcription and quantitative real-time polymerase reaction for analysis of VEGF mRNA. Total RNA was extracted using TRI-ZOL reagent (Gibco BRL). The amount of VEGF mRNA was quantified by the reverse transcription and polymerase chain reaction (RT-PCR). Total RNA was reverse transcribed using a TaqMan Gold RT-kit (P-E Applied Biosystems, Foster City, CA) and random hexamer. RT was performed for 30 min at 42°C followed by 5 min at 96°C. The synthesized cDNA was quantified by TaqMan PCR and PCR reagents and an Applied Biosystems Prism 7700 (P-E Applied Biosystems) according to the manufacturer's protocol. The primers used were a forward primer for mouse VEGF164 (5′-GAGCAGAAGTCCCATGAAGT G-3′) and a reverse primer (5′-GTCTCAATTGGACGGCAGTAG-3′). A mouse VEGF164 oligonucleotide was used as a probe (Fam-TCAAGTTCATGGACGTCTACC AGCGAA) (synthesized by P-E Applied Biosystems). We analyzed the expression of ribosomal RNA using a ribosomal control kit (P-E Applied Biosystems) as an internal control. The PCR conditions were as follows: 5 min at 95°C, followed by 40 cycles of 1 min at 94°C, 1 min at 60°C, and 1 min at 72°C.

Analysis of VEGF protein. VEGF protein was assayed in culture media of the podocytes using a commercial solid phase enzyme-linked immunosorbent assay (ELISA) (R&D Systems, Oxford, UK). This assay is specific for mouse VEGF164 (23), the main VEGF peptide synthesized and secreted by podocytes (13).

Assay for PKC activity. Podocytes were rinsed twice with PBS (−) and homogenized in ice-cold buffer A (20 mM, Tris–HCl (pH 7.5), 2 mM EDTA, 0.5 mM EGTA, 1 mM phenylmethylsulfonyl fluoride, 1 mM DTT, 0.3 M sucrose, 25 μg/ml leupeptin, 25 μg/ml aprotinin) with a Dounce homogenizer (60 strokes). The homogenates were ultracentrifuged at 100,000*g* for 1 h at 4°C. The supernatant was retained as the cytosolic fraction and pellet were resuspended with buffer B (buffer A without sucrose) and solubilized with 1% Triton X-100. After rehomogenization and incubation on ice for 30 min, the soluble membrane fraction was obtained by ultracentrifugation at 100,000*g* for 30 min. Protein concentration was quantified using the Bradford method (Bio-Rad, Hercules, CA) and each fraction was aliquoted and stored at −80°C until determination of PKC activity and Western blotting analysis. PKC activities of the membrane and cytosolic fractions were measured by PKC enzyme assay system (Amersham Pharmacia Biotech, UK), and defined as the Ca^{2+}, phosphatidylserine and PMA-stimulated transfer of ^{32}P from [γ-^{32}P]ATP into the PKC-specific substrate (RKRTLRRL). All reactions were performed at 37°C for 15 min, and the reaction was terminated by spotting the sample onto binding papers. The papers were immediately washed 3 times with 75 mM orthophosphoric acid and placed into scintillation vials, and radioactivity was counted.

Western blotting analysis for PKC isoforms. Proteins from each fraction were separated on 7.5% SDS gels under reducing conditions and transferred onto microporous polyvinylidene fluoride (PVDF) membranes (Immobilon-P, Millipore, Tokyo). Membranes were blocked overnight with 2% skimmed milk powder in PBS-T (PBS with 0.05% Tween 20, pH 7.0). PKC-α was detected by a monoclonal antibody (Transduction Laboratories) and horseradish peroxidase-linked anti-rabbit IgG antiserum. PKC-βI, -βII and -δ were detected by polyclonal anti-peptide antibodies (Santa Cruz, CA) and horseradish peroxidase-linked anti-rabbit IgG antiserum. PKC isoforms were quantified by a chemiluminescence technique using ECL reagent and ECL hyperfilm (Amersham Pharmacia Biotech.).

Assay for ERK1/2 activity. ERK1/2 activity was measured as reported (18) using p42/p44 MAP kinase enzyme assay system (Amersham Pharmacia Biotech.). Briefly, cells were rinsed twice with PBS (−) followed by addition of 0.5 ml of ice-cold lysis buffer containing 25 mM Tris–HCl (pH 7.5), 25 mM NaCl, 25 mM sodium phosphate, 25 mM NaF, 2 mM EGTA, 1 mM phenylmethylsulfonyl fluoride, 10 μg/ml leupeptin, 10 μg/ml aprotinin, 1% Triton X-100. Cell lysates were incubated on ice for 20 min and centrifuged at 15,000*g* for 20 min at 4°C. ERK1/2 activity was assayed by addition of kinase buffer containing 1.0 μCi of [γ-^{32}P]ATP and a substrate peptide containing the phosphorylation site of epidermal growth factor receptor followed by incubation at 30°C, 30 min.

Assay for AP-1 activity. To study AP-1-dependent transcriptional activation, the mouse podocyte cell line was stably transfected with a *cis*-reporter plasmid containing a placental alkaline phosphatase (PLAP) reporter gene (24) linked to the mouse plasminogen activator inhibitor 1 (PAI-1) promoter with tandem repeats of the AP-1 binding site (25). About 1.3 kb of mouse PAI-1 promoter region DNA (1 to 1320 nucleotides of GenBank Accession No. M33961) was isolated by PCR and inserted in pUC18. After checking the DNA sequence, the adapter including three AP-1 elements (5′-AATTCTGACTCAAG-CTTATGAGTCAGACACCTCTGGC TTTCGGAAGGGCTGAGTC-3′ and 5′-AATTGACTCAGCCCTTCCGAAGCCAG AGGTGTCTGAC-TCATAAGCTTGAGTCAG-3′) were inserted at the *Eco*RI site of this promoter. The TNF-α promoter region of TNF-α-PLAP plasmid (24) replaced with this modified mouse plasminogen activator protein-1 (PAI-1) promoter region was designated as plasmid 3 AP-1-PLAP. Podocytes were transfected with Lipofectamine (Gibco BRL) and selected with DMEM containing 1 mg/ml G418 medium under permissive conditions for about one month. G418-resitant cells were seeded at 2×10^3 cells per type I-collagen coated microplate (Iwaki Glass) under nonpermissive conditions for 4 days. The cells were starved for 48 h, and then divided into 4 experiment groups as defined above (NG, HG, MN, PMA). The cells were pretreated with

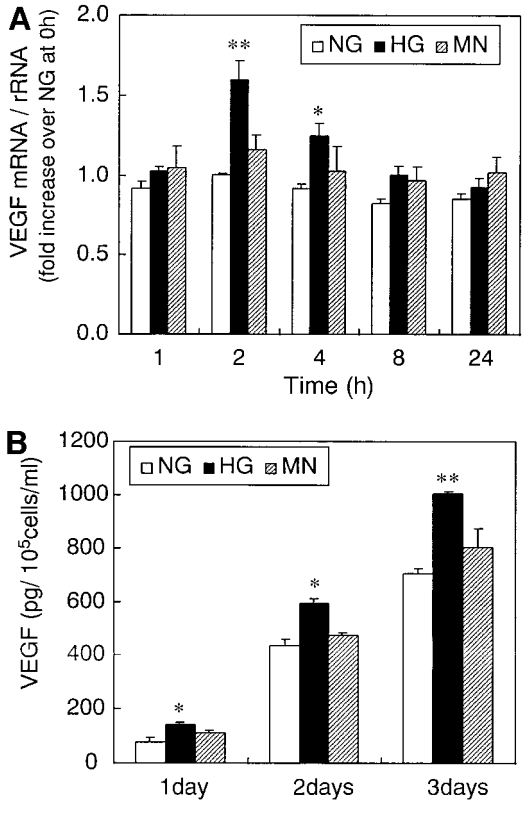

FIG. 1. Effects of normal glucose (5.5 mM; NG), high glucose (25 mM; HG) or normal glucose with mannitol (5.5 mM glucose + mannitol 19.5 mM; MN) on the expression of VEGF (A) mRNA and protein (B) in podocytes. (A) Podocytes were cultured under NG, HG, or MN. VEGF mRNA was quantified by TaqMan PCR analysis. Results are shown as VEGF mRNA levels normalized to that of rRNA and expressed as the ratio to NG. (B) MPC was cultured under NG, HG, or MN for up to 3 days. VEGF protein levels in culture media were determined by specific mouse VEGF164 ELISA. $n = 4$, $^{**}P < 0.01$, $^{*}P < 0.05$ vs NG.

inhibitors for 2 h before changing to each medium. Culture supernatants were collected after 2 days of cultivation, and alkaline phosphatase activity was measured with a microplate luminometer (EG & G Berthold, Germany) (22).

Statistical analysis. Results are expressed as means ± SEM. The significance of differences among experimental groups was determined by ANOVA. When a significant difference was detected, the data were further analyzed by Dunnett's multiple range test. Statistical significance was assumed at $P < 0.05$ and $P < 0.01$.

RESULTS

Time Course of HG-Induced VEGF mRNA and Protein Expression in Podocytes

In NG, podocytes constantly expressed VEGF mRNA by low levels. The levels of VEGF mRNA were unchanged under MN. By contrast, HG induced a time-dependent increase in VEGF mRNA levels (Fig. 1A). Quantitative analysis of HG-induced VEGF mRNA revealed 1.60 ± 0.13-fold at 2 h ($P < 0.01$) and 1.24 ± 0.08-fold at 4 h ($P < 0.05$) compared to the baseline of NG at 0 h. The podocytes continuously secreted VEGF protein into the culture media up to 3 days under NG. In HG condition, podocytes showed a significant increase in VEGF protein secretion compared to that of NG by 1.82 ± 0.11-fold at 1 day. This increased production was sustained for 3 days. No difference was observed between the NG and MN groups (Fig. 1B).

Increase of Protein Kinase C (PKC) Activity by High Glucose in Podocytes

Following culture in HG media for 3 days, podocytes showed a significant increase of PKC activity in the membrane fraction (1.43 ± 0.09, $P < 0.05$) (Fig. 2A), whereas the activity of PKC in the cytosolic fraction was unchanged. MN had no effect on the PKC activity. Western blotting analysis with specific antibodies against PKC isoforms revealed that PKC-α, -βI, -βII, and -δ isoforms were detected in the podocyte membrane and cytosolic fractions, while PKC-ϵ and -θ isoforms were undetectable (data not shown). PKC-α isoform was found preferentially in the cytosolic fraction. By contrast, PKC-βI and -βII isoforms were found pref-

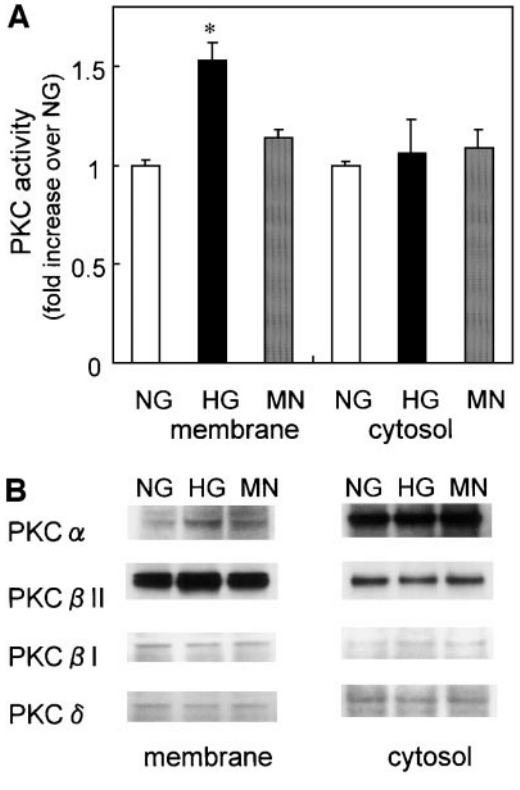

FIG. 2. Protein kinase C (PKC) activity (A) and PKC isoform expression (B) in podocytes exposed to NG, HG or MN. (A) PKC activity in membrane and cytosolic fractions. Results represent means ± SEM of four independent experiments. $^{*}P < 0.05$ vs NG. (B) Representative Western blot analysis with PKC isoform-specific antibodies in membrane and cytosolic fractions.

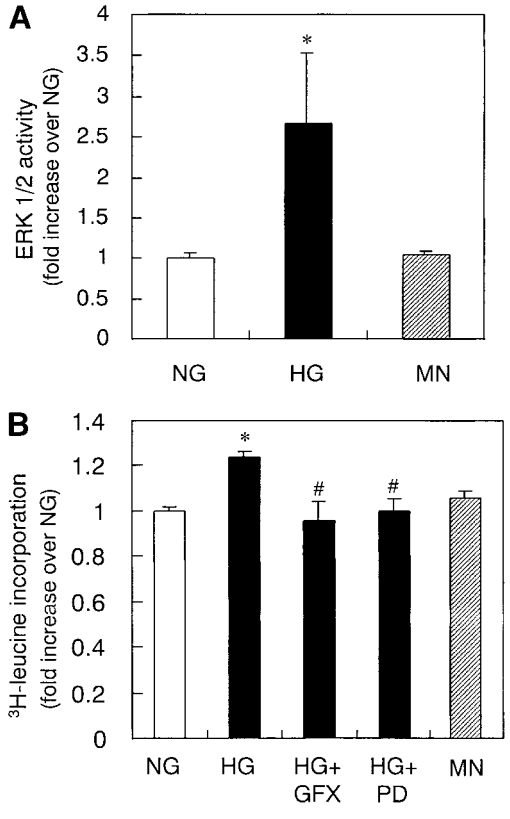

FIG. 3. Increases in ERK1/2 activity (A) and [^{3}H]leucine incorporation (B) by HG in mouse podocytes. (A) ERK1/2 activity in podocytes exposed to NG, HG, or MN for 3 days. Results represent means ± SEM of four independent experiments. (B) [^{3}H]Leucine incorporation in podocytes incubated for 18 h in the presence of PKC inhibitor, GF1090203X (3 μM; GFX) or MEK inhibitor; PD98059 (3 μM, PD) containing HG. Results represent means ± SEM of four independent experiments. *$P < 0.05$ vs NG. #$P < 0.05$ vs HG.

erentially in membrane fraction. Levels of membranous PKC-α and -βII isoforms, which are the active forms of PKC, were increased in the presence of high glucose conditions (Fig. 2B).

Increase of ERK 1/2 Activity by High Glucose in Podocytes

Podocytes cultured with HG showed a significant increase in ERK 1/2 activity compared with those cultured with NG (2.66 ± 0.87, $P < 0.05$), whereas MN had no effect on ERK 1/2 activity (Fig. 3A).

Increase of [^{3}H]Leucine Incorporation by High Glucose and Effects of GF109203X and PD98059 in Podocytes

[^{3}H]Thymidine incorporation was unchanged among the three groups of NG, HG, and MN (data not shown). By contrast, [^{3}H]leucine incorporation in HG was significantly elevated compared to the cells treated with NG or MN (NG; 14929 ± 180 dpm, HG; 18458 ± 339 dpm, MN; 15841 ± 381, NG vs HG; $P < 0.05$, $n = 4$). Increase of [^{3}H]leucine incorporation by HG was significantly blocked by GF109203X or PD98059 ($P < 0.05$) (Fig. 3B).

Time Course of PMA-Induced VEGF mRNA and Protein Expression in Podocytes

PMA significantly elevated VEGF mRNA and protein expression compared with that of NG. Time course changing of VEGF mRNA and protein expression by PMA are shown in Figs. 4A and 4B, which were similar to those under HG as shown in Fig. 2. VEGF mRNA expression was increased to 1.84 ± 0.21-fold after 1 h ($P < 0.01$), 4.18 ± 0.16-fold after 2 h ($P < 0.01$), 2.22 ± 0.22-fold after 4 h ($P < 0.01$) and 1.20 ± 0.08-fold after 8 h ($P < 0.05$) compared with the baseline levels under NG at 0 h. VEGF protein secretion was significantly increased after 1 day exposure to PMA medium compared with NG.

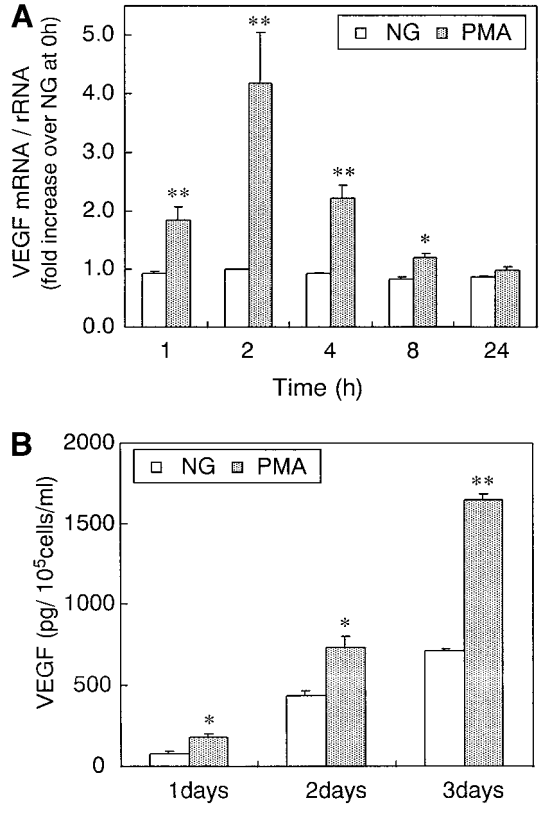

FIG. 4. Effects of PMA on expression of VEGF mRNA (A) and protein (B) in podocytes. (A) Podocytes were cultured under PMA (100 nM) for various periods. The mRNA was quantified by TaqMan PCR analysis. Results are shown as VEGF mRNA levels normalized to that of rRNA and expressed as the ratio to NG. (B) Podocytes were cultured under PMA for up to 3 days. VEGF protein levels in culture media were determined by a specific mouse VEGF 164 ELISA. $n = 4$, **$P < 0.01$, *$P < 0.05$ vs NG.

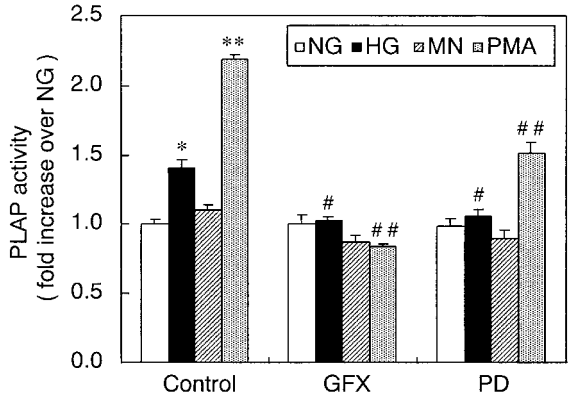

FIG. 5. Effects of HG, MN, or PMA, and PKC or MEK inhibitors on AP-1-dependent transcriptional activity in podocytes. Podocytes transfected with AP-1-dependent PLAP construct were pretreated with PKC inhibitor, GF1090203X (3 μM; GFX) or MEK inhibitor, PD98059 (3 μM, PD) for 2 h, and exposed to NG, HG, MN, or PMA for 2 days. Determinations were performed independently five times. Results represent means ± SEM as fold of NG. $^{**}P < 0.01$, $^{*}P < 0.05$ vs NG; $\#\#P < 0.01$, $\#P < 0.05$ vs corresponding values without inhibitors.

Effects of GF109203X and PD98059 on AP-1-Dependent Transcriptional Activity under HG and PMA in Podocytes

As shown in Fig. 5, HG or PMA significantly increased AP-1 activity compared with NG (HG; 1.41 ± 0.05-fold, $P < 0.05$, PMA; 2.19 ± 0.03-fold, $P < 0.01$), whereas MN had no effect. Pretreatment with GF109203X and PD98059 diminished the HG-induced ($P < 0.05$) or PMA-induced AP-1 activation ($P < 0.01$). Neither GF109203X nor PD98059 had any effect on AP-1 activity under NG.

Effects of GF109203X and PD98059 on HG- and PMA-Induced VEGF mRNA Expression in Podocytes

Preincubation with GF109203X or PD98059 for 2 h significantly suppressed HG or PMA-induced VEGF mRNA expression at 2 h (Fig. 6A) and VEGF protein secretion at 3 days (Fig. 6B). GF109203X or PD98059 alone had no effect on VEGF expression under NG conditions.

DISCUSSION

The roles of VEGF in diabetic nephropathy are still unknown. VEGF is a potent cytokine enhancing microvascular permeability in the skin, peritoneal wall, subcutaneous tissue and skeletal muscles. In diabetes, expression of VEGF was increased in the kidney in association with hyperfiltration and that it was suppressed by blocking of VEGF (4, 6, 8, 26). Hence, it is likely to speculate that VEGF acts to promote vascular permeability in the diabetic kidneys and is involved in its nephropathy.

The initial and one of the important symptoms in diabetic nephropathy is proteinuria, which is the result of deregulation of glomerular permeability. Glomerular permeability is determined by glomerular endothelial cells, basement membrane and largely by podocytes (27); the latter of which are the predominant sites of VEGF production (9). Of note, expression of VEGF was increased in podocytes in diabetic rats and human diabetes. In addition, Del Prete *et al.* proposed that diabetes increased glomerular permeability by VEGF resulting in albuminuria and in matrix accumulation in the glomeruli (28). Thus, it is tempting to speculate that podocytes are responsible for an up-regulation of VEGF in the diabetic millieu and involved in the increase of glomerular permeability, i.e., microalbuminuria or proteinuria as nephropathy.

Despite their role as the major sites of VEGF production and that they are the most important regula-

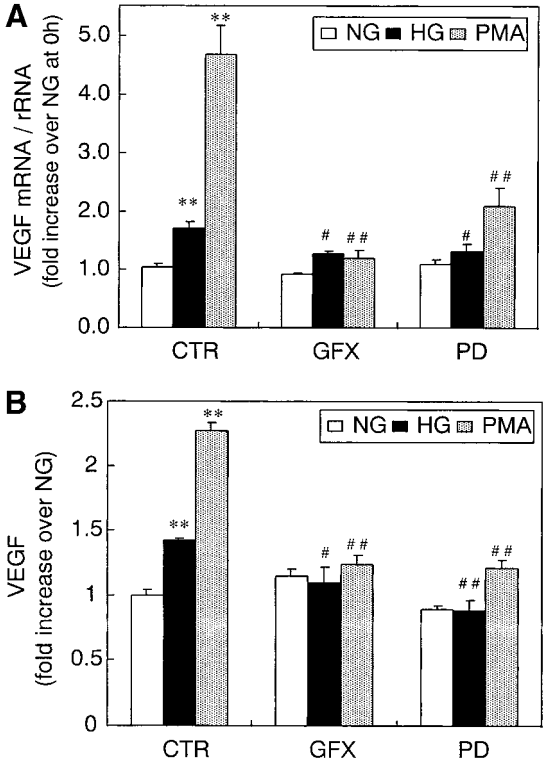

FIG. 6. Effects of PKC inhibitor or MEK inhibitor on HG-induced and PMA-induced VEGF mRNA (A) and protein expression (B). (A) Podocytes were pretreated with GF1090203X (3 μM; GFX) or PD98059 (3 μM, PD) for 2 h, and exposed to HG or PMA for 2 h. Results are shown as VEGF mRNA levels normalization to that of rRNA and expressed as the ratio to NG. $n = 4$, $^{**}P < 0.01$ vs NG; $\#\#P < 0.01$ vs corresponding values without inhibitor. (B) Podocytes were pretreated with GF1090203X (3 μM; GFX) or PD98059 (3 μM, PD) for 2 h, and exposed to HG, MN, or PMA for 3 days. Results are expressed as the ratio of the NG. $n = 4$, $^{**}P < 0.01$ vs NG; $\#\#P < 0.01$, $\#P < 0.5$ vs corresponding values without inhibitors.

tors of glomerular permeability, the role of podocytes in VEGF expression and intracellular signaling pathway in the diabetic milieu has not been investigated. The present study showed that high glucose media up-regulated VEGF164 homologue mRNA and protein levels in podocytes in a time-dependent manner. In addition, high glucose-induced VEGF mRNA and protein expression were completely abolished by a specific inhibitor of PKC. These results indicated that high glucose-induced VEGF production in podocytes is a PKC-dependent mechanism. This cellular response of PKC dependent VEGF up-regulation under high glucose was similar to that of human vascular smooth muscle cells (29) and mesangial cells (26).

PKC is not a single molecular entity, but consists of a family of closely related isoenzymes that differ in their structure, cofactor requirements, and substrate specificity (30). The family of PKCs includes at least eleven isoforms. Among the various PKC isoforms, PKC-β and -δ appeared to be preferentially activated in the aorta and the heart in diabetic rats (31), and in cultured vascular smooth muscle cells under high glucose conditions (32). On the other hand, levels of PKC-α, -βII and -ϵ isoforms were increased in the retina of diabetic rats (33). In addition, levels of PKC-α, -βI and -δ were shown to be elevated in the glomeruli of diabetic rats and mesangial cells in high glucose medium (34), indicating that activation of PKC isoforms in the diabetic milieu is cell type-specific. In this context, the present study showed for the first time that high glucose increased PKC activity in the podocytes compared to the controls cultured with NG. Furthermore, we found that levels of DAG-sensitive PKC-α and -βII isoforms were increased in the membrane fraction under high glucose conditions, whereas PKC-βI and -δ isoforms were unchanged. PKC-ϵ and -θ isoforms were not detected in the podocytes by Western blotting analysis. Thus, PKC-α and -βII are the specific isoforms expressed in podocytes in the setting of high glucose environment. Interestingly, a specific inhibitor of PKC-β prevented hyperfiltration and proteinuria in the early phase of streptozotocin-induced diabetes (35). This supports our notion that PKC-α and -β isoforms play some roles in diabetic nephropathy.

The mechanism responsible for up-regulation of PKC under high glucose conditions in podocytes is unknown. Previously, King *et al.* proposed that levels of diacylglycerol (DAG), an endogenous PKC activator, were increased in cells exposed to high glucose through *de novo* synthesis, which involves the progressive stepwise acylation of glycolytic intermediates (36, 37). Since anatomical localization of podocytes is well likely exposed to urine, which contains high glucose concentrations in diabetic patients, the hypothesis of DAG-PKC pathway may be appropriate for podocytes.

The intracellular signaling cascade between PKC activation and VEGF expression in podocytes under diabetic milieu is an additional important point to be elucidated. ERK is a member of the mitogen-activated protein kinase (MAP) family (38). The activation of ERK is involved in regulating proliferation and protein synthesis by stimulating transcription factors that induce the activation of AP-1 and other growth-responsive genes (39, 40). Moreover, PKC and ERK are known to regulate AP-1 (c-fos/c-jun) activation (39–41). In this context, our results indicated that high glucose-induced PKC and ERK activation in podocytes did not result in cell proliferation, but cell hypertrophy with VEGF production. In addition MEK inhibitor, PD98059 completely abolished high glucose-induced VEGF expression. These results indicated that the PKC-ERK pathway plays an essential role in high glucose-induced VEGF expression in podocytes. Furthermore, PMA-induced AP-1 activation and VEGF expression were significantly suppressed by PD98059, suggesting that ERK and AP-1 were activated mainly through the PKC pathway. Importantly, the promoter region of the VEGF gene contains the AP-1 binding consensus sequence (23, 42). These observations support the notion that the PKC- ERK-mediated AP-1 pathway plays an important role in VEGF regulation in podocytes.

In conclusion, the present study demonstrated for the first time that high ambient glucose induced VEGF mRNA and protein through a PKC-ERK dependent mechanism in podocyte cell line. In addition, we found that PKC-α and -βII were the isoforms activated by high glucose in podocytes. These observations may facilitate the development of novel strategies for treatment of diabetic nephropathy targeting PKC pathway.

ACKNOWLEDGMENTS

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