Transcriptional coactivator p300 regulates glucose-induced gene expression in endothelial cells

Shali Chen, Biao Feng, Biju George, Rana Chakrabarti, Megan Chen, and Subrata Chakrabarti

Department of Pathology, University of Western Ontario, Schulich School of Medicine, London, Ontario, Canada

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Chen S, Feng B, George B, Chakrabarti R, Chen M, Chakrabarti S. Transcriptional coactivator p300 regulates glucose-induced gene expression in endothelial cells. Am J Physiol Endocrinol Metab 298: E127–E137, 2010. First published November 10, 2009; doi:10.1152/ajpendo.00432.2009.—Sustained hyperglycemia in diabetes causes alteration of a large number of transcription factors and mRNA transcripts, leading to tissue damage. We investigated whether p300, a transcriptional coactivator with histone acetyl transferase activity, regulates glucose-induced activation of transcription factors and subsequent upregulation of vasoactive factors and extracellular matrix (ECM) proteins in human umbilical vein endothelial cells (HUVECs). HUVECs were incubated in varied glucose concentrations and were studied after p300 small interfering RNA (siRNA) transfection, p300 overexpression, or incubation with the p300 inhibitor curcumin. Histone H2AX phosphorylation and lysine acetylation were examined for oxidative DNA damage and p300 activation. Screening for transcription factors was performed with the Luminex system. Alterations of selected transcription factors were validated. mRNA expression of p300, endothelin-1 (ET-1), vascular endothelial growth factor (VEGF), and fibronectin (FN) and its splice variant EDB·FN and FN protein production were analyzed. HUVECs in 25 mmol/l glucose showed increased p300 production accompanied by increased binding of p300 to ET-1 and FN promoters, augmented histone acetylation, H2AX phosphorylation, activation of multiple transcription factors, and increased mRNA expression of vasoactive factors and ECM proteins. p300 overexpression showed a glucose-like effect on the mRNA expression of ET-1, VEGF, and FN. Furthermore, siRNA-mediated p300 blockade or chemical inhibitor of p300 prevented such glucose-induced changes. Similar mRNA upregulation was also seen in the organ culture of vascular tissues, which was prevented by p300 siRNA transfection. Data from these studies suggest that glucose-induced p300 upregulation is an important upstream epigenetic mechanism regulating gene expression of vasoactive factors and ECM proteins in endothelial cells and is a potential therapeutic target for diabetic complications.

transcription factors; vasoactive factors

GLUCOSE-INDUCED ENDOTHELIAL CELL (EC) abnormalities in diabetes are manifested as increased production of multiple vasoactive factors and extracellular matrix (ECM) proteins, leading to functional and structural alterations in the organs affected by chronic diabetic complications (6, 32, 46). Key vasoactive factors, augmented in chronic diabetic complications, include endothelin-1 (ET-1) and vascular endothelial growth factor (VEGF) (8, 12, 13, 48, 49). Furthermore, in diabetes, increased production and impaired degradation of ECM proteins like fibronectin (FN) and collagen lead to structural alterations such as basement membrane thickening and ECM protein deposition in tissues (6, 31, 46). Biochemical abnormalities in diabetes such as increased polyl pathway activity, protein kinase C (PKC) activation, nonenzymatic glycation, and oxidative stress affect gene expression of several of these molecules (6, 31, 46). We (9, 10, 15, 31) and others (44) have demonstrated that diabetes leads to increased synthesis of ECM protein FN in all organs affected by chronic diabetic complications. In addition, in diabetes there is augmented production of a functionally active embryonal splice variant of FN, EDB·FN, which is normally present in mature adult tissues (29, 30, 31). We (9, 10, 29) and others have shown that glucose increases expression of FN and vasoactive factors in ECs and in tissues of diabetic animals that occur through the activation of transcription factors such as nuclear factor-κB (NF-κB) and activating protein-1 (AP-1).

At the chromosomal level, however, histone-dependent packaging of genomic DNA is a key mechanism of gene regulation that is controlled by the degree of acetylation/deacetylation (21, 53), allowing transcription factor access to the DNA. Histone acetylation is a reversible process and is regulated by groups of histone acetyl transferases (HATs), which promote acetylation, and histone deacetylases (HDACs), which cause deacetylation. HATs and HDACs act in opposing manners, controlling access of transcriptional activators and gene induction (37, 38, 53). The transcriptional coactivator p300 was initially described as a protein associated with adenovirus E1a oncoprotein (2, 21, 53). However, p300 is involved in regulating a number of transcription factors including AP-1 and NF-κB (1, 3, 7). The association of transcription factors such as NF-κB with p300 is essential for their transcriptional activation (55). p300 plays an important role in regulating other transcription factors such as myocyte enhancer factor 2 (MEF2) and GATA binding protein 4 (GATA4) (19). p300 activation allows RNA polymerase II access, promoting formation of the preinitiation complex as well as DNA-protein and protein-protein interactions by acting as an adapter (19, 21, 38). The genes regulated by p300, in turn, mediate several cellular functions (21, 38). We previously demonstrated (28) that p300 is upregulated in the retina and in the heart of diabetic animals.

From a therapeutic perspective, because hyperglycemia causes alterations of multiple transcription factors affecting mRNA and protein expression of multiple effector molecules, it is extremely difficult to prevent diabetic complications by targeting individual vasoactive factors. Hence, an upstream transcriptional coactivator may provide an attractive therapeutic target. However, it must be established that the transcriptional coactivator indeed regulates multiple transcription factor activation and gene expression and that blocking of such factor may prevent specific abnormalities. The purpose of this study was to identify whether, in the context of diabetic complica-
tions, p300 regulates activation of multiple transcription factors and mRNAs and whether p300 may lend itself as a potential therapeutic target for chronic diabetic complications.

**MATERIALS AND METHODS**

**Cell culture.** Human umbilical vein endothelial cells (HUVECs; American Type Culture Collection, Rockville MD), in which we have previously shown glucose-induced increased vasoactive factor production and FN synthesis, were plated at 2,500 cells/cm² in endothelial growth medium (EGM) (Clonetics, Rockland, ME) (10). EGM was supplemented with 10 µg/l human recombinant epidermal growth factor, 1.0 mg/l hydrocortisone, 50 µg/l gentamicin, 50 µg/l amphotericin B, 12 mg/l bovine brain extract, and 10% fetal bovine serum. Appropriate concentrations of glucose were added to the medium when the cells were 80% confluent. All experiments were carried out after 24 h of glucose incubation unless otherwise indicated. The inhibitors were added 30 min before addition of glucose. L-Glucose (25 mmol/l) was used as osmotic control. At least three different batches of cells, each in triplicate, were used for each experiment. All reagents were obtained from Sigma (Oakville, ON, Canada) unless otherwise specified.

To investigate the effects of p300 signaling we used p300 gene silencing and chemical blockade of p300 with curcumin at a dose of 20 µmol/l. This dose is based on previous studies by us (16) and others (42). Curcumin is a known antioxidant and is thought to work by blocking p300's association with transcription factors (36, 42). Because p300’s action is balanced by deacetylases, we used general HDAC blockers 100 nmol/l trichostatin A (TSA) and 5 µmol/l suberoylanilide hydroxamic acid (SAHA) (Cayman Chemical, Ann Arbor, MI) as controls in acetylation experiments. To elucidate the signaling pathways, cells were pretreated with the following: PKC inhibitor chelerythrine (1 µmol/l); Akt inhibitor ML-9 (100 µmol/l; Cedarlane Laboratories, Hornby, ON, Canada); mitogen-activated protein kinase (MAPK) inhibitor U0126 (10 µmol/l, Promega, Pittsburgh, PA); phosphatidylinositol 3-kinase (PI 3-kinase) inhibitor LY-294002 (25 µmol/l); and poly(ADP-ribose) polymerase (PARP) inhibitor 3-aminobenzamide (ABA) (5 mmol/l, MP Biomedical). These concentrations are based on previous studies by us (28, 29) and others.

**p300 gene silencing.** We used small interfering RNA (siRNA) to specifically silence the p300 expression in ECs. siRNAs were constructed to target p300 mRNA with a siRNA construction kit (Silencer; Ambion, Austin, TX) as described previously (28). 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 (28). siRNA concentration was determined by

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**Fig. 1.** A, left: real-time RT-PCR analysis showing glucose-induced dose-dependent upregulation of p300 mRNA in endothelial cells (ECs). Right: no alterations were seen in cAMP response element binding protein (CREB) binding protein (CBP) mRNA expression by 25 mmol/l glucose (HG) or 25 mmol/l L-glucose (LG; osmotic control). NG, 5 mmol/l glucose. B: Western blot of nuclear protein (left) and quantitative analysis (right) showing glucose-induced increased p300 protein. C: immunofluorescence staining showing increased p300 immunoreactivity [evidenced by increased staining intensity in the nucleus (arrowheads)] in human umbilical vein endothelial cells (HUVECs) exposed to HG for 24 h. DAPI, 4’6’-diamidino-2-phenylindole. mRNA levels are expressed as ratio to 18S rRNA relative to NG; protein densitometric analysis in B is expressed as ratio to β-actin (loading control) relative to NG. *Significantly different from NG.
measuring absorbance at 260 nm. Endothelial cells were transfected with p300 siRNAs (100 nmol/l) with the siRNA transfection reagent siPORT Lipid (1 μl/500 μl transfection volume; Ambion). siRNA transfection efficiency was indirectly assessed by measuring p300 mRNA expression by real-time RT-PCR. Cells were incubated with glucose for 24 h after transfection.

**Plasmids and transfections.** The expression vectors containing the wild-type p300 (pCI-p300) and its HAT-deletion mutant (pCI-p300 HATΔ1472-1522) were generously provided by Dr. Joan Boyes (Institute of Cancer Research, London, UK) (4, 14). Plasmids were transformed into DH5α Escherichia coli cells and grown in bacteria for 16–18 h. Plasmids were extracted with the QIApret Spin Miniprep Kit (Qiagen Sciences) and stored at −20°C for later use. Cells were transfected with 2 μg of plasmids per flask with Lipofectamine 2000 (Invitrogen, Burlington, ON, Canada) according to the manufacturer’s protocol. Cells were collected 24 h after transfection, and transfection efficiency was assessed by real-time RT-PCR.

**Cell viability and cell proliferation.** Cell viability and proliferation were determined by 2-(4-isolophenyl)-3-(4-nitrophenyl)-5-(2,4-disulphophenyl)-2H-tetrazolium (WST-1; Roche, Laval, QC, Canada) assay. This colorimetric assay for the quantification of cell proliferation and cell viability is based on the cleavage of the tetrazolium salt WST-1 by mitochondrial dehydrogenases. Briefly, HUVECs were seeded onto 96-well plates at a density of 1.0 × 10^5 cells per well in 100 μl of culture medium with or without incubation with specific reagents for 24 h. Ten microliters of WST-1 was added per well, and the cells were incubated for 4 h at 37°C. Absorbance at 450 nm was measured (50).

**Organ culture of aorta.** We used a well-established method for these experiments (39). All animals were cared for according to the American Physiological Society’s “Guiding Principles in the Care and Use of Animals.” All experiments were approved by the University of Western Ontario Council on Animal Care Committee. This investigation conforms to the Western Ontario Council on Animal Care Committee. This investi- gation conforms to the Guide for the Care and Use of Laboratory Animals published by the National Institutes of Health (NIH Pub. No. 85-23, revised 1996). Briefly, adult male Sprague-Dawley rats (250–300 g) were anesthetized, and aortas were obtained under aseptic conditions. Thoracic aortas were cut into ~1-mm pieces and transferred to a 12-well plate (BD, Mississauga, ON, Canada) containing 1 ml of medium 199 (Invitrogen, Carlsbad, CA) with 10% fetal bovine serum per well. Media were supplemented with 100 units/ml penicillin, 100 units/ml streptomycin, and 2 mmol/l L-glutamine (39). The cultures were maintained in a humidified incubator at 37°C with 5% carbon dioxide. These tissues in culture were exposed to 5 or 25 mmol/l glucose for 24 h after transfection. They were fixed with 10 min at 37°C. The cells were lysed and sonicated to shear DNA. Chromatin immunoprecipitations were performed with antibodies against human NF-κB p65, p300, or acetylated H3 lysine (Santa Cruz Biotechnology, Santa Cruz, CA). Nonimmune serum was used as control. The immunoprecipitated DNA was detected by real-time PCR using promoter-specific primers for ET-1 and FN. p300-induced promoter acetylation may lead to recruitment of NF-κB in the target gene and cause transcription of specific genes. The primers were designed in the promoter regions of ET-1 [forward: 5′-TGTCT-GGGGCTGGAATAAAAG-3′ (position 378–397), reverse: 5′-CCTT-
TAACGGGGAGAAAGG-3’ (position 518–537) and FN [forward: 5’-CTGACGCACACTCTTCTG-3’ (position 164–183), reverse: 5’-AGTGGCACCAGTTTTGCTT-3’ (position 370–389)] (40).

Confocal microscopy. Cells were plated on eight-chamber tissue culture slides and incubated for 24 h. After treatment with glucose (25 mmol/l) and other inhibitors, the cells were fixed with methanol. The cells were then stained with p300 antibody (Santa Cruz Biotechnology,) or phospho-H2AX (Abcam, Cambridge, MA). Goat IgG labeled with FITC (Vector Laboratories, Burlingame, CA) was used for detection. Slides were mounted in Vectashield fluorescence mounting medium with 4’,6’-diamidino-2-phenylindole (DAPI; 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).

Transcription factor analysis. We initially used a Luminex bead-based system to simultaneously assess multiple transcription factors through the Marligen multiplex transcription factor assays testing services (http://www.marligen.com/multiplex-assays.html; Ijamsville, MD). This assay is based on specific binding of transcription factors to cognate DNA sequences on labeled beads. The sequences are mined from protein binding sequences in the TRANSFAC database (http://www.gene-regulation.com/pub/databases.html). Such testing is based on X-MAP technology. Within the analyzer, lasers excite the internal dyes that identify each microsphere particle and also capture any reporter dye during the assay. In this way, the technology allows multiplexing of up to 100 unique assays within a single sample. The assay is more sensitive than enzyme-linked immunosorbent assay (ELISA) or gel shift assay (23, 47). We screened for 50 different transcription factors from the isolated nuclear proteins (see below), using this technique after 24 h of glucose exposure. The specific transcription factor activation was further confirmed by Western blot or electrophoretic mobility shift assay (EMSA) (see below) as needed.

Western blotting and ELISA. Approximately 200 μg of protein was extracted from each flask. Twenty micrograms per lane of cellular or nuclear proteins (see below) was resolved by 5–10% sodium dodecyl sulfate-polyacrylamide gel electrophoresis and analyzed by Western blotting using p300 and β-actin antibody (Santa Cruz Biotechnology), MEF2, GATA1, GATA4, and acetylated H3 lysine antibody (Abcam). The signals were detected with horseradish peroxidase-conjugated secondary antibody (Santa Cruz Biotechnology) and developed with the chemiluminescent substrate (Amersham Pharmacia Biotechnology, Amersham, UK). The blots were analyzed by densitometry. ELISA for FN was performed with a commercially available ELISA kit for human FN (Chemicon, Temecula, CA) according to the manufacturer’s instructions.

Nuclear protein extraction and electrophoretic mobility shift assay. Nuclear extracts from HUVECs were prepared as described previously (9, 10, 28). From each 25-cm² tissue culture flask ~50 μg of nuclear protein was obtained. NF-κB, CAMP response element binding protein (CREB), and AP-1 consensus oligonucleotide (Promega, Madison, WI). DNA probes were prepared by end-labeling with [γ-32P]ATP (Amersham, Quebec, QC, Canada) using T4 polynucleotide kinase as previously described by us (9, 10, 28). 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 [32P]-labeled consensus oligonucleotides for 30 min at room temperature in a buffer containing 10 mmol/l Tris (pH 7.5), 50 mmol/l NaCl, 1 mmol/l MgCl2, 5% glycerol, 0.05% Nonidet P-40, 0.5 mmol/l EDTA, 0.5 mmol/l DTT, and 0.5 μg of poly(dI-dC). Protein-DNA complexes were resolved on a standard 6% (NF-κB), 4% (AP-1), and 5% (CREB) nondenaturing polyacrylamide

Fig. 3. A: chromatin immunoprecipitation (ChIP) assay showing increased acetylation as well as augmented binding of p300 and nuclear factor-κB (NF-κB) (p65) to endothelin-1 (ET-1) and fibronectin (FN) (2 molecules of importance in chronic diabetic complications) in HG compared with NG by real-time PCR. B: quantitative analysis of real-time PCR. Numbers in parentheses in A represent the crossing points. Chromatin immunoprecipitations were performed with antibodies against NF-κB p65, p300, and acetylated H3 lysine. Immunoprecipitated DNA was detected by real-time PCR using promoter-specific primers for ET-1 and FN. Cells in HG showed left shift of the crossing points compared with NG. ***Significantly different from corresponding NG.
gel in 0.5× Tris-borate-EDTA (TBE) running buffer. After electrophoresis, the gels were dried onto Whatman paper and subjected to autoradiography (9, 10). The specificity of binding was further confirmed by incubation with unlabeled oligonucleotides. The blots were quantified by densitometry.

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, where appropriate. Differences were considered significant at P values of <0.05.

RESULTS

Glucose causes p300 upregulation and increased histone acetylation. Initially, we confirmed that glucose causes increased p300 mRNA and protein expression in this system. Glucose caused significant dose-dependent upregulation of p300 mRNA, peaking at 25 mmol/l (Fig. 1A). On the basis of these data and our previous studies, in subsequent experiments we treated the cells with 25 mmol/l glucose (high glucose) for 24 h. No effect of 25 mmol/l l-glucose (osmotic control) was observed on p300 mRNA. As demonstrated by Western blots on the nuclear proteins of HUVECs after high glucose exposure for various durations, a significant increase in nuclear p300 was seen after 24 h of high glucose (Fig. 1B). In keeping with this finding, increased p300 protein was present in the nucleus of the HUVECs exposed to glucose (Fig. 1C). To examine whether such changes also affect related CREB binding protein (CBP), we examined CBP mRNA expression. We found no alteration of CBP mRNA expression (Fig. 1A). We
then examined whether this increase in p300 leads to functional alteration. We used Western blot with an antibody against acetylated H3 lysine. High glucose concentration caused increased histone acetylation, indicating that upregulated p300 is functionally active (Fig. 2C). Similar increases in histone acetylation were seen in parallel experiments with two HDAC blockers. Glucose-induced p300 mRNA upregulation, increased p300 protein production, and subsequent histone acetylation were prevented with p300 siRNA transfection (Fig. 2, A and C). In all experiments p300 transfection efficiency was separately assessed by RT-PCR. Approximately 80–90% reduction of p300 mRNA was seen after siRNA transfection. No effect was seen with a scrambled control. No effects were observed on CBP mRNA (Fig. 2B). In parallel, we used a chemical blocker of p300, curcumin. Similar to p300 siRNA, curcumin prevented glucose-induced p300 mRNA upregulation and augmented p300 protein production and p300-mediated acetylation (Fig. 2, A and C). No effects were observed on CBP mRNA (Fig. 2B).

We further examined whether the observed effects of p300 and transcription factors (see below) were mediated through binding to the promoters of specific transcripts altered by glucose. Hence we performed ChIP assays using promoter-specific primers on two key molecules, namely, ET-1 and FN. These transcripts are known to be increased in all organs affected by chronic diabetic complications, causing functional and structural alterations in these tissues (9, 31). DNA-protein complex immunoprecipitation was performed with anti-p300 antibody, acetylated H3 lysine antibody, as well as NF-κB (p65) antibody. Such experiments demonstrated augmented binding of p300 and p65 on the promoter region of these two genes after glucose exposure (Fig. 3). Furthermore, ChIP assay after precipitation with acetylated H3 antibody confirmed acetylation at these promoter sites (Fig. 3). No binding was demonstrated in nonimmune serum (data not shown).

**p300 blockade prevents glucose-induced nuclear DNA damage.** Because glucose-induced oxidative stress causes DNA damage leading to alteration of transcription factors, we examined the effects of glucose on DNA stability, using immunofluorescence stains with an antibody against H2AX (phospho), a marker for double-stranded DNA breakage (43). Because phospho-H2AX is activated in response to DNA damage, this antibody recognizes oxidative nuclear damage. Twenty-five millimoles per liter of glucose caused increased nuclear staining in these cells compared with the cells subjected to low glucose (5 mmol/l) after 24 h, which is indicative of increased double-stranded DNA breaks (Fig. 4A). p300 silencing and curcumin by reducing glucose-induced augmented p300 production prevented such DNA damage, indicating that p300 inhibition may limit double-stranded DNA breaks resulting from oxidative stress in hyperglycemia (Fig. 4). Furthermore, Western blot analysis on nuclear proteins using phospho-H2AX antibody confirmed the microscopic findings (Fig. 4B). Such direct demonstration of the involvement of p300 in glucose-induced oxidative DNA damage has not been shown previously.

**p300 mediates glucose-induced activation of multiple transcription factors.** Because high glucose initiates multiple biochemical and signaling pathways, multiple transcription factors are activated. To establish a pathogenetic role of p300 in glucose-induced transcription factor activation, we assessed a large number of transcription factors in the nuclear extracts with a Luminex bead-based assay system. Incubation of HUVECs in high glucose for 24 h caused activation of several transcription factors from isolated nuclear proteins from HUVECs exposed to NG, HG, NG with p300 siRNA, and HG with p300 siRNA. A: constitutive transcription factors. B–E: regulatory (conditional) transcription factors. Regulatory factors include steroid receptor and intracellular ligand dependent factors (B), cell-specific developmental factors (C), cell-membrane receptor-ligand-dependent latent cytoplasmic factors (D), and cell-membrane receptor-ligand-dependent resident nuclear factors (E). Glucose-induced activation of several transcription factors from all groups was prevented by p300 siRNA. **Median fluorescence intensity relative to NG; *HG values that are significantly different from NG; †HG/H11001 sip300 values that are significantly different from HG.**
factors of interest including NF-κB, AP-1, MEF2, and GATA. Several other transcription factors such as NF1, CRE-ATF1, and CEBPγ were not altered by high glucose (Fig. 5). From a functional standpoint, the altered transcription factors included both constitutively active and conditional factors. The latter group consisted of cell-specific developmental factors and signal-dependent factors such as steroid receptor- and intracellular ligand-dependent factors, cell-membrane receptor-ligand-dependent latent cytoplasmic factors, and cell-membrane receptor-ligand-dependent resident nuclear factors (5). We further confirmed activation of several transcription factors that are of importance in diabetic complications, using EMSA as previously described (10, 28, 50) or Western blot (GATA1, GATA4, MEF2) (Fig. 6). These factors are known to mediate glucose-induced upregulation of vasoactive factors, ECM proteins, and structural changes in the organs affected by chronic diabetic complications such as retinopathy, nephropathy, or cardiomyopathy (9, 10, 17, 28, 50). These experiments confirmed glucose-induced activation of these transcription factors. In the Luminex assay p300 siRNA blocked activation of the majority of the glucose-induced transcription factors [except for peroxisome proliferator-activated receptor (PPAR) and heat shock factor 1 (HSF1)]. Assessment of selected transcription factors by EMSA (NF-κB, AP-1, CREB) and Western blot (GATA1, GATA4, MEF2) also showed that p300 blockade using siRNA (~90% efficiency, data not shown) as well as curcumin treatment prevented glucose-induced activation of these factors. Although both were effective, in general curcumin appeared to have more pronounced effects (Fig. 6B). No effects were seen with scrambled siRNA on specific transcription factors, further indicating specificity of the transfection. These results are a direct demonstration that p300 regulates a large number of transcription factors that are activated by glucose.

**p300 blockade prevents glucose-induced upregulation of vasoactive factors and ECM proteins.** In the context of diabetic complications, it is important to find out whether p300 indeed regulates production of specific transcripts that are important in mediating tissue damage in diabetes. To address this issue, we focused on specific vasoactive factors and ECM proteins. We investigated mRNA expression of ET-1 and VEGF, major factors regulating structure and function of the vasculature in diabetes, as well as structural proteins such as FN and EDB+FN. Glucose, as expected, upregulated mRNA expression of ET-1, VEGF, FN, and EDB+FN (9, 10, 15, 29, 30, 31, 44). p300 siRNA prevented basal (except for VEGF) and glucose-induced overexpression of all vasoactive factors and ECM proteins. Curcumin demonstrated a similar and in most cases a more robust action (Fig. 7). We further expanded this study to examine whether similar changes occur in vascular tissues. We used an organ culture system using thoracic aorta. We exposed

![Fig. 6](https://www.ajpendo.org/)

**Fig. 6.** Electrophoretic mobility shift assay (EMSA; A) and Western blot (B) showing that glucose-induced activation of NF-κB, AP-1, and CREB (A) and monocyte enhancing factor 2 (MEF2), GATA binding protein 1 (GATA1), and GATA4 (B) was prevented by p300 siRNA and curcumin. Binding specificity of transcription factors was demonstrated by incubation with excess unlabeled oligonucleotides (cold probes). C: quantitative densitometric analysis of B, expressed as ratio to β-actin (loading control), relative to NG. − Control, no protein; + Control, phorbol ester-stimulated cells in NG. *Significantly different from HG; †significantly different from NG.
the tissues to glucose and transfected them with p300 siRNA. Similar to the cell culture model, these vascular tissues showed glucose-induced mRNA upregulation of p300, ET-1, VEGF, FN, and EDB/FN. Furthermore, p300 siRNA prevented overexpression of all these transcripts (Fig. 7F).

Multiple glucose-activated pathways cause p300 upregulation and p300 overexpression produces glucoselike effects. To further explore mechanism of glucose-induced p300 upregulation, we used multiple chemical blockers. These pathways are known to be activated in ECs after glucose exposure (28, 31, 50). Glucose-induced p300 upregulation was blocked after exposure of the cells to PKC, Akt, MAPK, PI 3-kinase, as well as PARP blockers (Fig. 8A). Hence, multiple signaling pathways may converge on p300 to produce deleterious effects of hyperglycemia. However, if p300 plays a major role in mediating the effects of hyperglycemia, then p300 overexpression under normal glucose conditions should produce glucoselike effects. Hence, we used p300 expression plasmid (wild type, pCI-p300) to overexpress of p300 in ECs. Overexpression of p300 after transfection resulted in upregulation of vasoactive factors such as ET-1, VEGF, and ECM protein FN. Such upregulations were not seen after transfection of p300 deletion mutant (Fig. 8B).

**DISCUSSION**

The present study has shown that, in ECs, glucose causes p300 upregulation. p300 binds to promoters of vasoactive factors and ECM proteins, mediates histone acetylation, activates multiple transcription factors, and causes increased production of downstream vasoactive factors and ECM proteins. We have further shown a similar mechanism in an organ culture model of vascular tissue.

Association of a transcriptional coactivator such as p300 with transcription factors is essential for gene transcription (17, 27, 37, 38). Simultaneous interaction of multiple transcription factors with p300 has been proposed to contribute to the transcriptional synergy (17, 27, 37, 38). The mechanism could underlie the ability of p300 to act as a bridge or as a scaffold or by acetylation of core histone tails leading to increased access to DNA, weakening of internucleosomal interaction, and destabilization of chromatin structure (26, 27). In this study we have shown that glucose induces p300-mediated lysine acetylation and binding of p300 on promoter regions of ET-1 and FN genes. Because the related CBP was not altered after glucose exposure, p300 may represent the main mediator of glucose-induced histone acetylation. However, we do recognize possible effects of other acetylators that were not investigated in this study. We have also demonstrated that p300-mediated histone acetylation leads to activation of multiple transcription factors and vasoactive factors. Hence, histone acetylation plays a significant role in controlling transcription factor activation and augmented vasoactive factor and ECM protein expression causing alteration of vascular structure and function in diabetes.
We previously demonstrated (17) p300-induced MEF2 calmodulin-dependent protein kinase (CaM kinase) (22, 25, 38, 39). We included PKC, working via PKD, MAPK(ERK1/2), as well as the association of MEF2 with p300 (18, 35, 54). HDAC activators of Class II HDAC's activation and translocation allows the pathogenesis of chronic diabetic complications (9, 28, 38). One of the interesting findings of the study was the demonstration of glucose-induced oxidative DNA damage and its prevention by nullifying the effects of p300. Oxidative stress is a key mechanism of cellular DNA damage in diabetes (6). It is also interesting to note that we have previously demonstrated regulation of the DNA repair enzyme PARP by p300 (28). This is also in keeping with other studies with respect to the role of PARP and p300 in transcription factor activation (24).

As a pathophysiological consequence of increased p300 levels, a large number of transcription factors were altered by glucose. As expected, several others such as NF1 or CRE-ATF1 were not altered by glucose. The majority, but not all, of the glucose-induced activated transcription factors were prevented by p300 silencing. We have further confirmed the expression of specific transcription factors such as NF-κB, CREB, AP-1, GATAs, and MEF2. All of these are important if they mediate alterations at the chromosome level of are important if they mediate alteration of effector molecules and if by blockade of these changes we can effectively prevent alteration of the effector molecules. Hence, we investigated mRNA transcripts of vasoactive factors and structural proteins such as ET-1, VEGF, FN, and EDB. FN, which are important mediators of tissue damage in chronic diabetic complications (6, 9, 10, 12, 13, 15, 30, 31, 44). Blockade of p300 by siRNA or by chemical inhibitor was effective in preventing glucose-induced upregulation of these vasoactive factors and ECM proteins. Other investigators have also demonstrated that p300 overexpression may lead to increased ET-1 promoter activity and NF-κB activity (34, 52). It is of interest to note that, in several of the parameters studied, curcumin treatment had more pronounced effects compared with p300 siRNA. Curcumin is a known p300 inhibitor (41). It has been demonstrated that curcumin reduces p300's acetylation by binding to p300 and causing its degradation (20, 41). In this study, however, we have demonstrated a downregulation of p300 expression in endothelial cells by curcumin. In keeping with these data we (11, 17) and others have previously demonstrated such inhibition of p300 synthesis in other systems. Curcumin has a large number of actions and is effective in the prevention of a significant number of oxidative stress- and inflammation-associated changes in several diseases (16, 20). We have recently demonstrated (11) that curcumin treatment prevents oxidative stress, p300 upregulation, NF-κB activation, increased ECM production, and structural abnormalities in the kidneys of diabetic rats. We and other investigators have also demonstrated that curcumin can prevent diabetes-induced abnormalities in the heart and the retina (16, 33).

In summary, our studies provide direct evidence that activation of p300 after oxidative nuclear damage is an important upstream event causing glucose-induced histone acetylation, transcription factor activation, and vasoactive factor ECM production in ECs and in vascular tissues. Hence, p300 may represent a novel target for the development of therapeutic modalities for chronic diabetic complications. However, long-term preclinical studies in animal models are necessary to establish such a notion.

**GRANTS**

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DISCLOSURES

No conflicts of interest are declared by the author(s).

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