High Glucose Stimulates Hyaluronan Production by Renal Interstitial Fibroblasts Through the Protein Kinase C and Transforming Growth Factor-β Cascade

Masanobu Takeda, Tetsuya Babazono, Kosaku Nitta, and Yasuhiko Iwamoto

Deposition of hyaluronan has been implicated in the pathogenesis of diabetic glomerulosclerosis. We hypothesized the involvement of hyaluronan in diabetic tubulointerstitial fibrosis. We investigated high-glucose effect on hyaluronan production by rat renal interstitial fibroblasts (normal rat kidney [NRK] cells) and examined the role of hyaluronan in NRK cell proliferation. The involvement of protein kinase C (PKC) and transforming growth factor-β (TGF-β) in this response was also examined. After 24 hours of incubation in medium containing 25.6 mmol/L glucose, production of hyaluronan by NRK cells was significantly increased compared with medium containing 5.6 mmol/L glucose ($P < .01$). L-glucose and mannitol had no effect on hyaluronan production. High glucose enhanced basal in situ PKC activity ($P < .01$), and both an activator of PKC (phorbol 12-myristate 13-acetate; [PMA]) and TGF-β 1 were able to increase hyaluronan production by NRK cells ($P < .01$). The effect of high glucose on hyaluronan production was diminished by coincubating cells with PKC inhibitors (staurosporine [Stp] or calphostin C [CpC]) or with an anti–TGF-β neutralizing antibody. Stimulation of hyaluronan production by PMA was also normalized by anti–TGFβ neutralizing antibody, but the effect of TGF-β1 was not affected by inhibition of PKC. Finally, incubating quiescent NRK cells with 50 or 100 ng/mL hyaluronan for 24 hours significantly increased NRK cell number ($P < .01$). In conclusion, high glucose stimulates hyaluronan production through the PKC/TGF-β cascade. Increased hyaluronan promotes NRK cell proliferation. These results suggest that hyaluronan may play a role in the pathogenesis of interstitial fibrosis in diabetic kidney disease.

DIABETIC NEPHROPATHY IS characterized histologically by mesangial expansion and glomerular basement membrane thickening.[1-3] Tubulointerstitial fibrosis and other extraglomerular alterations are also critical components of renal injury in diabetic nephropathy.[4-6] Progressive expansion of the renal interstitium leads to elevation of vascular resistance in the pre- and postglomerular vessels, potentially contributing to intraglomerular hypertension.[6] Hyaluronan, a ubiquitous polyanionic glycosaminoglycan, is an important component of the extracellular matrix [reviewed in Laurent and Fraser[7]]. Hyaluronan is synthesized in the plasma membrane and secreted into the extracellular space by fibroblasts and a number of other cell types.[7] Although the physiologic and pathophysiologic roles of hyaluronan in normal and diseased kidneys are not well established, accumulation of hyaluronan in renal interstitium has been associated with several renal pathologies, including ischemic injury,[8] allograft rejection,[9] and interstitial nephritis.[10] Recently, Mahadevan et al[11,12] found increased hyaluronan biosynthesis in glomeruli from diabetic animals and in mesangial cells cultured in high glucose. In addition, hyaluronan is reported to stimulate mesangial cell proliferation, possibly implicating this glycosaminoglycan in the pathogenesis of diabetic nephropathy.[13]

Several investigators have demonstrated increased protein kinase C (PKC) activity and altered subcellular localization of specific PKC isoforms in mesangial cells cultured in the presence of high glucose[14-16] and in glomeruli isolated from diabetic animals.[17-19] These phenotypic changes appear to be dependent on glucose because of the close association between high extracellular glucose levels, elevated intracellular glucose, and the increase in de novo synthesis of diacylglycerol from glycolytic intermediates, which results in activation of PKC.[14] PKC activation triggers mitogen-activated protein kinase (MAPK),[20] leading to upregulation of transforming growth factor-β (TGF-β) and ultimately to enhanced gene expression and synthesis of extracellular matrix proteins, including type I and IV collagens, fibronectin, and laminin.[21,22] Exposure to high-glucose concentrations also increases TGF-β mRNA expression in proximal tubular cells[23] and in renal cortical fibroblasts.[24] Hyaluronan synthesis is stimulated by several intrinsic mediators, including TGF-β, platelet-derived growth factor (PDGF), PKC, and low-density lipoprotein (LDL), depending on the cell type studied.[7,25-27] On the basis of these experimental observations, we hypothesized that hyaluronan synthesis by renal interstitial fibroblasts could be stimulated by exposure to high glucose during diabetes, and that this stimulation might be mediated through activation of the PKC/TGF-β cascade. To test this hypothesis, we examined the effects of high-glucose concentration on hyaluronan production in rat renal interstitial fibroblasts (normal rat kidney [NRK] cells), and assessed whether PKC/TGF-β signaling was associated with this effect. The effect of hyaluronan on NRK cell proliferation was also explored to determine whether increased hyaluronan production might contribute to interstitial fibrosis.

MATERIALS AND METHODS

Materials

Dulbecco's modified Eagle's medium (DMEM) and other culture reagents were obtained from Gibco Life Technologies (Grand Island, NY). Fetal bovine serum was obtained from ICN Biomedical (Costa Mesa, CA); hyaluronan, phorbol 12-myristate 13-acetate (PMA), stau-

From the third Department of Medicine, Diabetes Center, and the fourth Department of Medicine, Kidney Center, Tokyo Women's Medical University School of Medicine, Tokyo, Japan.

Submitted August 1, 2000; accepted January 24, 2001.

Address reprint requests to Tetsuya Babazono, MD, PhD, Diabetes Center, Tokyo Women's Medical University, 8-1 Kawada-cho, Shinjuku-ku, Tokyo 162-8666, Japan.

0026-0495/01/5007-0014$35.00/0
doi:10.1053/meta.2001.24207

rosporine (Stp), and calphostin C (CpC) were obtained from Sigma Chemicals (St Louis, MO), and [γ-^{32}P] adenosine triphosphate (ATP) was purchased from Amersham Pharmacia Biotech (Tokyo, Japan). TGF-β1 and anti–TGF-β neutralizing antibody were obtained from R&D Systems (Minneapolis, MN). The assay kit for hyaluronan was obtained from Reads Medical Products (Westminster, CO), and the kit for determining cell numbers (Cell Counting Kit-8; WST-8) was obtained from Dojin Kagaku (Tokyo, Japan).

Cell Culture

The normal rat kidney fibroblast cell line (NRK 49F) was obtained from the Japanese Cancer Research Resources Bank (Tokyo, Japan). NRK cells were grown in DMEM containing 5% fetal bovine serum, 2.38 mg/mL HEPES, 100 IU/mL penicillin, and 100 μg/mL streptomycin. Confluent NRK cells were incubated for 24 hours in DMEM without serum containing either 5.6 mmol/L or 25.6 mmol/L D-glucose. In studies designed to test the specificity of D-glucose and to rule out any osmotic effect of high-glucose, 20 mmol/L mannitol or L-glucose were added to DMEM containing 5.6 mmol/L D-glucose (to achieve a final concentration of 25.6 mmol/L) and incubated with NRK cells for 24 hours. PMA, PKC inhibitors (Stp, CpC), TGF-β1, and/or anti–TGF-β neutralizing antibody were added at the concentrations described in the text.

Determination of Hyaluronan Synthesis

Hyaluronan production was assessed by measuring the concentration of hyaluronan in the culture medium using a commercially available kit. This kit is based on a sandwich-type binding assay using hyaluronic acid binding protein (HABP). Briefly, diluted sample medium was incubated in HABP-coated microtiter plates for 60 minutes. After washing the wells with wash buffer, 3 μL of peroxidase-conjugated HABP (30 μL/mL) was added. Following an additional 3 washes, substrate solution (200 μg/mL 3,3′,5,5′-tetramethylbenidine, 0.33 μL/mL 30% H_2O_2) was added for 30 minutes. The reaction was stopped by adding 18 μmol H_2SO_4 followed by measurement of absorbance at 450 nm using a microplate reader. Although de novo synthesis of hyaluronan was not measured directly, an increase in hyaluronan concentration in the culture medium was considered to be reflective of an increase in hyaluronan production by the cells.

For cell protein determination, NRK cells were first dissolved in 0.5 mol/L NaOH, and total cell protein was quantified using Bio Rad DC protein assay (Hercules, CA). The hyaluronan content was expressed as nanogram per milligram protein.

In Situ PKC Assay

PKC activity in NRK cells was determined by in situ phosphorylation of the PKC-specific substrate derived from the epidermal growth factor (EGF) receptor (VRKRTLRRL) as described previously.[28] Briefly, confluent NRK cells were incubated with digitonin-based permeabilization buffer, containing 137 mmol/L NaCl, 5.4 mmol/L KCl, 10 nmol/L $MgCl_2$, 0.3 mmol/L Na_2HPO_4, 0.4 mmol/L KH_2PO_4, 25 mmol/L β-glycerophosphate, 5.6 mmol/L glucose, 5 mmol/L EGTA, 2.5 mmol/L $CaCl_2$, 20 mmol/L HEPES, pH 7.2, 50 μg/mL digitonin, 100 μmol/L [γ-^{32}P] ATP (specific activity ~1,000 cpm/pmol), and 300 mmol/L PKC-specific peptide substrate. After a 10-minute incubation at 30°C, the reaction was terminated by adding ice-cold trichloroacetic acid (final concentration, 5%). Aliquots (50 μL) of the acidified reaction mixture were spotted onto 2.1-cm phosphocellulose paper circles (Whatman P81, Maidstone, UK), and washed using 3 changes of 75 mmol/L phosphoric acid, and a final rinse with 75 mmol/L Na_2HPO_4 pH 7.5. The radioactivity retained on the circles was counted in a liquid scintillation counter. All ^{32}P cpm values were normalized for the corresponding total cell protein.

Cell Proliferation Assay

The effect of hyaluronan on NRK cell proliferation was determined by indirect cell counting using a commercial kit. This method is based on the colorimetric conversion of a tetrazolium compound [2-(2-methoxy-4-nitrophenyl)-3-(4-nitro-phenyl)-5-(2,4-disulfophenyl)-2H-tetrazolium, monosodium salt: WST-8] to formazan. The resulting absorbance is directly proportional to the number of living cells, and the method is well correlated with [^{3}H] thymidine incorporation methods.[29]

Subconfluent NRK cells were grown in a 96-well plate and growth arrested by incubation in serum-free medium for 24 hours. Quiescent cells were stimulated with hyaluronan (0 to 100 ng/mL) for 24 hours. At the end of the incubation period, WST-8 was directly added into each well, and absorbance at 450 nm was measured using a microplate reader.

Statistical Analysis

Results are expressed as mean ± SD with the number of experimental determinations indicated as n. Statistical comparisons were performed using 1-way analysis of variance (ANOVA) followed by Fisher's protected least significant difference test.[30] A P value < 0.05 was considered statistically significant. All statistical analyses were performed using the Statview Software on a Macintosh computer.

RESULTS

Exposure of NRK cells to high-glucose concentration resulted in an increase in hyaluronan production (Fig 1). Hyaluronan production by NRK cells, normalized for cell protein values, was increased by 62% ± 21% when cells were incubated for 24 hours in medium containing 25.6 mmol/L D-glucose compared with medium containing 5.6 mmol/L glucose ($P < .01$). Incubation of NRK cells with L-glucose or mannitol for 24 hours did not cause any increase in hyaluronan production (Fig 2), indicating that the response to high glucose was not due to an osmotic effect.

To test whether PKC activation was involved in the glucose-induced increase in hyaluronan production by NRK cells, an activator of PKC (PMA), as well as PKC inhibitors, was used. For cells cultured in normal glucose, incubation with 100 nmol/L PMA for 24 hours caused a 151% ± 21% increase in hyaluronan production (Fig 3). Correspondingly, the increased production of hyaluronan by cells incubated with high glucose was completely inhibited when PKC inhibitors, Stp, 0.5 nmol/L or CpC, 50 nmol/L were added to the medium. These PKC inhibitors did not inhibit hyaluronan production by NRK cells incubated in normal glucose (Fig 3), suggesting that the inhibition of hyaluronan production by the PKC inhibitors was not due to reduced cell viability.

To further investigate the possibility that PKC activation could play a role in the stimulation of hyaluronan production by high glucose, we studied the effect of PMA on PKC activity in NRK cells preincubated in normal and high-glucose medium using an in situ phosphorylation assay. As shown in Fig 4, PMA significantly increased PKC activity in NRK cells preincubated in medium containing normal or high glucose. There was a slight, but significantly greater, basal PKC activity in NRK cells cultured for 24 hours in high glucose to normal glucose (31.5 ± 1.4 v 38.0 ± 0.5 pmol/min/mg protein, $P < .01$). Addition of PMA, as expected, further increased the activation of PKC, while the modest difference in PKC activity

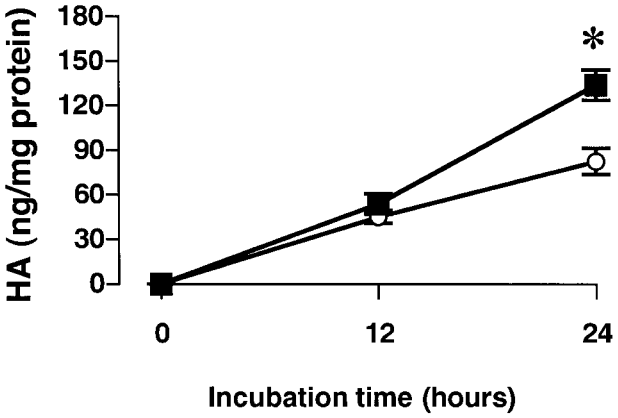

Fig 1. Effect of high glucose on hyaluronan production by NRK cells. NRK cells were incubated for 24 hours in medium containing high glucose (25.6 mmol/L, HG) or normal glucose (5.6 mmol/L, NG), and hyaluronan was measured in the conditioned medium as described in Materials and Methods. Data are expressed as mean ± SD (n = 4). Asterisk (*) indicates a significant difference from NG ($P < .01$) (○, NG; ■, HG).

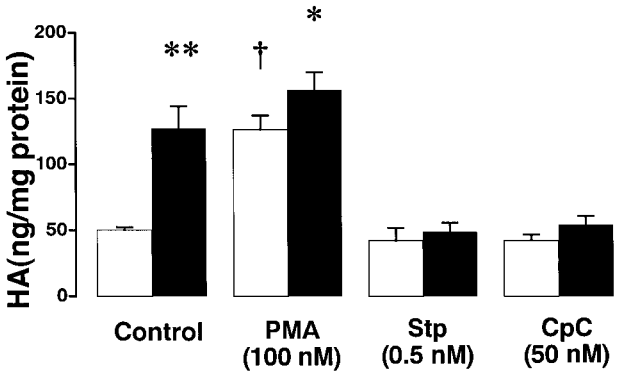

Fig 3. Effect of PKC activation and inhibition on hyaluronan production by NRK cells. The PKC activator PMA (100 nmol/L) stimulated hyaluronan production in the presence of both 5.6 mmol/L (NG) and 25.6 mmol/L glucose (HG). The effect of high glucose on hyaluronan production was inhibited by the PKC inhibitors: Stp, 0.5 nmol/L and CpC, 50 nmol/L. Data are expressed as mean ± SD (n = 4). Significant differences from 5.6 mmol/L glucose (NG) are indicated by *$P < .01$, **$P < .001$, and significant difference from control is indicated by † $P < .01$ (□, NG; ■, HG).

between high glucose and normal glucose-treated cells was maintained or slightly amplified. These data support the hypothesis that PKC activation may play a role in the stimulation of hyaluronan production by high glucose.

In mesangial cells[17] and tubulointerstitial cells,[23,24] high glucose provokes an increase in the synthesis of TGF-β. To ascertain whether TGF-β participates in the high–glucose-induced hyaluronan production by renal interstitial fibroblasts, the effects of TGF-β and an anti–TGF-β neutralizing antibody were investigated. Incubation of NRK cells with 10 ng/mL TGF-β1 for 24 hours caused a 10.9-fold ($P < .01$) and 7.7-fold ($P < .01$) increase in hyaluronan production for conditions of normal and high glucose, respectively (Fig 5). Correspondingly, treating cells with 10 μg/mL anti–TGF-β neutralizing antibody partially attenuated the stimulation of hyaluronan production by high glucose (69% ± 9% of control, Fig 5). When cells cultured in normal glucose were coincubated with both PMA and anti–TGF-β antibody, the increased hyaluronan production by PMA was completely suppressed (Fig 6). In contrast, the PKC inhibitors Stp and CpC did not affect TGF–β1-induced hyaluronan overproduction by NRK cells cultured in normal glucose (Fig 7). These findings suggest that TGF-β acts downstream of PKC in the signal transduction pathway

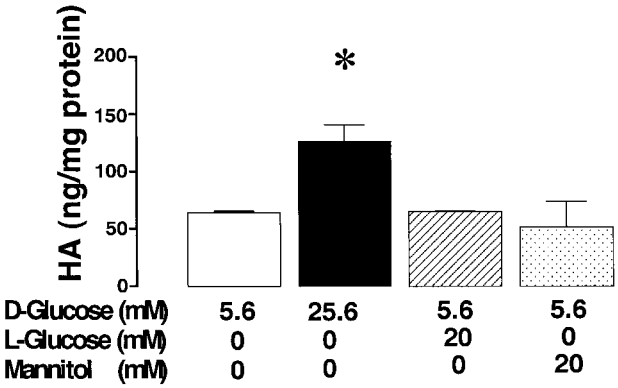

Fig 2. Effect of D-glucose (25.6 mmol/L), L-glucose (20 mmol/L), and mannitol (20 mmol/L) on hyaluronan production by NRK cells. NRK cells were incubated with 25.6 mmol/L D-glucose or 20 mmol/L L-glucose or 20 mmol/L mannitol in medium containing 5.6 mmol/L D-glucose (final carbohydrate concentration, 25.6 mmol/L). Hyaluronan concentrations in medium from cells incubated with 25.6 mmol/L D-glucose medium were significantly higher than from cells cultured in 5.6 mmol/L glucose, but no effect was observed for L-glucose or mannitol. Data are expressed as mean ± SD (n = 4). Significant difference from 5.6 mmol/L D-glucose is indicated by asterisk (*) ($P < .01$).

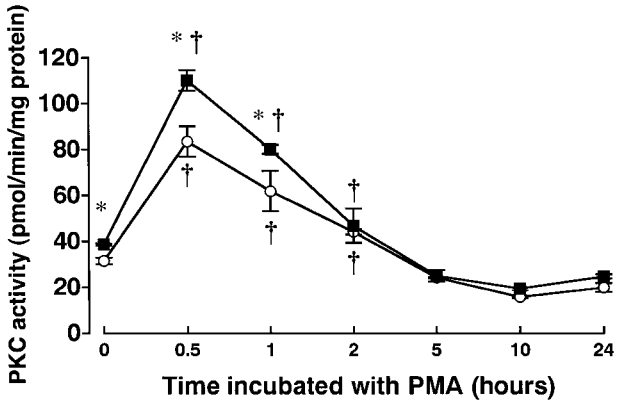

Fig 4. Time course of PKC activation following exposure to PMA. NRK cells were preincubated with high glucose (HG) or normal glucose (NG) medium for 24 hours. After stimulation with 100 nmol/L PMA, PKC activity immediately increased in both NG and HG-treated cells, and this effect was downregulated by 5 hours. Data are expressed as mean ± SD (n = 6). Significant difference from 5.6 mmol/L glucose (NG) is indicated by *$P < .01$. Significant difference from baseline (0 hour) is indicated by † $P < .01$ (○, NG; ■, HG).

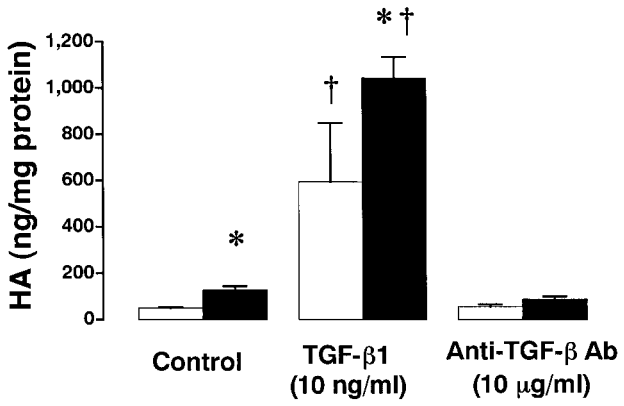

Fig 5. Effect of TGF-β and anti–TGF-β neutralizing antibody on hyaluronan production by NRK cells. Hyaluronan production in both normal glucose (NG) or high glucose (HG)-containing media was significantly increased by TGF-β1 (10 ng/mL). Anti–TGF-β antibody (10 μg/mL) partially attenuated overproduction of hyaluronan mediated by HG. Data are expressed as mean ± SD (n = 4). Significant difference from 5.6 mmol/L glucose (NG) is indicated by *P < .01, significant difference from control is indicated by † P < .01 (□, NG; ■, HG).

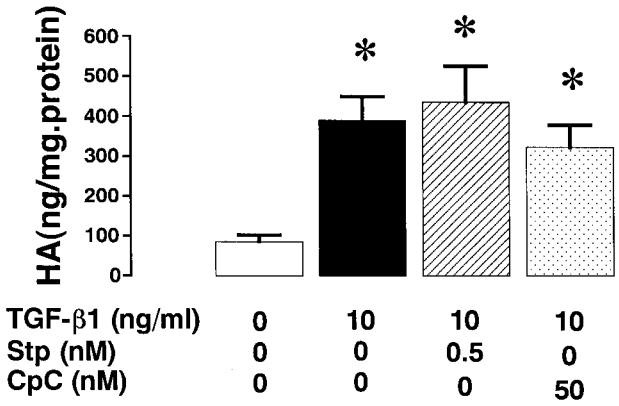

Fig 7. Effect of PKC inhibition on TGF-β–stimulated hyaluronan production. Neither Stp, 0.5 nmol/L nor CpC, 50 nmol/L were able to normalize the increased hyaluronan production caused by TGF-β1. Data are expressed as mean ± SD (n = 4). Significant difference from control is indicated by *P < .01.

that mediates the effect of high glucose on NRK cell hyaluronan production.

Finally, the effect of hyaluronan on NRK cell proliferation was explored to determine whether increased hyaluronan could contribute to interstitial fibrosis. Incubation of quiescent NRK cells with 50 and 100 ng/mL hyaluronan for 24 hours significantly increased NRK cell numbers by 46% ± 12% and 39% ± 11%, respectively ($P < .01$) (Fig 8). This result suggests that hyaluronan production induced by high glucose might lead to fibroblast proliferation, thus contributing to the pathogenesis of interstitial fibrosis.

DISCUSSION

The present study demonstrated that high D-glucose stimulates hyaluronan production in NRK cells. This effect was D-glucose–specific and not due to an osmotic effect of the glucose, as substituting L-glucose or mannitol did not cause a similar response. This study also showed that for NRK cells cultured in normal glucose, PMA-induced PKC activation and exposure to exogenous active TGF-β mimicked the high–glucose-induced increase in hyaluronan production. In addition, PKC inhibitors and an anti–TGF-β neutralizing antibody inhibited the increased production of hyaluronan by NRK cells cultured in high glucose. These results suggest that the effect of high glucose on hyaluronan production by NRK cells is mediated via PKC activation and upregulation of TGF-β

Both PKC and TGF-β have been implicated in the modulation of hyaluronan synthesis by several cell types including skin fibroblasts,[25] peritubular cells from immature rat testis,[31] and neuroblastoma cells.[32] In addition, increased hyaluronan production by glomerular cells from diabetic rats has been reported by Mahadevan et al.[11] They suggested a role of PKC in the high–glucose-induced hyaluronan overproduction by

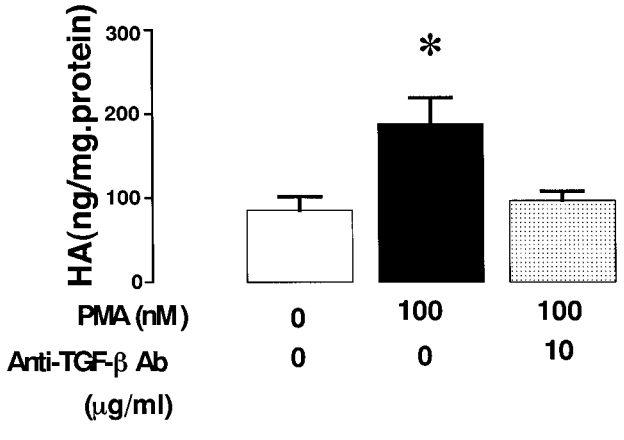

Fig 6. Effect of anti–TGF-β antibody on PMA-stimulated hyaluronan production. Anti–TGF-β antibody (10 μg/mL) normalized the increased hyaluronan production caused by PMA. Data are expressed as mean ± SD (n = 6). Significant difference from control is indicated by *P < .01.

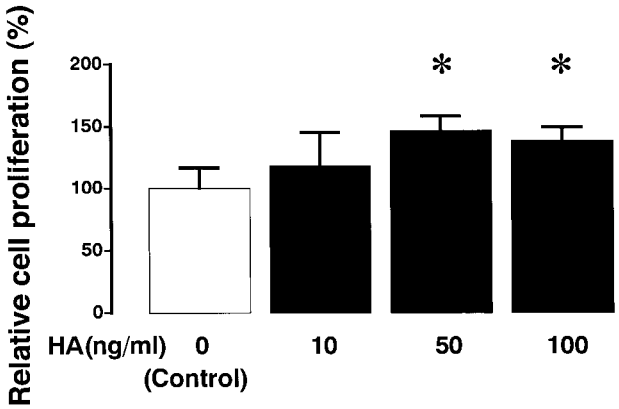

Fig 8. Effect of hyaluronan on NRK cell proliferation. NRK cell counts were significantly increased after incubating with 50 or 100 ng/mL hyaluronan for 24 hours. Data are expressed as mean ± SD (n = 6). Significant difference from control is indicated by *P < .01.

these cells.[12] In the present study, we demonstrated that in renal interstitial cells, upregulation of the PKC-TGF-β pathway is closely associated with hyaluronan production, suggesting that this pathway plays a crucial role in increased hyaluronan synthesis caused by high glucose.

PKC activation in glomeruli from diabetic animals and in mesangial cells exposed to high glucose has been reported by several groups.[14-19] Enhanced TGF-β expression due to high glucose has also been reported in diabetic nephropathy.[23,24,33] In the present study, we demonstrated that increased hyaluronan production caused by PKC activation (using PMA) was attenuated by a TGF-β neutralizing antibody, whereas PKC inhibitors did not affect TGF–β1-induced hyaluronan production. In addition, we showed that exposure to high glucose caused in situ PKC activation (Fig 4), but that incubation with TGF-β1 did not activate PKC (data not shown). These results suggest that TGF-β acts downstream of PKC in the signal transduction pathway evoked by high glucose in NRK cells. By contrast, in cultured vascular smooth muscle cells, TGF-β has been shown to directly activate PKC.[34] The reason for this difference is unclear, but may relate to differences in the cell types. PKC consists of a family of at least 12 isoforms classified according to their calcium-and phospholipid-dependence into 3 categories: classical, novel, and atypical.[18] In diabetic glomeruli or mesangial cells cultured in high glucose, selective PKC isoforms, especially diacylglycerol-sensitive classical and novel PKC isoforms are activated; however, controversy exists on the PKC isoform selectivity.[17-19] Thus, effects of high glucose on renal interstitial cell PKC isoform expression and subcellular distribution require further study.

The major hyaluronan receptor is CD44, a 90-kd molecule that is localized on the surface of many cell types.[32] Biological functions of hyaluronan mediated through interaction with the CD44 receptor are not well understood, however, several studies suggest that hyaluronan can promote cell proliferation through this receptor.[35-37] We also found a stimulatory effect of hyaluronan on NRK cell proliferation (Fig 8). It is therefore possible that increased hyaluronan production caused by high glucose could promote renal interstitial fibrosis, which is associated with renal fibroblast proliferation.[38] Cultured skin fibroblasts obtained from type 1 diabetics with nephropathy have been demonstrated to exhibit increased cellular proliferation and collagen synthesis compared with fibroblasts from diabetic patients without nephropathy.[39] Recent in vitro studies suggest that high glucose stimulates cell proliferation and collagen synthesis in renal cortical fibroblasts.[24,40] While we did not investigate the effect of high glucose on matrix protein production, our results suggest that increased hyaluronan production by interstitial fibroblasts could contribute to the deposition of interstitial matrix material in diabetic nephropathy.

Whether hyaluronan provides a promotional, defensive, or passive role in interstitial fibrosis remains to be determined. Several glycosaminoglycans have been reported to have a renoprotective role in the kidney. Gambaro et al[41,42] demonstrated that sulfated glycosaminoglycan inhibits PKC/TGF-β1 in experimental diabetic nephropathy. On the other hand, hyaluronan has been shown to suppress sulphation of matrix glycosaminoglycans, ie, proteoglycans,[43,44] which alter the charge barrier during glomerular filtration. Increased hyaluronan in the renal interstitium in diabetes may also affect tubulointerstitial matrix structure; however, its contribution to diabetic nephropathy is unestablished. Further investigation will be necessary to clarify the role of hyaluronan in diabetic kidney disease.

In conclusion, high glucose increases hyaluronan production by renal interstitial cells in culture through a mechanism independent of its osmotic effect. This increased production of hyaluronan is mediated by activation of the PKC/TGF-β cascade. In addition, increased hyaluronan levels stimulate proliferation of interstitial fibroblasts. These results suggest that increased hyaluronan production by renal interstitial fibroblasts caused by elevated glucose may play a role in the pathogenesis of interstitial fibrosis in diabetic kidney diseases.

ACKNOWLEDGMENT

We would like to thank James B. Moberly (Renal Division, Baxter Healthcare Corporation) for his valuable comments on the manuscript.

REFERENCES

1. Osterby R, Gundersen HJG: Glomerular size and structure in diabetes mellitus. I. Early abnormalities. Diabetologia 11:225-229, 1975

2. Osterby R: Morphometric studies of the peripheral glomerular basement membrane in early juvenile diabetes. I. Development of initial basement membrane thickening. Diabetologia 8:84-92, 1972

3. Chavers BM, Bilous RW, Ellis EN, et al: Glomerular lesions and urinary albumin excretion in type I diabetes mellitus without overt proteinuria. N Engl J Med 320:966-970, 1989

4. Mauer SM, Steffes MW, Ellis EN, et al: Structural-functional relationships in diabetic nephropathy. J Clin Invest 74:1143-1155, 1984

5. Lane PH, Steffes MW, Fioretto P, et al: Renal interstitial expansion in insulin-dependent diabetes mellitus. Kidney Int 43:661-667, 1993

6. Ziyadeh FN, Goldfarb S: The renal tubulointerstitium in diabetes mellitus. Kidney Int 39:464-475, 1991

7. Laurent TC, Fraser JRE: Hyaluronan. FASEB J 6:2397-2404, 1992

8. Johnson C, Tufveson G, Wahlberg J, et al: Experimentally induced warm renal ischemia induces cortical accumulation of hyaluronan in the kidney. Kidney Int 50:1224-1229, 1996

9. Wells A, Larsson E, Hanas E, et al: Increased hyaluronan in acutely rejecting human kidney grafts. Transplantation 55:1346-1349, 1993

10. Sibalic V, Fan X, Loffing J, et al: Upregulated renal tubular CD44, hyaluronan and osteopontin in kdkd mice with interstitial nephritis. Nephrol Dial Transplant 12:1344-1353, 1997

11. Mahadevan P, Larkins RG, Fraser JRE, et al: Increased hyaluronan production in the glomeruli from diabetic rats: A link between glucose-induced prostaglandin production and reduced sulphated proteoglycan. Diabetologia 38:298-305, 1995

12. Dunlop MJ, Clark S, Mahadevan P, et al: Production hyaluronan by glomerular mesangial cells in response to fibronectin and platelet-derived growth factor. Kidney Int 50:40-44, 1996

13. Mahadevan P, Larkins RG, Fraser JRE, et al: Effect of prostaglandin E2 and hyaluronan on mesangial cell proliferation. A potential contribution to glomerular hypercellularity in diabetes. Diabetes 45:44-50, 1996

14. Ayo SH, Radnik R, Garoni JA, et al: High glucose increases

diacylglycerol mass and activates protein kinase C in mesangial cell culture. Am J Physiol 261:F571-F577, 1991

15. Derbertis FR, Craven PA: Activation of protein kinase C in glomerular cells in diabetes. Mechanisms and potential links to the pathogenesis of diabetic glomerulopathy. Diabetes 43:1-8, 1994

16. Kikkawa R, Haneda M, Uzu T, et al: Translocation of protein kinase C α and ζ in rat glomerular mesangial cells cultured under high glucose conditions. Diabetologia 37:838-841, 1994

17. Koya D, Jirousek MR, Lin Y-W, et al: Characterization of protein kinase C β 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

18. Ishii H, Jirousek MR, Koya D, et al: Amelioration of vascular dysfunctions in diabetic rats by an oral PKC β inhibitor. Science 272:728-731, 1996

19. Babazono T, Kapor-Drezgic J, Whiteside C: Glomerular cell localization of protein kinase C isoforms and altered expression in diabetes. Diabetes 47:668-676, 1998

20. Haneda M, Araki S, Togawa M, et al: 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

21. Roberts AB, McCune BK, Sporn MB: TGF-β1: Regulation of extracellular matrix. Kidney Int 44:948-958, 1993

22. Border WA, Noble NA: Transforming growth factor-β in tissue fibrosis. N Engl J Med 331:1286-1292, 1994

23. Rocco M, Chen Y, Goldfarb S, et al: Elevated glucose stimulates TGF-β gene expression and bioactivity in proximal tubule. Kidney Int 41:107-114, 1992

24. Han DC, Isono M, Hoffman BB, et al: High Glucose stimulates proliferation and collagen type I synthesis in renal cortical fibroblasts: Mediated by autocrine activation of TGF-β. J Am Soc Nephrol 10: 1891-1899, 1999

25. Suzuki M, Asplund T, Yamashita H, et al: Stimulation of hyaluronan biosynthesis by platelet-derived growth factor-BB and transforming factor-β1 involves activation of protein kinase C. Biochem J 307:817-821, 1995

26. Heldin P, Laurent TC, Heldin CH: Effect of growth factors on hyaluronan synthesis in cultured human fibroblasts. Biochem J 258: 919-922, 1989

27. Chana RS, Wheeler DC, Thomas GJ, et al: Low-density lipoprotein stimulates mesangial cell proteoglycan and hyaluronan synthesis. Nephrol Dial Transplant 15:167-172, 2000

28. Kapor-Drezgic J, Zhou X, Babazono T, et al: Effect of high glucose on mesangial cell protein kinase C-δ and -ε is polyol pathway-dependent. J Am Soc Nephrol 10:1193-1203, 1999

29. Ishiyama M, Miyazono Y, Sasamoto K, et al: A water-soluble tetrazolium salt useful for colorimetric cell viability assay. Anal Commun 36:47-50, 1997

30. Dowdy S, Wearden S: Statistics for Research, ed 2. New York, NY, Wiley, 1995

31. Thiébot B, Langris M, Bonnamy P-J, et al: Activation of protein kinase C pathway by phorbol ester results in a proteoglycan synthesis increase in peritubular cells from immature rat testis. Biochim Biophys Acta 1426:151-167, 1999

32. Fichter M, Hinrichs R, Eissner G, et al: Expression of CD44 isoforms in neuroblastoma cells is regulated by P13-kinase and protein kinase C. Oncogene 14:2817-2824, 1997

33. 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

34. Dalton ML, Gadson PF, JR, Wrenn RW, et al: Homocysteine signal cascade: Production of phospholipids, activation of protein kinase C, and the induction of c-fos and c-myb in smooth muscle cells. FASEB J 11:703-711, 1997

35. Hronowski L, Anassiades TP: The effect of cell density on net rates of glycosaminoglycan synthesis and secretion by cultured fibroblasts. J Biol Chem 255:10091-10099, 1980

36. Matsuoka K, Mitsui Y, Murota S, et al: Actions of exogenous heparan sulphate and hyaluronic acid on growth and thymidine incorporation of normal and transformed human fibroblasts. Cell Biol Int Resp 9:815-824, 1985

37. Prehm P: Synthesis of hyaluronate in differentiated tetracarcinoma cells. Characterization of the synthase. Biochem J 211:181-189, 1983

38. Struzt F: Novel aspects of renal fibrogenesis. Nephrol Dial Transplant 10:1526-1532, 1995

39. Trvisan R, Li LK, Messent J, et al: Na^+/H^+ antiport activity and cell growth in cultured skin fibroblasts of IDDM patients with nephropathy. Diabetes 41:1239-1246, 1992

40. Jones SC, Saunders HJ, Qi W, et al: Intermittent high glucose enhances cell growth and collagen synthesis in cultured human tubulointerstitial cells. Diabetologia 42:1113-1119, 1999

41. Gambaro G, Ceol M, Facchin S, et al: Glycosaminoglycan prevents hyperglycemia-induced renal TGF-β1 gene expression. Nephrol Dial Transplant 14:20-21, 1999 (suppl 4)

42. Ceol M, Gambaro G, Sauer U, et al: Glycosaminoglycan therapy prevents TGF-β1 overexpression and pathologic changes in renal tissue of long-term diabetic rats. J Am Soc Nephrol 11:2324-2336, 2000

43. Handley CJ, Lowther DA: Inhibition of proteoglycan biosynthesis by hyaluronic acid in chondrocytes in cell culture. Biochim Biophys Acta 444:69-74, 1986

44. Shimazu A, Jikko A, Iwamoto M: Effect of hyaluronic acid on the release of proteoglycan from the cell matrix in rabbit chondrocyte cultures in the presence and absence of cytokines. Arthritis Rheum 36:247-253, 1993