Original Article

Diabetes-Induced Extracellular Matrix Protein Expression Is Mediated by Transcription Coactivator p300

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Increased fibronectin expression is a key feature of diabetic angiopathy. We have previously shown that nuclear factor-κB (NF-κB) mediates fibronectin expression in endothelial cells and in organs affected by diabetes complications. p300, a transcription coactivator, may regulate NF-κB activity via poly(ADP-ribose) polymerase (PARP) activation. Hence, we examined the role of p300 in fibronectin expression in diabetes. High glucose induced fibronectin expression in the endothelial cells, which was associated with increased p300, PARP activity, and NF-κB activation. This p300 alteration is mediated by mitogen-activated protein kinase and protein kinase C and B. We then used p300 small interfering RNA (siRNA) and showed decreased fibronectin and PARP expression, as well as NF-κB activation, in the endothelial cells. Examination of the heart tissues of streptozotocin-induced diabetic mice revealed increased fibronectin and p300 mRNA. Intravenous injection of p300 siRNA resulted in decreased p300 levels and normalized fibronectin expression in the heart. We further investigated retinal tissues from streptozotocin-induced diabetic rats treated with intravitreal p300 siRNA injection. Similar to the heart, p300 siRNA inhibited fibronectin expression in the retina of the diabetic animals. These results indicate that transcriptional coactivator p300 may regulate fibronectin expression via PARP and NF-κB activation in diabetes. *Diabetes* 55:3104–3111, 2006

Imbalance in the production and the degradation of extracellular matrix (ECM) proteins like fibronectin and collagen may lead to structural alterations such as basement membrane thickening and ECM protein deposition in the tissues in chronic diabetes complications (1,2). We have shown that diabetes leads to increased fibronectin in all target organs of secondary complications (3–6). The mechanism of increased ECM synthesis is of great interest for the development of therapeutic modalities, as ECM deposition remains the single most common finding in chronic diabetes. We have previously reported that transcription factors nuclear factor-κB (NF-κB) and activating protein-1 (AP-1) mediate diabetes-induced fibronectin expression in the heart, kidney, and retina (5). Expression of fibronectin in vascular endothelial cells exposed to high ambient glucose levels is also through the activation of both NF-κB and AP-1 (7).

Transcription factor NF-κB encompasses a family of factors that are involved in cell survival, cancer, and inflammation (8). The specificity of NF-κB transcriptional activity is dependent on homo- and heterodimerization and a growing list of coactivators and repressors (9–12). An important transcripton coactivator p300, and its homologue cAMP-responsive element–binding protein (CREB)-binding protein (CBP), has been shown to directly interact with the p65 subunit of NF-κB (9,13). In addition to NF-κB, p300 is involved in the regulation of a large number of transcription factors, nuclear receptors, and DNA repair enzymes (14). The genes regulated by p300 mediate cell growth, proliferation, and differentiation and DNA replication, tissue differentiation, and cell cycle checkpoints (14). Recently, the binding of p300 to NF-κB was shown to be facilitated by poly(ADP-ribose) polymerase (PARP) (15). PARP is a homodimeric nuclear protein associated with the chromatin (16,17). The function of PARP is to transfer the ADP-ribose units from NAD^+ to itself and to other nuclear chromatin–associated proteins (16,17). Interestingly, reports indicate that PARP-deficient mice are protected against myocardial infarction and streptozotocin-induced diabetes (16,18). Furthermore, it has been shown that NF-κB is regulated by PARP in diabetic retinopathy (19).

PARP activation and p300/NF-κB–mediated transcriptional machinery may represent a novel signaling pathway in the pathogenesis of chronic diabetes complications. Hence, in the present study, we have investigated the role of p300 in diabetic angiopathy, in modulating PARP, and in augmented expression of fibronectin in diabetes. To test the hypothesis, we have utilized both cultured endothelial cells and animal models of chronic diabetes.

RESEARCH DESIGN AND METHODS

Cell culture. Human umbilical vein endothelial cells (American *Type Culture* Collection, Rockville, MD), in which we have previously shown glucose-induced increases in fibronectin synthesis, were plated at 2,500 cells/cm^2 in

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AP-1, activating protein-1; CBP, CREB-binding protein; CREB, cAMP-responsive element–binding protein; ECM, extracellular matrix; MAPK, mitogen-activated protein kinase; NF-κB, nuclear factor-κB; PARP, poly(ADP-ribose) polymerase; PKB, protein kinase B; PKC, protein kinase C; siRNA, small interfering RNA.

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TABLE 1
Oligonucleotide sequences for PCR

Gene and oligonucleotide sequences
FN
Human
GATAAATCAACAGTGGGAGC
CCCAGATCATGGAGTCTTTA
Mouse
CGGTAGGACCTTCTATTCCT
GATACATGACCCCTTCATTG
Rat
CCAGGCACTGACTACAAGAT
CATGATACCAGCAAGGAGT
p300
Human/mouse/rat
GGGACTAACCAATGGTGGTG
ATTGGGAGAAGTCAAGCCTG
PARP
Human
ACACCCCTTGCACGTACTTC
GATGGGTTCTCTGAGCTTCG
CBP
Human
ATGATCTTCCTGATGAGCTG
AGCCCCACTTGCTTTTGT
β-actin
Human/mouse
CCTCTATGCCAACACAGTGC
CATCGTACTCCTGCTTGCTG

endothelial growth medium (Clonetics, Rockland, ME). Endothelial growth medium was supplemented with 10 μg/l human recombinant epidermal growth factor, 1.0 mg/l hydrocortisone, 50 mg/l gentamicin, 50 μg/l amphotericin B, 12 mg/l bovine brain extract, and 10% fetal bovine serum. Cells were grown in 25-cm^2 tissue culture flasks. Appropriate concentrations of glucose were added to the medium when the cells were 80% confluent.

To investigate the signaling pathways leading to altered p300 expression, we used well-established pharmacological inhibitors (Sigma-Aldrich, Oakville, ON, Canada) of pathways known to be altered in diabetes. Chelerythrine, a protein kinase C (PKC) inhibitor, was used at 1 μmol/l. ML-9, a protein kinase B (PKB) inhibitor, was used at 100 μmol/l. Mitogen-activated protein kinase (MAPK) inhibitor U0126 was used at 10 μmol/l. Lastly, phosphatidylinositol 3-kinase was inhibited by LY294002 (25 μmol/l). We and others have previously reported the working concentrations of these inhibitors (20–22). All experiments were carried out after 24 h of incubation, unless otherwise indicated. The inhibitors were added 30 min before the addition of glucose. Three different batches of cells, each in duplicate, were investigated.

RNA isolation and cDNA synthesis. Trizol reagent (Invitrogen, Burlington, ON, Canada) was used to isolate total RNA as described (21,22). First-strand cDNA synthesis was performed using Superscript-II system (Invitrogen). The resulting cDNA products were stored at −20°C.

Real-time RT-PCR. Real-time quantitative RT-PCR was performed using the LightCycler (Roche Diagnostic Canada, Laval, QC, Canada). For a final reaction volume of 20 μl, the following reagents were added: 10 μl SYBR (Sigma-Aldrich), 1.6 μl $MgCl_2$, 1 μl forward/reverse primer (Table 1), 4.4 μl H_2O, and 2 μl cDNA. Melting curve analysis was used to determine melting temperature (T_m) of specific amplification products and primer dimers (23). For each gene, the specific T_m values were used for the signal acquisition step (2–3°C below T_m). The data were normalized to β-actin to account for differences in reverse-transcription efficiencies and amount of template in the reaction mixtures.

Western blotting. Approximately 200 μg protein was extracted from each flask. For Western blotting, 20 μg protein was loaded in each lane. Total proteins were resolved by 8% SDS-PAGE and analyzed by Western blotting using the polyclonal p300 antibody (Santa Cruz Biotehnology, Santa Cruz, CA). The signals from Western blots were detected using horseradish peroxidase–conjugated secondary antibody (Santa Cruz Biotechnology) and developed using the chemiluminescent substrate (Amersham Pharmacia Biotechnology, Amersham, U.K.). The membranes were stripped and reincubated with β-actin antibody to control for protein loading. The blots were analyzed by densitometry.

TABLE 2
Oligonucleotide sequences for siRNA synthesis and electrophoretic mobility shift assay

siRNA synthesis
p300 siRNA1
AATTCGCCGCAGCGGACCGGC CCTGTCTC
AAGCCGGTCCGCTGCGGCGAATTCCTGTCTC
p300 siRNA2
AAGCGGCCTAAACTCTCATCTCCTGTCTC
AAAGATGAGAGTTTAGGCCGCTTCCTGTCTC
p300 siRNA3
AATCAGCTTCAGACAAGTCTTCCTGTCTC
AAAAGACTTGTCTGAAGCTGATTCCTGTCTC
p300 siRNA4
AAAACAGGTATGATGAACAGTCCCCTGTCTC
AAGGACTGTTCATCATACCTGTTCCTGTCTC
EMSA
NF-κB
AGTTGAGGGGACTTTCCCAGGC
TCAACTCCCCTGAAAGGGTCCG
AP-1
CGCTTGATGAGTCAGCCGGAA
GCGAACTACTCAGTCGGCCTT
CREB
AGAGATTGCCTGACGTCAGAGAGCTAG
TCTCTAACGGACTGCAGTCTCTCGATC

EMSA, electrophoretic mobility shift assay.

Confocal microscopy. Cells were plated on eight-chamber tissue culture slides and incubated for 24 h. Following treatment with glucose (25 mmol/l) and other inhibitors, the cells were fixed with methanol. The cells were then stained with polyclonal p300 antibody. Goat IgG labeled with Texas red or flurescein isothiocyanate (Vector Laboratories, Burlingame, CA) was used for detection. Slides were mounted in Vectashield fluorescence mounting medium with DAPI (4′,6′-diamino-2-phenylindole; Vector Laboratories) for nuclear staining. Microscopy was performed by an examiner unaware of the identity of the sample using a Zeiss LSM 410 inverted laser scan microscope equipped with fluorescein, rhodamine, and DAPI filters (Carl Zeiss Canada, North York, ON, Canada).

p300 gene silencing. We used a small interfering RNA (siRNA)-based technique to specifically silence the p300 expression in the endothelial cells. siRNAs were constructed to target the p300 mRNA using the siRNA construction kit (Silencer; Ambion, Austin, TX) as described previously (23). The potential sites in p300 were identified by scanning the domain for (AA) dinucleotide sequences. After identification of the target sequences, oligonucleotides were synthesized for in vitro transcription and siRNA generation (Table 2). The oligonucleotides were synthesized by addition of a dinucleotide AA sequence at the 5′ end and an 8-nucleotide 5′-CCTGTCTC-3′ leader sequence at the 3′ end. After synthesis, siRNA concentration was determined by measuring absorbance at 260 nm. Endothelial cells were transfected with p300 siRNAs (100 nmol/l) using siRNA transfection reagent (1 μl reagent per 500 μl transfection volume; siPORT Lipid; Ambion). siRNA transfection efficiency was determined by real-time RT-PCR.

Nuclear protein extracts and electrophoretic mobility shift assay. Nuclear extracts from the endothelial cells were prepared as described previously (5,7). From each 25-cm^2 tissue culture flask, ~50 μg nuclear protein was obtained. NF-κB, CREB, and AP-1 consensus oligonucleotide (Promega, Madison, WI) DNA probes were prepared by end labeling with [γ-^{32}P]ATP (Amersham, Baie D' Urfe, QC, Canada) using T4 polynucleotide kinase. The probes were purified by ethanol precipitation and resuspended in 10 mmol/l Tris and 1 mmol/l EDTA (pH 7.6). Nuclear proteins (5 μg) were incubated with 100,000 cpm of ^{32}P-labeled consensus oligonucleotides for 30 min at room temperature. The incubation was carried out in a buffer containing 10 mmol/l Tris (pH 7.5), 50 mmol/l NaCl, 1 mmol/l $MgCl_2$, 5% glycerol, 0.05% nonidet P-40, 0.5 mmol/l EDTA, 0.5 mmol/l dithiothreitol, and 0.5 μg poly(dI-dC). Protein-DNA complexes were resolved on a standard 6% (NF-κB), 4% (AP-1), and 5% (CREB) nondenaturing polyacrylamide gel in 0.5× Tris-Borate EDTA running buffer. After 30 min of electrophoresis at 350 V, gels were dried under a heated vacuum onto Whatman paper and subjected to autoradiography (5,7). The specificity of binding was further confirmed by incubation with 100-fold unlabeled oligonucleotides. The blots were quantified by densitometry. The analyses for comparison of effects of various reagents were carried out after 4 h of incubation.

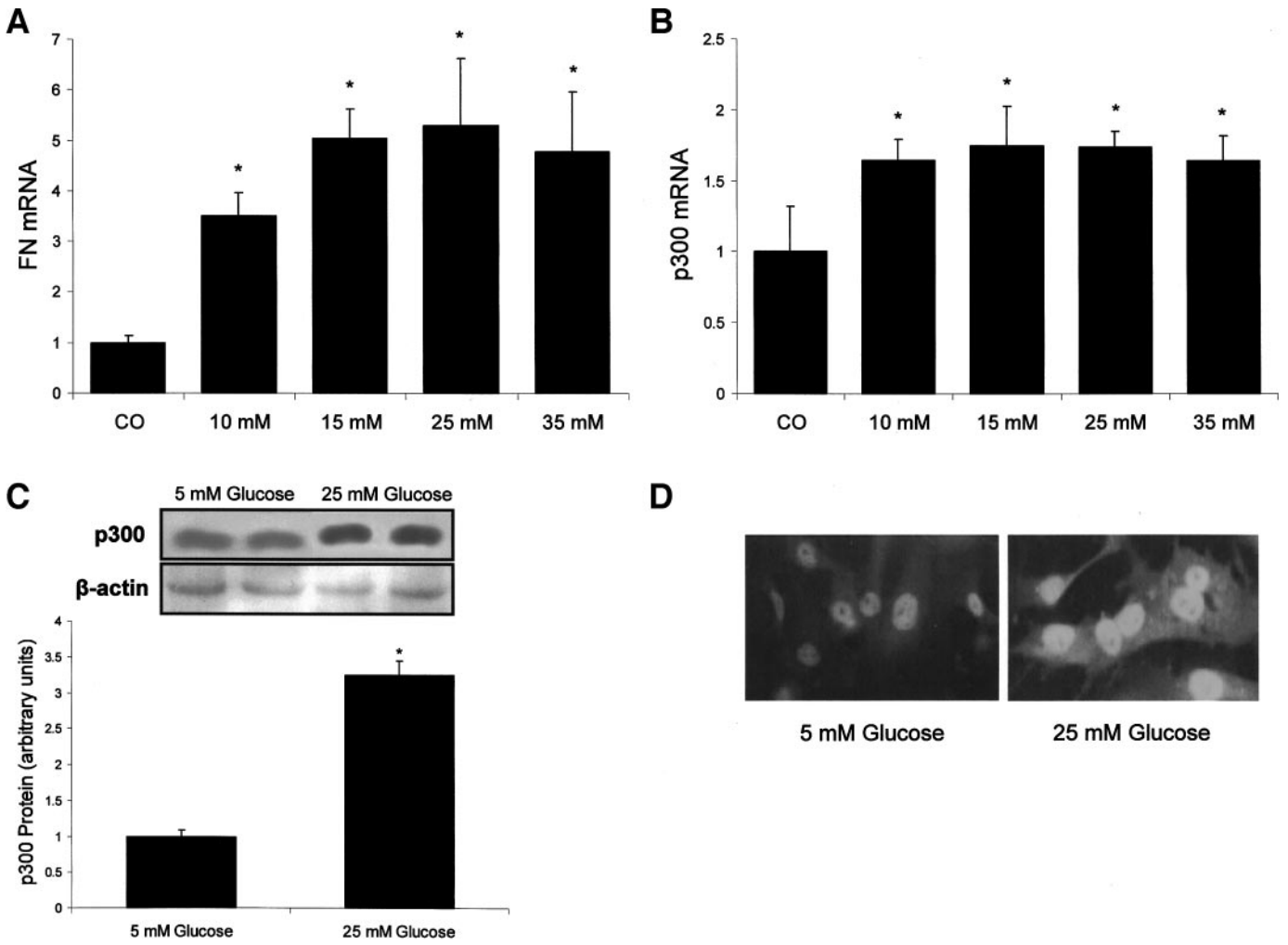

FIG. 1. Dose-dependent upregulation of fibronectin (FN) (*A*) and p300 mRNA (*B*) as assessed by real-time RT-PCR. High levels of glucose also increased p300 protein (*C*) and nuclear immunoreactivity (*D*). Control (CO) = 5 mmol/l glucose. mRNA levels are expressed as a ratio of target to β-actin (relative to control); the Western blots were analyzed by densitometry. *$P < 0.05$ compared with controls.

PARP activity assay. The PARP activity assay (R&D Systems, Minneapolis, MN) was performed based on the measurement of incorporated radiolabeled ADP from NAD^+. The use of radiolabeled NAD^+ allows detection of ADP incorporated into the poly(ADP-ribose) polymer. The acid-insoluble counts were used for quantitative assessment of PARP activity in 20 μg cell extract. Purified recombinant PARP was used as positive control. Timed enzymatic reactions were set in the microcentifuge tubes with either recombinant PARP or the cell extract. At the end of the incubation, ice-cold 20% trichloroacetic acid was added. Following centrifugation of samples at 12,000*g* for 10 min, the supernatant was discarded safely. One milliliter of 10% trichloroacetic acid was added to the tubes along with the scintillation cocktail and counted.

Animal studies. Male B6 mice were diabetes induced at 6 weeks of age by multiple (three) intraperitoneal injections of streptozotocin (50 mg/kg, in citrate buffer, pH 5.6). Age- and sex-matched littermates were used as controls and given equal volume of citrate buffer. For in vivo siRNA-mediated gene silencing (following induction of diabetes), one group of diabetic animals received 150 μg p300 siRNAs (four siRNAs; Table 2) weekly for 3 weeks by intravenous route (tail vein) using siPORT lipid transfection reagent. The other diabetic group received a similar dose of scrambled/negative control siRNA. The animals' blood glucose, urine glucose and ketones, and body weight were monitored. The animals were killed after 4 weeks of diabetes. Heart tissues were harvested and assayed for p300 mRNA levels by real-time RT-PCR.

To investigate the role of p300 in the retina, we used male Sprague-Dawley rats (Charles River, Saint-Constant, Baie D' Urfe, Canada) weighing 200–250 g. The rats were diabetes induced by single intravenous injection of streptozotocin (65 mg/kg, in citrate buffer, pH 5.6). Age- and sex-matched rats were used as controls and given equal volume of citrate buffer. The animals were monitored for glucosuria and ketonuria (Uriscan Gluketo; Yeong Dong, Seoul, South Korea) (3–6). Diabetic animals received three weekly intravitreal injections of 75 μg p300 siRNA in siPORT lipid transfection reagent in the left eye. The right eye received the same dose of scrambled/negative control siRNA. The animals were killed 1 week after the third injection, and the retinal tissues were collected.

All animal care adhered to the Association for Research in Vision and Opthalmology statement for the use of animals in ophthalmic and vision research. The University of Western Ontario Council on Animal Care formally approved all experimental protocols.

Statistical analysis. All experimental data are expressed as means ± SE and were analyzed by ANOVA, followed by Student's *t* test with Bonferroni corrections or by Student's *t* test only, when appropriate. Differences were considered significant at *P* values <0.05.

RESULTS

Glucose upregulates fibronectin expression in association with increased p300 level. As reported by us previously (7,23), glucose increased fibronectin mRNA expression in the endothelial cells in a dose-dependent manner (Fig. 1*A*). The most significant effect on fibronectin expression was observed with 25 mmol/l glucose. Hence, subsequent experiments were performed with cells exposed to either 5 mmol/l glucose (control) or 25 mmol/l glucose (high glucose). Real-time RT-PCR (Fig. 1*B*) also revealed an increase in glucose-induced p300 mRNA expression. Similarly, Western blot analysis showed that p300 protein levels paralleled mRNA levels (Fig. 1*C*). Furthermore, 25 mmol/l glucose showed increased nuclear

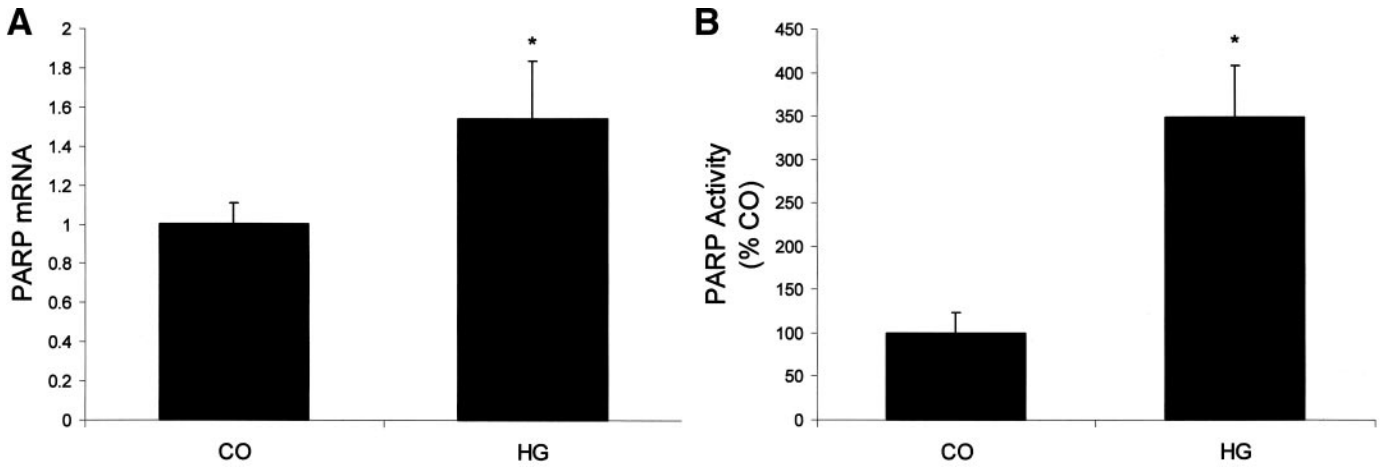

FIG. 2. High levels of glucose increased PARP mRNA (*A*) and PARP activity (*B*) (control [CO] = 5 mmol/l glucose; high glucose [HG] = 25 mmol/l glucose). PARP activity was measured as cpm/20 μg protein and expressed relative to control. **P* < 0.05 compared with controls.

and cytoplasmic immunoreactive p300 protein (Fig. 1*D*). Increased cytoplasmic immunoreactivity may reflect increased synthesis, which is also evident by Western blotting (Fig. 1*C*). These data further show that a relatively small increase in glucose may trigger p300 and upregulation of fibronectin.

PARP has been reported to facilitate the interaction between the p300 coactivator and the NF-κB transcription factor subunits (15). We determined whether high glucose induced p300 upregulation and whether fibronectin expression is associated with PARP alteration and NF-κB activity. Our results show that high glucose caused increased PARP mRNA expression and protein activity level in the endothelial cells (Fig. 2*A* and *B*).

p300 inhibition prevents glucose-induced fibronectin expression, PARP alteration, and transcription factor activity. To investigate the functional significance of increased p300 level in high glucose, we performed gene silencing to specifically inhibit p300 expression. Transfection of the endothelial cells with p300 siRNA caused complete normalization of glucose-induced p300 mRNA upregulation (Fig. 3*A*). Furthermore, p300 siRNA did not affect the mRNA levels of p300 homologue, CBP (data not shown). Interestingly, PARP and fibronectin mRNA expression was significantly downregulated in p300 siRNA-transfected cells, indicating a significant role of p300 in regulating the expression of PARP and fibronectin (Fig. 3*B*). We next tested NF-κB, AP-1, and CREB transcription factor activity in transfected cells followed by exposure to high glucose levels by electrophoretic mobility shift assay. The results show that p300 inhibition decreased NF-κB, AP-1, and CREB activity (Fig. 4).

p300 may be regulated by signaling molecules MAPK, PKC, and PKB. Our results indicate that high glucose levels lead to increased transcriptional upregulation of p300. The signaling mechanisms of this glucose-induced effect were examined following treatment of the endothelial cells to pharmacological inhibitors of signaling pathways known to be altered in diabetes (21,22,24). The results showed that p300 mRNA expression was downregulated by inhibitors of the MAPK, PKC, and PKB pathway (Fig. 5*A*). Interestingly, the same inhibitors also lead to reduction in PARP activity (Fig. 5*B*).

In vivo p300 siRNA prevents diabetes-induced fibronectin expression in the heart and retina. The role of p300 in fibronectin expression in diabetes-induced fibronectin expression was examined in an animal model of chronic diabetes complications. Streptozotocin-induced diabetic mice showed increased p300 and fibronectin expression following 1 month of diabetes follow-up (Fig. 6*A*). To explore the role of p300 in fibronectin expression in vivo, we inhibited p300 expression in the diabetic

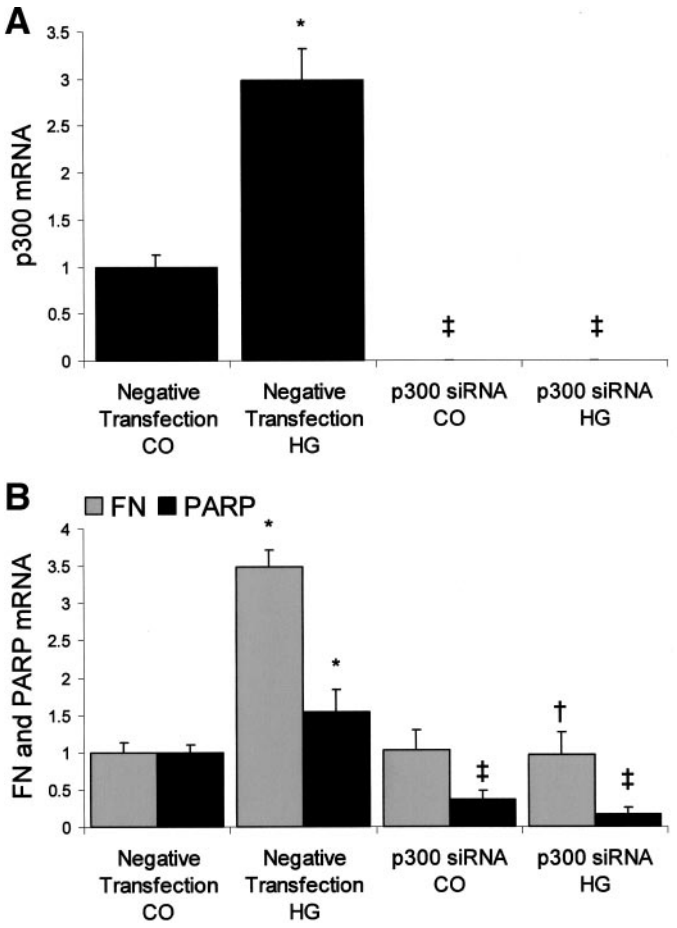

FIG. 3. Effect of p300 gene silencing on endothelial cells showing p300 mRNA and knockdown efficiency (*A*) and fibronectin (FN) and PARP mRNA levels (*B*) (high glucose [HG] = 25 mmol/l glucose). **P* < 0.05 compared with negative transfection controls (CO); †*P* < 0.05 compared with negative transfection high glucose; and ‡*P* < 0.05 compared with negative transfection controls and high glucose.

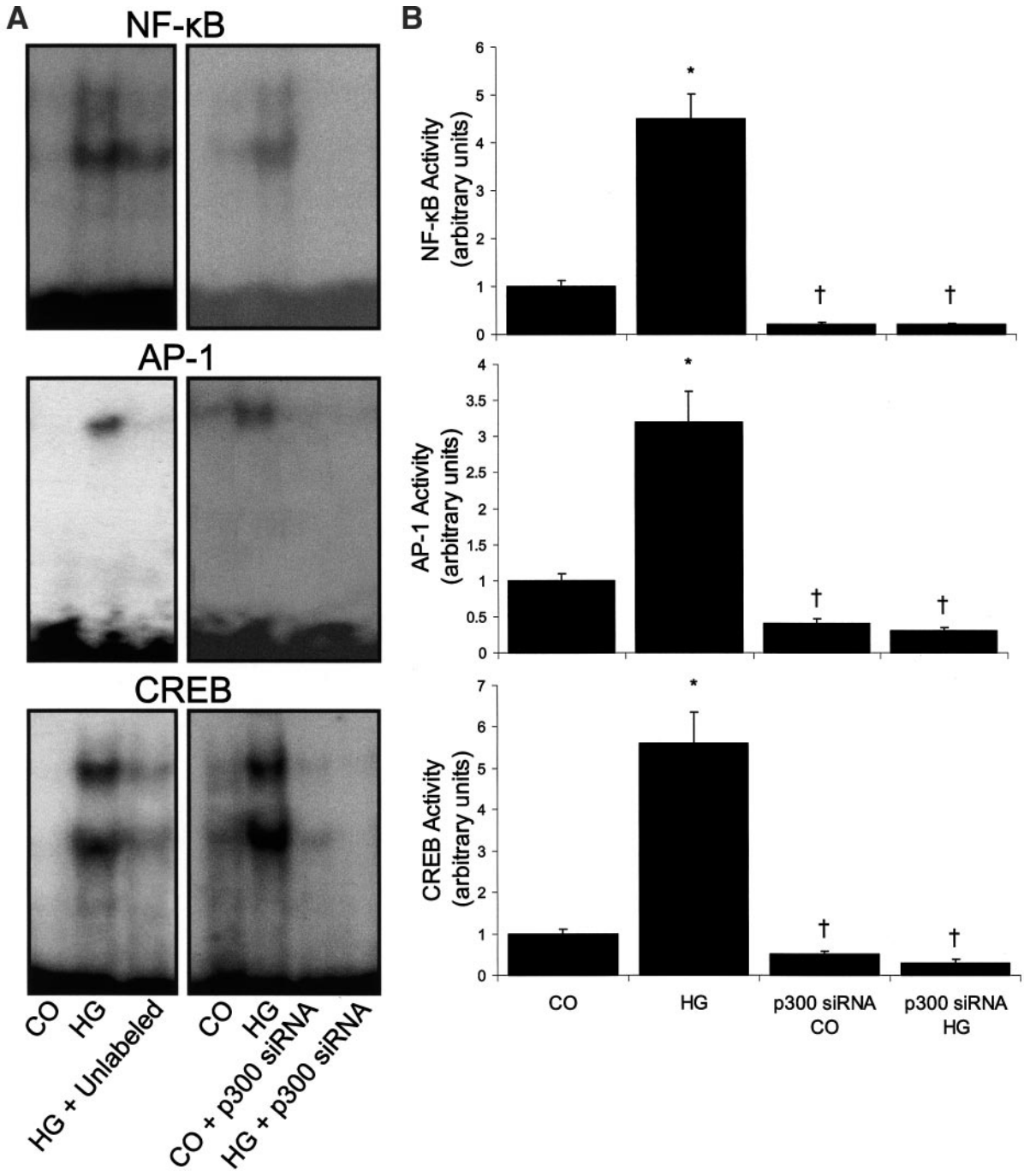

FIG. 4. Transcription factor activity and the effect of p300 inhibition. *A*: Specificity of the activation was demonstrated by competition experiments performed using 100-fold excess unlabeled nucleotides corresponding to specific binding sequences binding sequences (HG + Unlabled). *B*: Semiquantitative analysis of the transcription factor activity. *$P < 0.05$ compared with controls; †$P < 0.05$ compared with respective negative control transfections. CO, control; HG, high glucose.

animals by weekly intravenous injections of nascent p300 siRNA. Our results show that siRNA injection reduced the transcript level of p300 in the heart (Fig. 6*A*). As expected, p300 inhibition also caused a marked decrease in fibronectin expression (Fig. 6*B*). No effects of p300 siRNA injection on body weight or blood glucose levels were observed (Table 3).

We next examined the role of p300 in fibronectin expression in the retina. Our results show that diabetes increases p300 and fibronectin mRNA expression in the retina. p300 siRNA injection completely inhibited diabetes-induced p300 and fibronectin mRNA upregulation (Fig. 7). Similar to intravenous p300 siRNA injections in the diabetic mice, no significant effects of intravitreal p300 siRNA injections were observed on body weight or blood glucose levels (Table 3).

DISCUSSION

We have, for the first time, shown an important role of transcription coactivator p300 in glucose- and diabetes-induced fibronectin expression. Endothelial cells cultured in high levels of glucose increase p300, PARP, and activation of transcription factors (NF-κB, AP-1, and CREB) and

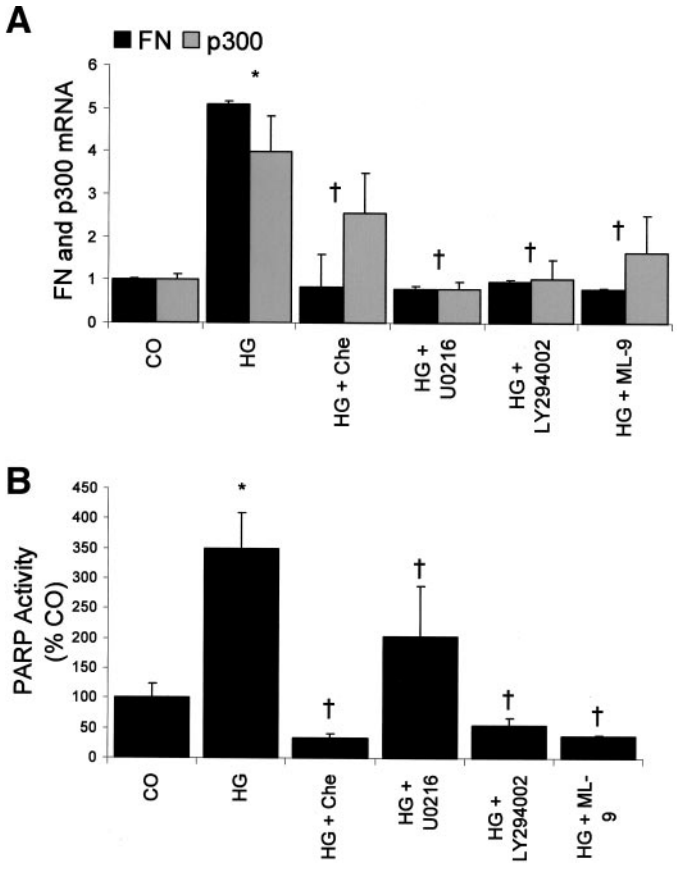

FIG. 5. Role of signaling pathway inhibitors on mRNA expression of fibronectin (FN) and p300 (*A*) and PARP activity (*B*). **P* < 0.05 compared with controls (CO); †*P* < 0.05 compared with high glucose (HG).

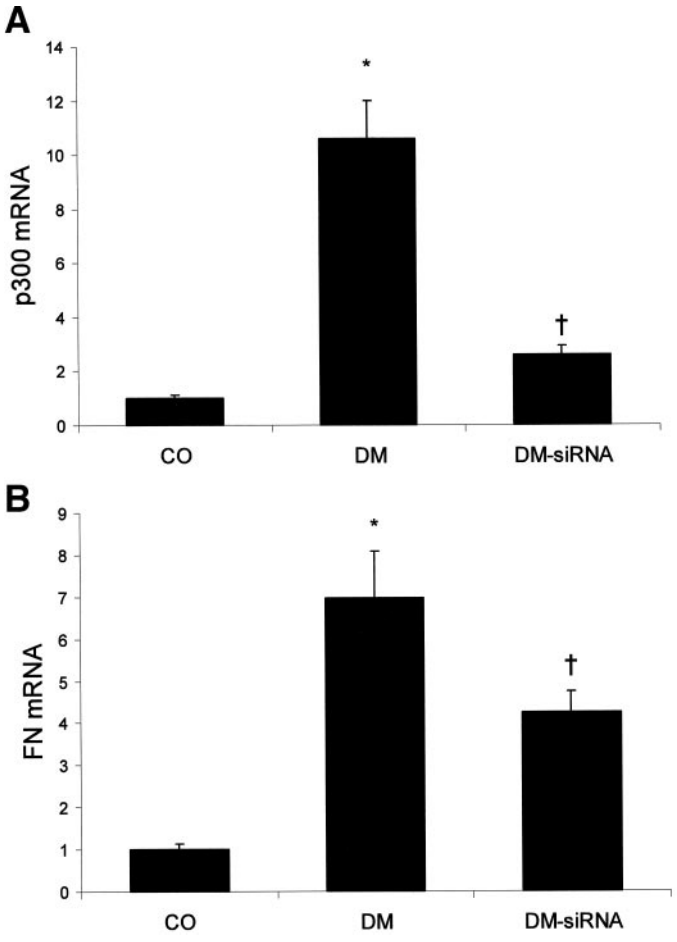

FIG. 6. Diabetes-induced cardiac p300 (*A*) and fibronectin (FN) (*B*) mRNA levels and the effect of intravenous p300 siRNA injection. CO, nondiabetic controls; DM, diabetic animals treated with scrambled siRNA; DM-siRNA, diabetic animals treated with p300 siRNA. **P* < 0.05 compared with controls; †*P* < 0.05 compared with DM.

mediate fibronectin expression. Specific p300 gene silencing caused marked attenuation of both PARP and fibronectin levels. The upstream mechanism of glucose-induced p300 possibly involves activation of MAPK, PKC, and PKB pathways. Furthermore, we have demonstrated that diabetes-induced p300 alterations in the heart and retina regulate fibronectin expression.

The biochemical mechanisms that govern increased ECM protein expression in diabetes are still not completely understood. Recent studies have indicated an important role of protein kinase pathways including MAPK, PKC, PKB, and a homologue of PKB, serum- and glucocorticoid-regulated protein kinase (21,22,24,25). The findings of the current study identify p300 and PARP as the downstream mediators of these protein kinase pathways. We have shown that inhibition of MAPK, PKC, and PKB leads to downregulation of p300 and fibronectin expression. In addition, PARP paralleled these changes. As multiple pathways may regulate PARP and p300, variability in the responses to the inhibitors were observed. U0216 was more effective in reducing p300 levels compared with PARP activation, whereas chelerythrine was less effective in preventing p300 mRNA compared with PARP activation. Hence, multiple signaling pathways may converge and produce the diabetes-induced changes; the relative role may be variable.

Transcriptional coactivator p300 is being increasingly recognized as an important element in the pathogenesis of various diseases (14,26). Our findings have further corroborated such a notion. The simultaneous interaction of multiple transcription factors (14) with p300 has been proposed to contribute to the transcriptional synergy. The

TABLE 3
Clinical parameters of experimental groups

Groups	Body weight (g)	Blood glucose (mmol/l)	Urine glucose	Urine ketones
Control mice	26.19 ± 3.23	6.38 ± 0.55	—	—
Diabetic mice	21.76 ± 2.52*	24.13 ± 2.81*	+++	Occasional trace
Diabetic-siRNA mice	20.42 ± 2.38*	23.73 ± 2.32*	+++	Occasional trace
Control rats	403.34 ± 21.52	5.87 ± 0.52	—	—
Diabetic rats	327.17 ± 18.34*	23.76 ± 2.83*	+++	Occasional trace

Data are means ± SE. *Significantly ($P < 0.05$) different from the corresponding control group. +++, high positive; —, negative.

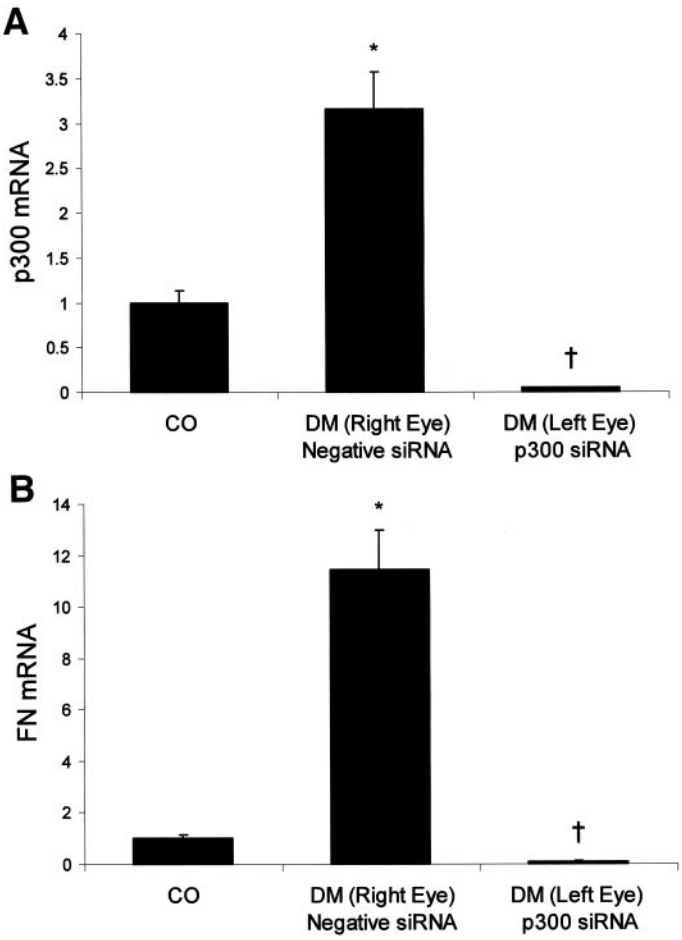

FIG. 7. Diabetes-induced retinal p300 (*A*) and fibronectin (FN) (*B*) mRNA levels and the effect of intravitreal p300 siRNA injection. **P* < 0.05 compared with controls (CO); †*P* < 0.05 compared with negative control siRNA. DM, diabetic animals.

mechanism could underlie the ability of p300 to act as a bridge or a scaffold or by acetylation of core histone tails leading to increased DNA access, weakening of internucleosomal interaction, and destabilization of chromatin structure (27,28). Association of p300 with the transcription factors is essential for gene transcription. In the context of diabetes complications, the present study demonstrated, both in vitro and in vivo, that such an association may play a significant role in ECM protein expression, which is instrumental in basement membrane thickening in diabetes.

One of the interesting findings of the study was the demonstration of regulation of PARP activation by p300. PARP, a ubiquitous nuclear enzyme, may be activated in diabetes as a result of hyperglycemia-induced oxidative stress, advanced glycation end products, PKC activation, and augmented polyol pathway (29–31). All of these biochemical anomalies have increased generation of superoxide anions as a common theme. Oxidative stress–induced DNA damage, as reported by us previously (32,33), may result in PARP activation. Activated PARP, in turn, results in formation of poly(ADP-ribose) polymers that bind to PARP itself and other proteins including transcription factors and DNA repair enzymes (16,17). Hence, it appears that p300 may play a role in sensing oxidative stress–induced DNA damage and PARP activation. Some studies in other systems have also indicated that PARP may be regulated by p300 (34). Altered p300 levels may have pathophysiologic consequences as glucose-induced increases in ECM protein expression in the endothelial cells, as well as diabetes-induced fibronectin upregulation in the heart and retina, were prevented by p300 gene silencing.

In conclusion, our studies provide evidence of an important role of p300 in glucose- and diabetes-induced fibronectin synthesis. Signaling molecules PKC, PKB, and MAPK may modulate p300 levels in the context of diabetes. These studies further suggest that p300 upregulation may be a common upstream event leading to increased PARP and transcription factor activation and resulting in fibronectin synthesis. This novel mechanistic pathway may provide greater insight of the pathogenesis of diabetes complications. Furthermore, p300 may represent a novel target for the development of therapeutic modalities for diabetes complications and other fibrotic diseases.

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