Regulation of the Laminin C1 Promoter in Cultured Mesangial Cells

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Expression of the genes encoding several matrix proteins, including the laminin γ1 and β1 subunits, is increased in glomeruli or renal cortex from diabetic animals or in mesangial cells cultured in high concentrations of glucose. Transforming growth factor (TGF)-β1 and IGF-1 have been implicated as mediators of this response. In the present study, we assessed the influence of high glucose concentrations and the roles of TGF-β1 and IGF-1 in the regulation of laminin C1 gene expression in cultured mesangial cells. Culture of normal rat mesangial cells (RMC) or SV40-transformed mouse mesangial (MES-13) cells in 500 mg/dl D-glucose for 2 days to 3 weeks significantly increased laminin C1 mRNA abundance compared with cells cultured in 100 mg/dl D-glucose. IGF-1 also increased laminin C1 mRNA abundance in RMC or MES-13 cells, whereas TGF-β1 was without effect. The influence of raising the medium glucose concentration on laminin C1 promoter activity was further studied in MES-13 cells that had been stably transfected with a reporter gene containing the promoter linked to luciferase. Culture in 500 mg/dl D-glucose for 4 h to at least 1 week increased laminin C1 promoter activity compared with cells maintained in 100 mg/dl glucose. In contrast, culture of cells in medium that contained 400 mg/dl mannitol or 400 mg/dl L-glucose in addition to 100 mg/dl D-glucose did not increase laminin C1 promoter activity. The ability of high glucose to increase laminin C1 promoter activity was absolutely dependent on the presence of serum. Consistent with results obtained with mRNA, TGF-β1 had no influence on promoter activity in stable integrants. Whereas IGF-1 transiently increased promoter activity in stable integrants, the increase was not sustained (6 h). Moreover, neutralizing antibody to TGF-β or to IGF-1 receptor did not suppress increases in laminin C1 promoter activity induced by culture of stable integrants in high glucose. Several inhibitors of protein kinase C, including bisindolylmaleimide (GFX), myristoylated PKC inhibitor peptide, and LY333531, were also without effect on increases in laminin C1 promoter activity induced by culture in high glucose. Exposure to the NO donor (±)-s-nitroso-n-acetylpenicillamine (SNAP) blocked increases in laminin C1 promoter activity induced by serum and by culture in high glucose without influencing promoter activity in cells cultured in the absence of serum and in 100 mg/dl glucose. The ability of high glucose concentrations and IGF-1 to increase laminin C1 promoter activity in cultured mesangial cells, and the suppression of glucose actions by the NO donor SNAP, provide potential mechanisms whereby the synthesis of the laminin γ1 chain may be regulated in the glomerulus in diabetes. Of note, the mechanism by which high glucose increases laminin C1 promoter activity appears to differ from mechanisms previously described for some other glucose actions on matrix protein synthesis. In this regard, TGF-β and protein kinase C were not implicated as mediators of the effect of high glucose on laminin C1 promoter activity. *Diabetes* 48:2083–2089, 1999

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FBS, fetal bovine serum; ITS, 5 µg/ml insulin, 5 µg/ml transferrin, and 5 ng/ml sodium selenite; MES-13 cell, SV40-transformed mouse mesangial cell; PDGF, platelet-derived growth factor; PKC, protein kinase C; RMC, rat mesangial cell; SNAP, (±)-s-nitroso-n-acetylpenicillamine; TGF, transforming growth factor; TRE, phorbol ester response element.

Hyperglycemia and its metabolic consequences play major roles in the development and progression of diabetic nephropathy (1). Selective mesangial matrix expansion, resulting in obliteration of the capillary lumen and loss of filtration surface area, is the hallmark of diabetic nephropathy (2). Expression of the genes encoding the laminin γ1 and β1 subunits (3–5), fibronectin (5,6), and the α1(I) (4), α2(I) (7), α1(III) (4), and α1(IV) (3–7) chains of collagen are increased in glomeruli (4) or renal cortex (6) from diabetic animals or in mesangial cells cultured in high concentrations of glucose (3,5,7), suggesting that, through these actions on mesangial cells, hyperglycemia may contribute to the progressive expansion of the glomerular mesangium that occurs in diabetic nephropathy.

Transforming growth factor (TGF)-β1 has been implicated in the increased levels of α1(IV) collagen and fibronectin mRNA in renal cortex from diabetic animals (6) and of α1(IV) and α2(I) collagen chains and mRNA in mesangial cells cultured in high glucose (7). Moreover, TGF-β1 has been shown to increase the activity of the promoter of the fibronectin (8) and α2(I) collagen (9) genes. Platelet-derived growth factor (PDGF) (10,11) and IGF-1 (12,13) have also been implicated in the synthesis of mesangial matrix proteins. However, the influence of high glucose concentrations and the role of TGF-β1 and other growth factors in regulation of laminin C1 gene transcription have not been assessed.

Studies conducted in vivo in experimental animals (14,15) and in vitro in cultured mesangial cells (16–18) suggest that activation of protein kinase C (PKC), and in particular its β isozyme, may contribute to both functional and structural changes that occur in the glomerulus in diabetes. Similarly,

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studies in cultured mesangial cells from our own and other laboratories have implicated activation of PKC as a signal to increase the TGF-β and matrix protein synthesis that occurs in these cells in response to high glucose (19), TXA_2 (20), low-density lipoproteins (21), angiotensin II (16), and mechanical stretch (22).

In the present study, we demonstrate that high glucose concentrations activate the promoter of the laminin C1 gene in mesangial cells. The roles of TGF-β1, IGF-1, and PKC in mediating activation by high glucose of the laminin C1 promoter were assessed. Because studies conducted both in vitro (23) and in vivo (23,24) in our own and other laboratories have implicated NO in the suppression of glomerular and mesangial cell matrix protein synthesis, we also examined the influence of NO on activation of the laminin C1 promoter by high glucose concentrations.

RESEARCH DESIGN AND METHODS

Culture of rat mesangial cells. Glomeruli were prepared from rat renal cortex under sterile conditions by graded sieving as previously described in detail (19). Rat mesangial cells (RMCs) were cultured from collagenase-treated glomeruli as previously reported (19). RMCs had typical stellate morphology, stained positively for smooth muscle actin and thy1.1, and were negative for factor VIII. RMCs were maintained in RPMI 1640 containing 15% fetal bovine serum (FBS); penicillin (100 U/ml); streptomycin (100 µg/ml); 5 µg/ml insulin, 5 µg/ml transferrin, and 5 ng/ml sodium selenite (ITS); and 100 mg/dl glucose at 37°C in 5% CO_2, 95% air. Where indicated, the concentration of glucose in the medium was raised to 500 mg/dl. RMCs were passaged at weekly intervals, and medium was changed at 3- to 4-day intervals or when 80% confluent.

Culture of SV40-transformed mouse mesangial cells. SV40-transformed mouse mesangial (MES-13) cells were obtained from ATCC (Rockville, MD) and maintained in RPMI 1640 containing 5% FBS, penicillin (100 U/ml), streptomycin (100 µg/ml), and 100 mg/dl glucose at 37°C in 5% CO_2, 95% air. Cells were passaged three times per week. Where indicated, the concentration of glucose in the medium was raised to 500 mg/dl.

Extraction of RNA and Northern analysis. Northern analysis was performed on RNA extracted from RMCs or MES-13 cells with Ultraspec (Biotecx Laboratories, Houston, TX) (25). RNA (7 µg) was resolved on 1% agarose gels containing 3% formaldehyde. To assess sample loading, RNA was stained with ethidium bromide and photographed, and the film was subjected to quantitative densitometry. RNA was then transferred to nylon membranes (Du Pont-NEN, Boston, MA) by capillary blotting and cross-linked to the membrane by irradiation with ultraviolet light. Membranes were prehybridized for 4 h at 52°C in 50% formamide, 0.25 mol/l Na_2HPO_4 (pH 7.2), 0.25 mol/l NaCl, 1 mmol/l EDTA, 100 µg/ml denatured salmon sperm DNA, and 7% SDS and hybridized for 16 h at 42°C in fresh solution of the same composition containing 5 ng/ml heat-denatured radiolabeled cDNA probe. cDNA probes for α1(IV) collagen and laminin C1 mRNA were prepared from pCIV-1-PE16 (ATCC) and p16 (26), respectively, using restriction enzymes, purified by electrophoresis, and labeled with [^{32}P]dCTP to a specific activity of at least 1×10^9 dpm/µg by random-primed labeling (Boehringer Mannheim, Mannheim, Germany). Labeled cDNAs were separated from unincorporated nucleotides using Sephadex G-50 spin columns. The hybridized membranes were washed for 15 min at 52°C two times each in *1*) 2 × sodium chloride–sodium citrate (0.30 mol/l NaCl, 0.030 mol/l Na citrate), 0.1% SDS, pH 7.2; *2*) 25 mmol/l Na_2HPO_4, 1 mmol/l EDTA, 0.1% SDS, pH 7.2; and *3*) 25 mmol/l Na_2HPO_4, 1 mmol/l EDTA, 1% SDS, pH 7.2. mRNA abundance was measured by phosphorimager analysis and expressed as relative abundance (isotope content of an mRNA band divided by the relative amount of ethidium bromide–stained 28S rRNA).

Transfection of cells. Construction of the luciferase reporter gene *pHC1Luc*, which is driven by the laminin C1 promoter, has been described (27). Cells were grown to 60–80% confluence in a T75 flask, trypsinized, resuspended in RPMI plus 5% FBS, and counted. The cells were centrifuged, resuspended in cold HEPES-buffered saline (137 mmol/l NaCl, 5 mmol/l KCl, 0.7 mmol/l Na_2HPO_4, 6 mmol/l dextrose, 21 mmol/l HEPES, pH 7.0) at a concentration of ~10^6 cells/0.45 ml, and placed on ice. A total of 10 µg circular DNA was added to 0.4 ml of the cell suspension in an electroporation cuvette. The DNA mixture contained 5.3 µg pHC1Luc, 0.4 µg pRLneo for selection of stable integrants, and pUC18 to bring the final DNA mass to 10 µg. After electroporation at 500 V and 700 µF, the cell suspension was diluted to 12 ml with complete medium and distributed equally among the wells of a six-well plate. After cultivation for 3–5 days, stable integrants of pHC1Luc were selected by growth in 400 µg/ml G-418. After several weeks, individual or pooled clones were recovered and grown in medium containing G-418 at 200 µg/ml.

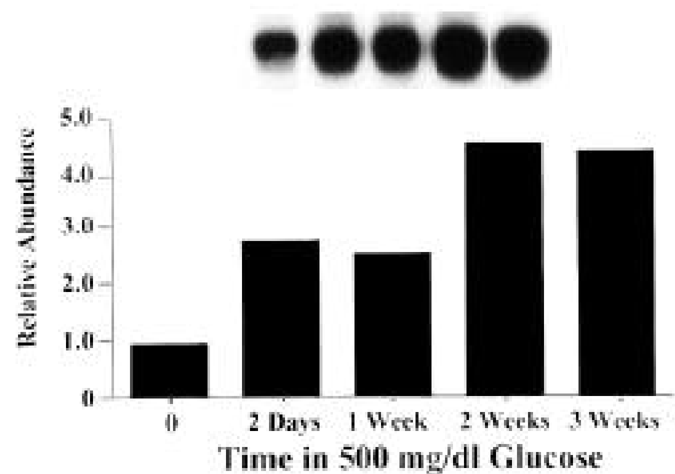

FIG. 1. Effects of culture of RMCs in high glucose on α1(IV) collagen mRNA. RMCs were cultured in complete medium containing 15% FBS and 100 mg/dl glucose. Medium was changed every 2–3 days, and cells were passaged once a week. The concentration of glucose in the medium was raised to 500 mg/dl for the times indicated. RNA was extracted and Northern analysis performed as described in METHODS. Data shown are from a single representative experiment replicated in a separate study.

Determination of luciferase activity. Cells were rapidly washed three times in phosphate-buffered saline and lysed by addition of 0.25 ml of 25 mmol/l Trisphosphate, pH 7.8, 2 mmol/l dithiothreitol, 1 mmol/l EDTA, 0.1% Triton X-100, and 10% glycerol. After incubation at room temperature for 10 min, the lysate was clarified by centrifugation for 1 min in a microcentrifuge at room temperature, and the supernatant was stored at –70°C. Luciferase activity was measured as described (27). Protein was determined by the Bradford procedure using a Bio-Rad assay kit (Richmond, CA).

Materials. Neutralizing pan-specific TGF-β antibody, neutralizing anti-human PDGF antibody, normal rabbit IgG, and recombinant human IGF-1 were obtained from R&D systems (Minneapolis, MN). IGF-1 receptor antibody, bisindolylmaleimide (GFX), myristoylated PKC inhibitor peptide, and (±)-s-nitroso-n-acetylpenicillamine (SNAP) were obtained from Calbiochem (La Jolla, CA). LY333531 was a gift from Lilly Research Laboratories (Indianapolis, IN).

RESULTS

Laminin C1 mRNA abundance. Figure 1 illustrates the effect of 500 mg/dl glucose on α1(IV) collagen mRNA abun-

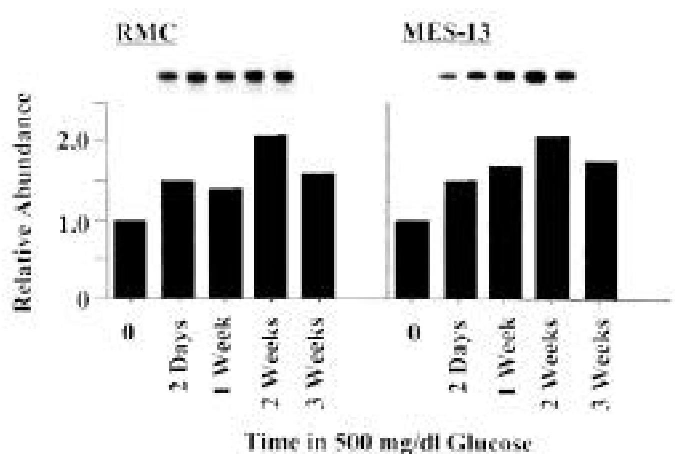

FIG. 2. Effects of high glucose on laminin C1 mRNA in RMCs or MES-13 cells. Studies were conducted as described in Fig. 1, except MES-13 cells were routinely cultured in 5% FBS and passaged three times a week. Data shown are from a single representative experiment replicated in a separate study.

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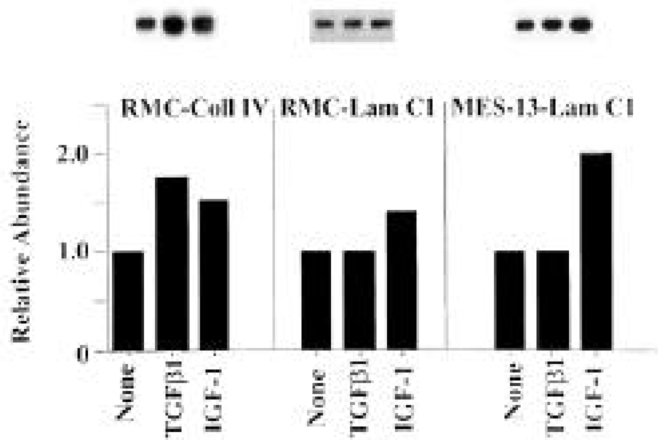

FIG. 3. Effects of TGF-β1 or IGF-1 on α1(IV) collagen and laminin C1 mRNA in RMCs and MES-13 cells. RMCs and MES-13 cells were cultured in complete medium containing 15 and 5% FBS, respectively. For RMCs, the FBS concentration was reduced to 0.5% and ITS was removed from the medium for the final 48 h. MES-13 cells were cultured in serum-free medium for the final 48 h. Where indicated, TGF-β1 (100 pmol/l) or IGF-1 (100 ng/ml) was added to the cultures for the final 48 h. Data shown are from a single representative experiment replicated in a separate study.

dance in primary RMCs. Culture in high glucose progressively increased α1(IV) collagen mRNA abundance beginning at 2 days, with a maximal four- to fivefold increase occurring at 2 and 3 weeks. As shown by a single representative experiment in Fig. 2, culture in 500 mg/dl glucose for 2 days to 3 weeks also progressively enhanced laminin C1 mRNA abundance in RMCs and MES-13 cells. When results from two experiments were averaged, culture in high glucose significantly increased laminin C1 mRNA in RMCs at 1, 2, and 3 weeks by 1.45 ± 0.05–, 1.95 ± 0.15–, and 1.5 ± 0–fold, respectively (mean ± SE, $P < 0.05$ vs. 100 mg/dl glucose). Culture in high glucose also significantly increased laminin C1 mRNA in MES-13 cells at 1, 2, and 3 weeks by 1.4 ± 0.1–, 1.9 ± 0.1–, and 1.8 ± 0.21–fold, respectively. The increases in laminin C1 mRNA were less than those observed for α1(IV) collagen mRNA.

Both TGF-β1 (7) and the IGF-1 system (28) have been shown to be activated in mesangial cells cultured in high glucose and have been implicated as mediators of the actions of high glucose on extracellular matrix protein synthesis. As illustrated by a single experiment in Fig. 3, addition of either TGF-β or IGF-1 to RMCs cultured in 100 mg/dl glucose increased collagen IV mRNA abundance. When results from two experiments were averaged, TGF-β or IGF-1 significantly increased collagen IV mRNA by 1.6 ± 0.1– and 1.4 ± 0.1–fold, respectively ($P < 0.05$). IGF-1 also increased laminin C1 mRNA in both RMCs and MES-13 cells (Fig. 3). When results from two experiments were averaged, IGF-1 significantly increased laminin C1 mRNA in RMCs and MES-13 cells by 1.4 ± 0.1– and 2.0 ± 0.1–fold, respectively ($P < 0.05$). In contrast to results obtained with α1(IV) collagen mRNA, however, addition of TGF-β had no effect on laminin C1 mRNA abundance in either RMCs or MES-13 cells (Fig. 3).

Stably integrated laminin C1 promoter. The mechanism by which culture in high glucose enhances laminin C1 mRNA abundance was further examined in MES-13 cells containing pHC1Luc stably integrated into chromatin. pHC1Luc is a luciferase reporter gene containing the laminin C1 promoter (27). As illustrated in Table 1, laminin C1 promoter activity in pooled G418-resistant clones increased 48 h after the glucose concentration was raised from 100 to 500 mg/dl. Moreover, the increase in promoter activity caused by high glucose concentrations was absolutely dependent on the presence of serum in the culture medium. Comparable increases in promoter activity were also detected in single clones (Table 1). Quantitative differences in reporter gene activity between the clones are thought to be due to differences in the number of integrated copies or differences in the chromatin environment at different sites of reporter gene integration. As is also shown in Table 1, serum deprivation reduced the activity of the stably integrated laminin C1 promoter in all of the cell lines.

Figure 4 illustrates the time course of laminin C1 promoter activation in stable integrants after the increase in glucose concentration. Where indicated, cells were deprived of serum for the final 48 h of culture. In the presence of 5% FBS, culture in 500 mg/dl glucose for 4 or 6 h increased laminin C1 promoter activity approximately twofold. Raising the glucose concentration did not increase promoter activity in the absence of serum. In other experiments, the increase in laminin C1 promoter activity was stable for at least 1 week after the increase in glucose concentration (data not shown). Moreover, addition of 400 mg/dl L-glucose or mannitol for 1, 6, or 48 h to cells cultured in 100 mg/dl D-glucose did not increase laminin C1 promoter activity beyond that seen in cells cultured in 100 mg/dl D-glucose (data not shown).

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TABLE 1
Laminin C1 promoter activity in stably integrated pHC1Luc: effects of culture in high glucose with and without serum

	Relative light units/μg protein ($\times 10^{-3}$)			
	Without FBS		With 5% FBS	
	100 mg/dl glucose	500 mg/dl glucose	100 mg/dl glucose	500 mg/dl glucose
Pooled clones	88 ± 14	100 ± 9.4	189 ± 20*	288 ± 20*†
Clone 1	28 ± 1	22 ± 4	40 ± 1*	52 ± 1*†
Clone 2	97 ± 2	68 ± 6	144 ± 6*	300 ± 4*†
Clone 3	61 ± 2	52 ± 2	156 ± 3*	314 ± 1*†
Clone 4	48 ± 0	53 ± 0	103 ± 3*	135 ± 1*†

Single G418-resistant clones containing stably integrated pHC1Luc were isolated with cloning cylinders. Pooled G418-resistant clones were harvested from culture dishes containing ~20 colonies. Cells were seeded in 24-well plates and allowed to attach overnight. Medium was changed to that containing the indicated concentration of FBS and glucose. Cells were lysed 48 h after the medium change for measurement of luciferase activity and protein. *$P < 0.05$ vs. without FBS; †$P < 0.05$ vs. with 100 mg/dl glucose.

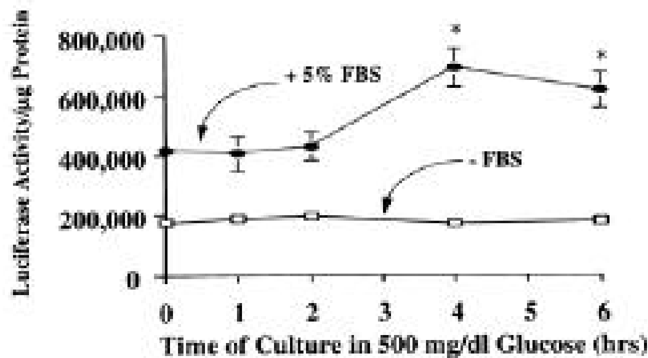

FIG. 4. Time course of laminin C1 promoter activation by high glucose in stably integrated pHC1Luc in the presence and absence of serum. Mixed clones of MES-13 cells stably transfected with pHC1Luc were seeded in 24-well plates and allowed to attach overnight. Medium was changed to fresh serum-free medium or fresh medium containing 5% FBS for the final 48 h of incubation. The concentration of glucose in the medium was raised to 500 mg/dl for the indicated times. Luciferase activity is expressed as relative light units per microgram of protein. Results are means ± SE from two separate experiments. *$P < 0.05$ vs. no FBS.

As illustrated in Fig. 5, neutralizing antibody to TGF-β did not influence laminin C1 promoter activity in stable integrants cultured in 100 mg/dl glucose or in cells cultured for 6 or 48 h in 500 mg/dl glucose. Moreover, in other experiments, exposure to TGF-β1 (10–100 pmol/l) for 48 h did not significantly increase laminin C1 promoter activity in cells cultured in serum-free medium with 100 or 500 mg/dl glucose (data not shown).

As illustrated in Fig. 6, IGF-1 (100 ng/ml) increased laminin C1 promoter activity when present for 6 h in serum-free mesangial cell cultures. Antibody to IGF-1 receptor significantly reduced laminin C1 promoter activity in cells grown in serum-free medium and blocked the stimulatory effect of IGF-1. In contrast, antibody to IGF-1 receptor was without effect on laminin C1 promoter activity in cells grown in the presence of serum and did not suppress the stimulatory effect of high glucose on promoter activity (Fig. 6). In other studies (not shown) the stimulatory effect of IGF-1 on promoter activity was transient and not sustained for 48 h.

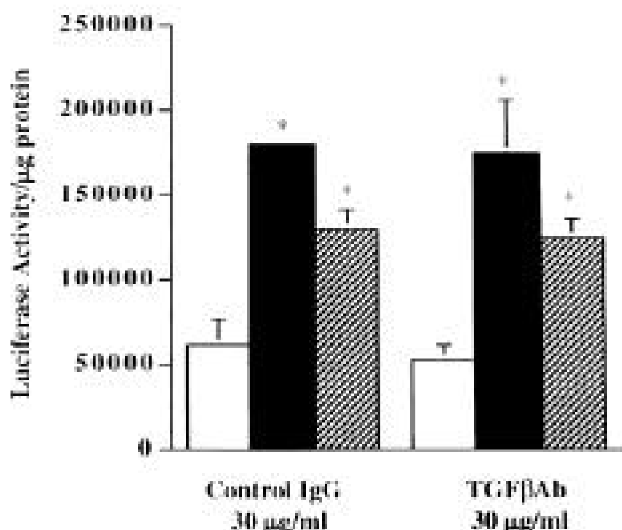

FIG. 5. Effects of TGF-β antibody on laminin C1 promoter activation in stably integrated pHC1Luc in high and low glucose. Studies were conducted as described in Fig. 4. Cells were maintained in medium containing 5% FBS for the entire experiment. Neutralizing pan-specific TGF-β antibody or control IgG was present where indicated for the final 48 h. The concentration of glucose in the medium was maintained at 100 mg/dl for the entire experimental period (□) or was raised to 500 mg/dl for the final 6 (■) or 48 (▨) h. Results are means ± SE from three separate experiments. *$P < 0.05$ vs. 100 mg/dl glucose.

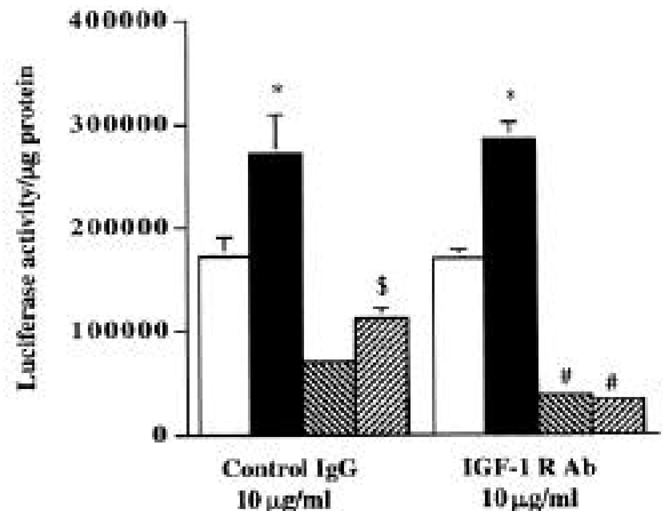

FIG. 6. Influence of IGF-1 receptor antibody on laminin C-1 promoter activity and responses to serum, high glucose, and IGF-1 in stably integrated pHC1Luc. Studies were conducted as described in Fig. 4. Cells were cultured in the presence of 5% serum (□, ■) or in serum–free medium (▨, ▨). The concentration of glucose in the medium was raised to 500 mg/dl (■) or IGF-1 (100 ng/ml) was added (▨) for the final 6 h of incubation. Where indicated, control IgG or IGF-1 receptor antibody was added 30 min before raising the concentration of glucose or adding IGF-1. *$P < 0.05$ vs. 100 mg/dl glucose; $\$P < 0.05$ vs. absence of IGF-1; #$P < 0.05$ vs. control IgG.

As illustrated in Fig. 7, exposure to the NO donor SNAP completely prevented increases in laminin C1 promoter activity induced by serum. SNAP also partially suppressed the increase in promoter activity induced by high glucose concentrations. Of note, SNAP was without significant effect on basal promoter activity observed in serum-free medium containing 100 mg/dl glucose. This finding argues against a generalized toxic effect of the NO donor.

In other experiments (data not shown), prior exposure to the PKC inhibitors, GFX (5 μmol/l), the selective inhibitor of the β isozyme of PKC LY379196 (50 nmol/l), or the myristoylated PKC inhibitor peptide (100 μmol/l) for 30 min did not affect increases in laminin C1 promoter activity induced by high glucose. The PKC inhibitors also did not alter promoter activity in the presence or absence of serum at a glucose concentration of 100 mg/dl.

DISCUSSION

Expression of laminin has been reported to be increased in glomeruli or renal cortex from diabetic animals (4) or in mesangial cells derived from human or rat glomeruli and cultured in high concentrations of glucose (3,29). In the present study, consistent with previous results, culture of mesangial cells in high glucose for 2 days to 3 weeks increased α1(IV) collagen (3–7) and laminin C1 (3) mRNA. Optimal effects on both transcripts were observed at 2 weeks. Since

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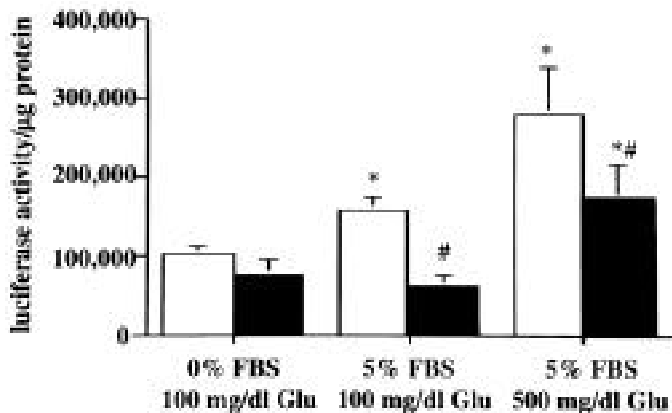

FIG. 7. Effects of SNAP on basal laminin C1 promoter activity and responses to serum and high glucose in stably integrated pHC1Luc. Studies were conducted as described in Fig. 4. Where indicated, cells were deprived of serum for the final 48 h. The concentration of glucose in the medium was maintained at 100 mg/dl for the entire experimental period where indicated or was raised to 500 mg/dl for the final 6 h. SNAP (500 µmol/l) (■) or vehicle (0.4% DMSO) (□) was added to the medium 30 min before raising the concentration of glucose or 6.5 h before harvest. Results are means ± SE from three separate experiments. *P < 0.05 vs. 0% FBS, 100 mg/dl glucose; #P < 0.05 vs. vehicle.

results obtained with MES-13 cells were qualitatively similar to those obtained with RMCs, we used the transformed cell line for subsequent studies. In contrast to increases in fibronectin (8), α2(I) collagen (7), and α1(IV) collagen (7) mRNA abundance (Fig. 3), the ability of high glucose to increase laminin C1 mRNA did not appear to be mediated, even in part, by TGF-β, since exposure to exogenous TGF-β1 for 48 h did not increase laminin C1 mRNA in RMCs or MES-13 cells. The present studies are consistent with those of Suzuki et al. (30), who found only transient increases in laminin C1 mRNA 2–4 h after addition of TGF-β1, increases that were not sustained for 6 h. Of note, TGF-β–independent effects of high glucose on α2(I) collagen expression have previously been observed within the first 24 h after exposure of mesangial cells to high glucose (7) and on fibronectin expression in umbilical vein endothelial cells that had been cultured in high glucose for several days (31).

The ability of high glucose to increase laminin C1 mRNA appeared to be, at least in part, related to activation of the laminin C1 promoter. Thus, culture in high glucose increased the activity of the laminin C1 promoter in MES-13 cells containing the stably integrated reporter gene. Increased promoter activity appeared at 4 h, persisted for at least a week, and was absolutely dependent on the presence of serum. The serum requirement led us to examine the role of TGF-β present in serum in the expression of glucose-induced increases in laminin C1 promoter activity. In this regard, previous work by Kreisberg et al. (8) demonstrated that increases in activity of the fibronectin gene promoter by culture in high glucose required TGF-β. However, our studies of the laminin C1 promoter using neutralizing pan-specific antibody to TGF-β suggest that the serum contribution to high glucose–mediated activation of the laminin C1 promoter is not due to TGF-β.

In contrast to TGF-β, IGF-1 did increase laminin C1 mRNA abundance in RMCs and MES-13 cells and α1(IV) collagen mRNA in RMCs. IGF-1 also activated the laminin C1 promoter in stable transfection assays. The effects of IGF-1 were observed at low concentrations that would not be expected to interact with the insulin receptor (32) and were blocked by neutralizing antibody to the IGF-1 receptor (33), implying mediation of IGF-1 actions on the laminin C1 promoter through the IGF-1 receptor. IGF-1 has been implicated early in the development of diabetic nephropathy in humans (34) and experimental animals (35). Thus the ability of IGF-1 to increase the activity of the promoter of the laminin C1 gene may contribute to the development of glomerular disease in diabetes.

Culture in high glucose has been reported to increase IGF-1 secretion, IGF-1 and IGF-2 binding, and IGF-1 receptor expression in mesangial cells (28). Moreover, exogenous IGF-1 increases expression of several extracellular matrix proteins, including laminin, in mesangial cells (12,13). In the present study, IGF-1 increased laminin C1 promoter activity transiently when stably integrated into chromatin. Nevertheless, the increase in promoter activity with IGF-1 was not sustained for 48 h, despite a clear and sustained (4 h to 1 week) stimulatory effect of high glucose. Moreover, antibody to IGF-1 receptor did not suppress laminin C1 promoter activity in mesangial cells cultured in the presence of serum and did not block the stimulatory effects of high glucose, implying that the effects of high glucose on laminin C1 transcription are not mediated by IGF-1. The observation that IGF-1 induces only a transient increase in promoter activity in stable integrants, whereas IGF-1 induces a sustained increase (48 h) in mRNA abundance, is consistent with the notion that *cis*-acting elements required for sustained transcription in a chromatin environment are missing from the promoter fragment in our reporter gene.

The laminin C1 promoter was also more active in continuous culture in 5% FBS than in cells deprived of serum for 48 h. Recently, a PDGF-induced increase in TGF-β was shown to mediate somewhat less than half the stimulation induced by serum of a reporter gene driven by the promoter and other *cis*-acting elements from the α1(IV) collagen gene (10). PDGF has also been implicated in advanced glycation end product–induced increases in the synthesis of collagen by mesangial cells (11). Nevertheless, in the present study TGF-β did not stimulate the laminin C1 promoter in cells deprived of serum for 48 h. Moreover, exposure to neutralizing antibody to TGF-β or the IGF-1 receptor for 48 h was without effect on laminin C1 promoter activity in cells continuously cultured in 5% FBS, implying that TGF-β and IGF-1 are not involved in the stimulation of laminin C1 promoter activity by serum. Of interest, serum has been shown to increase GLUT1 expression and protein as well as [^{3}H]2-deoxyglucose uptake in mesangial cells cultured in low glucose (36). It is not known whether a serum-induced increase in GLUT1 is responsible for the stimulatory effect of serum on laminin C1 promoter activity and increased promoter activity induced by culture in high glucose.

Several *cis*-acting elements in the promoters of genes encoding extracellular matrix proteins or TGF-β have been implicated in the activation of transcription commonly observed in response to high glucose concentration. c-*fos* and c-*jun* (AP-1) transcripts and proteins (37), as well as binding of AP-1 to DNA (38), were shown to be elevated in mesangial cells cultured in high glucose, raising the possibility that glucose actions may be mediated through a phorbol ester response element (TRE). Further, the consensus cAMP response element has been implicated in activation of the

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fibronectin promoter by high glucose (8). Recently, a glucose response element was identified in the TGF-β1 promoter (39). The laminin C1 promoter does not contain sequences identical to a TRE or this glucose response element; however, a sequence similar to the consensus cAMP response element is present (40). Additional studies involving deletion analysis or electrophoretic mobility shift assays will be necessary to delineate the sequences in the laminin C1 promoter that mediate the response to high glucose.

Previous studies from our own and other laboratories have identified a key role for PKC in the mediation of increases in TGF-β and extracellular matrix protein synthesis by high glucose in vitro and in diabetic animals in vivo (14–19). Results of the present study using inhibitors of PKC imply that other, non–PKC-mediated, signaling pathways are responsible for the influence of high glucose on the laminin C1 promoter.

Studies from our own and other laboratories support an antifibrotic role for NO in glomeruli in vivo (23,24) and in cultured mesangial cells (23,41–43). Thus, culture of mesangial cells with an exogenous NO donor or coculture of mesangial cells with endothelial cells, which produce NO, suppresses increases in TGF-β, collagen, or fibronectin synthesis induced by culture in high glucose (41,42) or exposure to the thromboxane/endoperoxide analog U46619 (43). Moreover, studies conducted in transgenic mice demonstrated that the NO synthetase inhibitor L-nitroarginine methyl ester (L-NAME) increased the activity of a reporter gene driven by the α2(I) collagen gene promoter and a far-upstream enhancer (24). Results of the present study demonstrate that SNAP also inhibits increases in transcriptional activity of the laminin C1 promoter induced by culture in high glucose or in serum. SNAP had no effect on the basal activity of the promoter in mesangial cells cultured in serum-free medium containing 100 mg/dl glucose, supporting the specificity of SNAP action. The ability of the NO donor SNAP to suppress transcriptional activation of the laminin C1 promoter in response to serum and culture in high glucose provides further support for a role for NO in the suppression of extracellular matrix production in the glomerulus in diabetes.

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