Glucose Enhances Endothelial LOX-1 Expression

Role for LOX-1 in Glucose-Induced Human Monocyte Adhesion to Endothelium

Ling Li,[1] Tatsuya Sawamura,[2] and Geneviève Renier[3]

Endothelial dysfunction is an early and key determinant of diabetic vascular complications that is elicited at least in part by oxidized LDL (oxLDL). The recent observation that lectin-like oxLDL receptor-1 (LOX-1) expression is increased in the vascular endothelium of diabetic rats suggests a role for LOX-1 in the pathogenesis of diabetic vascular dysfunction. Because postprandial plasma glucose has been recently proposed as an independent risk factor for cardiovascular diseases in patients with diabetes, we evaluated, in the current study, the in vitro effect of high glucose on LOX-1 expression by human aortic endothelial cells (HAECs) and the role of this receptor in glucose-induced human monocyte adhesion to endothelium. Exposure of HAECs to high D-glucose concentrations (5.6–30 mmol/l) enhanced, in a dose- and time-dependent manner, LOX-1 expression, both at the gene and protein levels. The stimulatory effect of glucose on LOX-1 gene expression in HAECs was abolished by antioxidants and inhibitors of nuclear factor (NF)-κB, protein kinase C (PKC), and mitogen-activated protein kinases (MAPKs). Electrophoretic mobility shift assay data demonstrated that high glucose enhanced, in HAECs, the nuclear protein binding to the NF-κB regulatory element of the LOX-1 gene. Finally, our results showed that incubation of HAECs with high glucose increased human monocyte adhesion to endothelium through a LOX-1–dependent signaling mechanism. Overall, these results demonstrate that high glucose induces endothelial LOX-1 expression. This effect appears to be exerted at the transcriptional level through increased oxidant stress and NF-κB, PKC, and MAPK activation. The study also suggests a role for LOX-1 as mediator of the stimulatory effect of high glucose on monocyte adhesion. *Diabetes* 52:1843–1850, 2003

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AGE, advanced glycation end product; DTT, dithiothreitol; EGM, endothelial growth culture medium; GAPDH, glyceraldehyde-3-phosphate deshydrogenase; HAEC, human aortic endothelial cell; ICAM-1, intercellular adhesion molecule-1; LOX-1, lectin-like oxLDL receptor-1; MAPK, mitogen-activated protein kinase; NAC, *N*-acetyl-L-cysteine; NF, nuclear factor; NP-40, Nonidet P-40; oxLDL, oxidized LDL; PKC, protein kinase C; PMSF, phenylmethylsulfonyl fluoride; ROS, reactive oxygen species; TNF, tumor necrosis factor; VCAM-1, vascular cell adhesion molecule-1.

Atherosclerotic cardiovascular disease is the leading cause of death and the major complication of diabetes (1–5). Endothelial dysfunction is a key, early, and potentially reversible event in atherogenesis that is commonly present in human diabetes (6–8) and plays a key role in the pathogenesis of diabetic vasculopathies (9). Several mechanisms may cause or contribute to endothelial dysfunction in diabetes. These include hyperlipidemia, oxidative stress, oxidized LDL (oxLDL), insulin resistance, formation of advanced glycation end products (AGEs), activation of protein kinase C (PKC), and hyperglycemia (10–16). Recently, a role has been proposed for lectin-like oxLDL receptor-1 (LOX-1), a novel endothelial cell receptor for oxLDL and AGEs (17,18), in vascular cell dysfunction and monocyte adhesion (19,20). The finding that LOX-1 expression is increased in the vascular endothelium of diabetic rats (21) suggests a role for this receptor in endothelial dysfunction associated with diabetes. The mechanisms responsible for the upregulation of vascular LOX-1 in diabetes are unknown. The pathophysiological stimuli relevant to atherosclerosis in diabetes that may contribute to this alteration include oxLDL, tumor necrosis factor (TNF)-α, and AGEs (21–23). Since postprandial plasma glucose has recently been proposed as an independent risk factor for cardiovascular disease in patients with diabetes (24), we sought to investigate in the present study the regulation of endothelial LOX-1 expression by high glucose and the molecular mechanisms involved in this effect. On the basis of previous observations showing that hyperglycemia increases leukocyte-endothelial interaction (25–28) and that LOX-1 supports adhesion of leukocytes to endothelium (19,20), we further examined the role for LOX-1 in glucose-induced monocyte adhesion.

RESEARCH DESIGN AND METHODS

Reagents. FCS was purchased from Wisent (St. Bruno, Quebec, Canada). RPMI-1640 medium, phenylmethylsulfonyl fluoride (PMSF), Nonidet P-40 (NP-40), Hank's balanced salt solution, lymphoprep, penicillin-streptomycin, glycine, SDS, and Trizol reagent were obtained from Gibco BRL (Burlington, Ontario, Canada). Human aortic endothelial cells (HAECs), endothelial growth culture medium (EGM), and EGM bullet kit were obtained from Clonetics (San Diego, CA). D-Glucose, L-glucose, mannitol, BSA fraction V, dianisinine dihydrochloride, hexadecyltrimethylamine ammonium bromide, dithiothreitol (DTT), DMSO, vitamin E, and vitamin C were purchased from

Sigma. Monoclonal antibodies against β-actin, p50, and p65 were bought from Santa Cruz Biotechnology (Santa Cruz, CA). Recombinant human TNF-α, IgG_1 neutralizing antibody, and monoclonal antibodies to human intercellular adhesion molecule-1 (ICAM-1), vascular cell adhesion molecule-1 (VCAM-1), and E-selectin were purchased from R&D Systems (Minneapolis, MN). Monoclonal antibody to human LOX-1 was kindly provided by Dr. Sawamura (National Cardiovascular Center Research Unit, Osaka, Japan). Calphostin C, BAY11–7,085, *N*-acetyl-L-cysteine (NAC), and PD98059 were obtained from Calbiochem (La Jolla, CA). LY379196 was kindly provided by Eli Lilly (Indianapolis, IN).

Endothelial cells. HAECs were grown to confluence in EGM under recommended conditions. The EGM was supplemented with 2% FBS containing 0.2 μg/ml cupric sulfate, 0.01 μg/ml human epidermal growth factor, 0.1% gentamicin sulfate amphotericin-B, 1 μg/ml hydrocortisone, and 12 μg/ml bovine brain extract protein content. Final LDL concentration in the EGM was ~40 μg/ml. Determination of TBARS content in the EGM demonstrated that LDL oxidation occurred in the supernatants of HAECs cultured in normoglycemic (TBARS content: 1.4 nmol · mg $protein^{-1}$ · ml^{-1}) and hyperglycemic conditions (TBARS content 4.4 nmol · mg $protein^{-1}$ · ml^{-1}). Confluent cells were used in all experiments at passages 3–5. Evidence that these cells express Von Willebrand factor, adhesion molecules, and cytokines and demonstrate PAI-1 activity supports the relevance of these cells as model for the study of native vascular endothelium.

Human monocytes. Human monocytes were isolated as previously described (29). Briefly, peripheral blood mononuclear cells were isolated from healthy control subjects by density centrifugation using Ficoll, allowed to aggregate in the presence of FCS, and further purified by the rosetting technique. After density centrifugation, highly purified monocytes (85–90%) were recovered. Human monocyte purity was assessed by flow cytometry (FACScan; Becton Dickinson) using phycoerythrin-conjugated anti-CD14 monoclonal antibody (Becton Dickinson).

Analysis of mRNA expression. Expression of the LOX-1 gene in human HAECs was measured by the PCR technique. Total RNA for use in the PCR was extracted from cells by an improvement of the acid-phenol technique of Chomczynski and Sacchi (30). Briefly, cells were lysed with Trizol reagent and chloroform was added to the solution. After centrifugation, the RNA present in the aqueous phase was precipitated and resuspended in diethyl pyrocarbonate water. cDNA was synthesized from RNA by incubating total cellular RNA with 0.1 μg oligodT (Pharmacia) for 5 min at 98°C and then by incubating the mixture with reverse transcription buffer for 1 h at 37°C. The cDNA obtained was amplified by using 0.8 μmol/l of two synthetic primers specific for human LOX-1 (5′-TTACTCTCCATGGTGGTGCC-3′ and 5′-AGCTTCTTCT DCTTGTTGCC-3′) and human glyceraldehyde-3-phosphate deshydrogenase (GAPDH) (5′-CCCTTCATTGACCTCAACTACATGG-3′ and 5′-AGTCTTCTGG GTGGCAGTGATGG-3′), which was used as an internal standard in the PCR mixture. A 193-bp human LOX-1 cDNA fragment and a 456-bp human GAPDH cDNA fragment were amplified enzymatically by 30 and 20 repeated cycles, respectively. An aliquot of each reaction mixture was then subjected to electrophoresis on 1% agarose gel containing ethidium bromide. The intensity of the bands was measured by an image analysis scanning system (Alpha Imager 2000; Packard Instruments, Meriden, CT). Titrating the cDNA samples ensured that the signal lies on the exponential part of the standard curve. To achieve better quantification of LOX-1 mRNA expression, levels of LOX-1 mRNA were also measured in some experiments by Northern blot analysis. Ten million HAECs were plated in plastic petri dishes (100 × 200 mm) (Falcon, Lincoln Park, NJ). After treatment, cells were lysed with Trizol reagent. Total RNA was isolated and separated in a 1.2% agarose gel containing 2.2 mol/l formaldehyde. The blots were prehybridized for 6 h. The mRNA expression was analyzed by hybridization with [^{32}P]dCTP-labeled human LOX-1 cDNA probe. Hybridization was detected by autoradiography with Kodak X-Omat-AR films (Rochester, NY). mRNA expression was quantified by high-resolution optical densitometry (Alpha Imager 2000).

DNA binding assay. The isolation of the nuclei was performed as previously described (31). Briefly, 5×10^7 HAECs were collected, washed with cold PBS, and lysed in 1 ml ice-cold buffer A (15 nmol/l KCl, 2 mmol/l $MgCl_2$, 10 mmol/l HEPES, 0.1% PMSF, and 0.5% NP-40). After a 10-min incubation on ice, lysed cells were centrifuged and the nuclei were washed with buffer A NP-40. The nuclei were then lysed in a buffer containing 2 mol/l KCl, 25 mmol/l HEPES, 0.1 mmol/l EDTA, and 1 mmol/l DTT. After a 15-min incubation period, a dialysis buffer (25 mmol/l HEPES, 1 mmol/l DTT, 0.1% PMSF, 2 μg/ml aprotinin, 0.1 mmol/l EDTA, and 11% glycerol) was added to the nuclei preparation. Nuclei were collected by centrifugation for 20 min at 13,000 rpm. Aliquots (50 μl) of the supernatants were frozen at −70°C, and protein concentration was determined. DNA retardation (mobility shift) electrophoresis assays were performed as previously described by Fried and Crothers (32). Briefly, 5 μg nuclear extracts were incubated for 15 min in the presence of five times binding buffer (125 mmol/l HEPES, pH 7.5, 50% glycerol, 250 mmol/l NaCl, 0.25% NP-40, and 5 mmol/l DTT) in the presence or absence of 200 ng anti-p50 and anti-p65 antibodies. End-labeled double-stranded consensus sequences of the LOX-1 promoter nuclear factor (NF)-κB–enhancing elements (20,000 cpm per sample) were then added to the samples for 30 min. Samples were analyzed on a 4% nondenaturating polyacrylamide gel (PAGE) containing 0.01% NP-40. The specificity of the nuclear protein binding was assessed by incubating the nuclear proteins isolated from HAECs with the labeled DNA probe in the presence of a 1,000-fold molar excess of unlabeled DNA probe.

DNA probes. Double-stranded oligonucleotides containing the NF-κB (5′-CGTCTGCCCTTTCCCCCTCT-3′ and 5′-GAGAAGAGGGGGAAAGG-3′) consensus sequence of the human LOX-1 gene promoter (33) were synthesized with the aid of an automated DNA synthesizer. After annealing, the oligonucleotides were labeled with [γ-^{32}P]ATP using the Boehringer-Mannheim 5′ end-labeling kit (Indianapolis, IN).

Western blot. HAEC protein extracts (15 μg) were applied to 10% SDS-PAGE and transferred to a nitrocellulose membrane using a Bio-Rad transfer blotting system at 100 V for 1 h. Nonspecific binding was blocked with 3% BSA for 1 h at room temperature. After washing with PBS-Tween 0.1%, blots were incubated overnight at 4°C with anti–LOX-1 or anti–β-actin antibody. After further wash, membranes were incubated for 1 h at room temperature with a horseradish peroxidase–conjugated donkey anti-mouse IgG (1/5,000). Antigen detection was performed with an enhanced chemiluminescence detection system (Amersham).

Adhesion assay. Confluent HAECs were exposed for 72 h to 5.6 or 30 mmol/l glucose and then treated for 1 h in the presence of antibodies to IgG_1, LOX-1, ICAM-1, VCAM-1, or E-Selectin. HAECs were then washed twice with Hank's balanced salt solution and incubated for 2 h with freshly purified human monocytes (280,000 cells/well) resuspended in serum-free RPMI medium. At the end of this incubation period, nonadherent monocytes were removed by washing the cells with PBS (pH 6.0). Monocyte adhesion to HAECs was quantitated by measuring monocyte myeloperoxidase activity (34).

Statistical analysis. All values were expressed as means ± SE. Data were analyzed by one-way ANOVA followed by the Tukey test. A P value <0.05 was considered statistically significant.

RESULTS

Effect of D-glucose on endothelial cell LOX-1 mRNA expression. Incubation of HAECs for 24–96 h with D-glucose (5.6–30 mmol/l) enhanced, in a time-dependent manner, LOX-1 mRNA levels in these cells. Maximal effect was observed from 48 to 96 h (Fig. 1*A*). Glucose-induced LOX-1 mRNA levels, normalized to the levels of GAPDH mRNA, are presented in Fig. 1*A*. Incubation of HAECs for 48 h with increasing D-glucose concentrations (5.6–30 mmol/l) enhanced, in a dose-dependent manner, LOX-1 mRNA levels in these cells (Fig. 1*B*). Maximal effect was observed with a concentration of 30 mmol/l glucose. LOX-1 mRNA levels, normalized to the levels of GAPDH mRNA, are presented in Fig. 1*B*. Incubation of HAECs with L-glucose or mannitol (30 mmol/l) did not induce LOX-1 mRNA expression (LOX-1 mRNA expression [% of control values]: L-glucose 102 ± 7; mannitol 97 ± 9). Quantification of LOX-1 mRNA levels by Northern blot analysis in HAECs exposed for 48 h to 5.6 or 30 mmol/l glucose is presented in Fig. 1*C*.

Effect of D-glucose on endothelial cell LOX-1 protein expression. Treatment of HAECs for 24–96 h with 5.6 or 30 mmol/l glucose increased LOX-1 protein expression in these cells. Maximal effect was observed from 72 to 96 h (Fig. 2*A*). LOX-1 protein levels normalized to the levels of β-actin are illustrated in Fig. 2*A*. Incubation of HAECs for 72 h with increasing D-glucose concentrations (5.6–30 mmol/l) enhanced, in a dose-dependent manner, LOX-1 protein expression in these cells (Fig. 2*B*). Maximal effect was observed with a concentration of 30 mmol/l glucose. LOX-1 protein levels normalized to the levels of β-actin are illustrated in Fig. 2*B*. No stimulatory effect of mannitol (30 mmol/l) on endothelial LOX-1 protein expression was

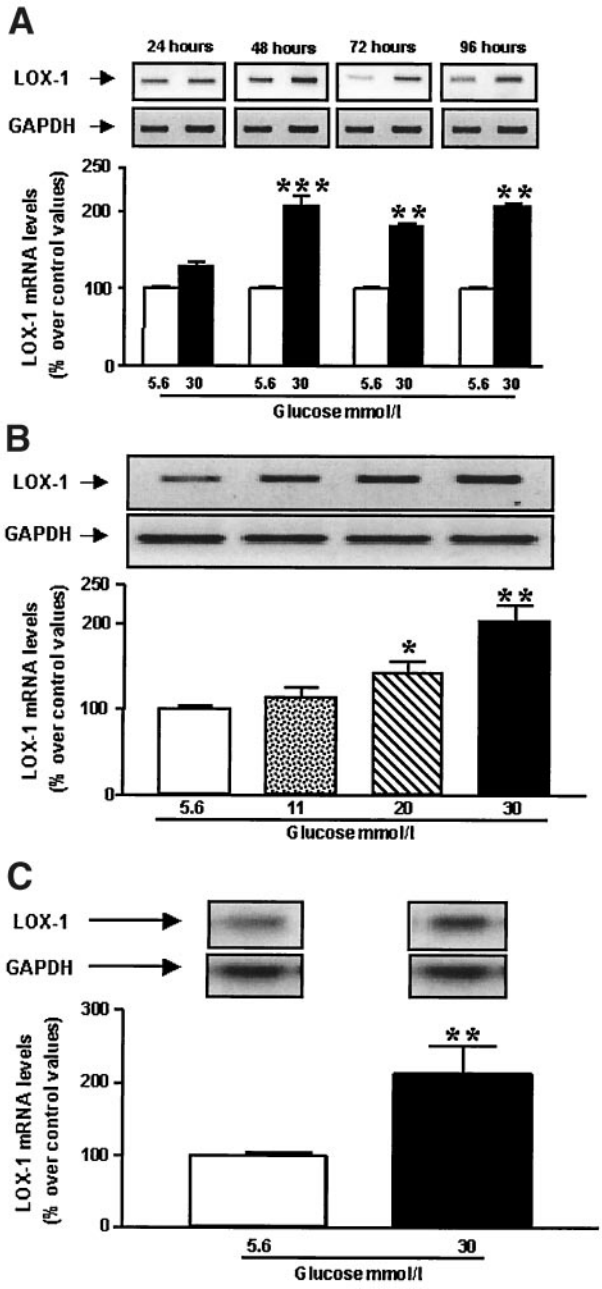

FIG. 1. Time- and dose-dependent effect of high glucose on LOX-1 mRNA levels in HAECs. Cultured HAECs were incubated for 24–96 h (*A*) or 48 h (*B* and *C*) with 5.6–30 mmol/l glucose. At the end of the incubation period, cells were lysed and LOX-1 mRNA was analyzed by RT-PCR (*A* and *B*) or Northern blot analysis (*C*). LOX-1 mRNA levels were normalized to the levels of GAPDH mRNA. Data illustrated on the graph bar represent the means ± SE of six (*A* and *B*) or three (*C*) different experiments. *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$ vs. 5.6 mmol/l glucose.

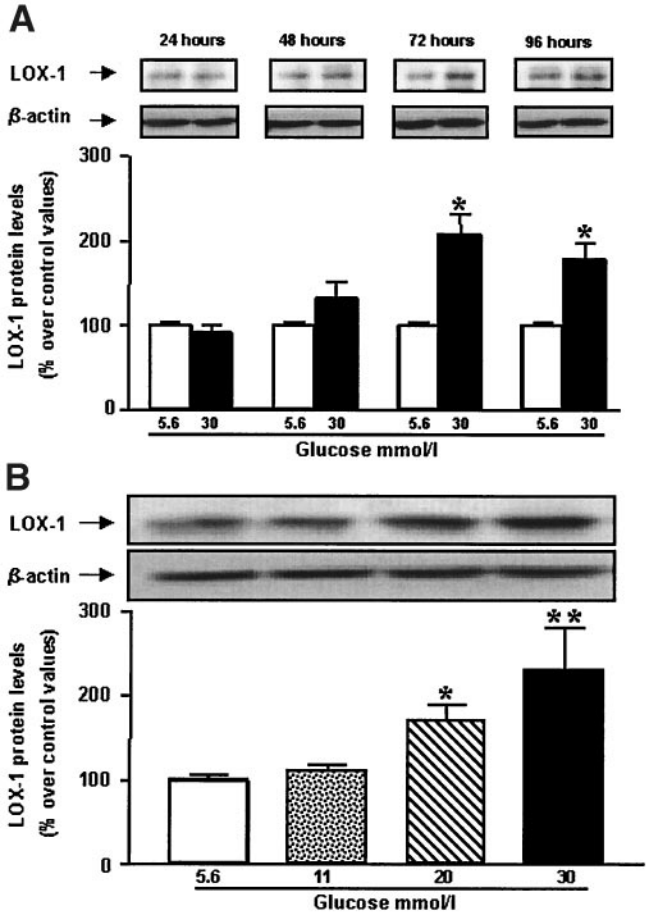

FIG. 2. Time- and dose-dependent effect of high glucose on LOX-1 protein expression in HAECs. HAECs were cultured for 24–96 h (*A*) or 72 h (*B*) with 5.6–30 mmol/l glucose. At the end of the incubation period, cells were lysed and LOX-1 membrane protein expression was determined by Western blot analysis. LOX-1 protein levels were normalized to the levels of β-actin protein. Data illustrated on the graph bar represent the means ± SE of four (*A*) and three (*B*) different experiments. *$P < 0.05$, **$P < 0.01$ vs. 5.6 mmol/l glucose.

observed (LOX-1 protein expression [% of control values]: mannitol 105 ± 10).

Since TNF-α is increased in diabetes and enhances endothelial LOX-1 expression in vitro, we next measured the levels of LOX-1 protein elicited by this cytokine in low or high glucose conditions. As shown in Fig. 3, TNF-α–treated endothelial cells cultured under normoglycemic conditions express similar LOX-1 levels to high glucose–treated cells. Levels of LOX-1 protein elicited by this cytokine were reduced in HAECs exposed to high glucose concentrations. Under these experimental conditions, no modulation of the β-actin protein levels, used as internal control, was observed. LOX-1 protein levels normalized to the levels of β-actin are illustrated in Fig. 3.

Role of PKC, mitogen-activated protein kinases, and NF-κB in the upregulation of endothelial cell LOX-1 gene expression by glucose.

To determine whether glucose induces endothelial LOX-1 gene expression via PKC- and/or mitogen-activated protein kinase (MAPK)-dependent pathways, HAECs were pretreated for 1 h in the presence or absence of the pan-specific PKC inhibitor, calphostin C (0.1 μg/ml), the PKCβ inhibitor, LY379196 (30 nmol/l), or the MAPK inhibitor, PD98059 (50 μmol/l), before exposure to high glucose. As shown in Fig. 4*A*, incubation of HAECs with these inhibitors completely prevented the stimulatory effect of high glucose on LOX-1 mRNA expression. The inhibitory effect of LY379196 was still observed when cells were exposed to 20 mmol/l glucose (LOX-1 mRNA expression [% of control values]: glucose [20 mmol/l] 150 ± 9; glucose + LY379196 113 ± 8; $P < 0.05$). Under these experimental conditions, no modulation of the mRNA expression of GAPDH, used

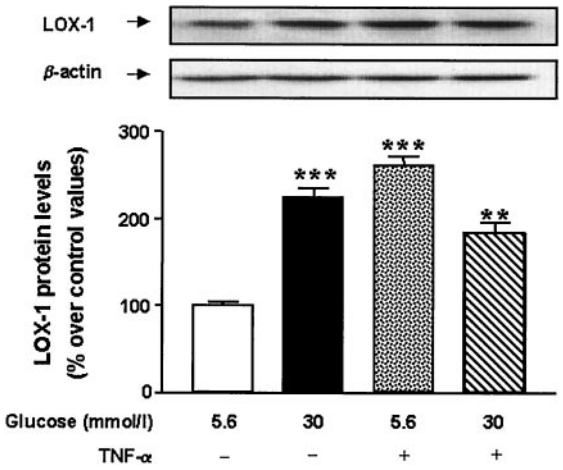

FIG. 3. Effect of high glucose and TNF-α on endothelial LOX-1 protein expression. HAECs were cultured for 48 h in low (5.6 mmol/l) or high (30 mmol/l) glucose environment and then treated for an additional 24-h time period with TNF-α (50 ng/ml). At the end of the incubation period, cells were lysed and LOX-1 membrane protein expression was determined by Western blot analysis. LOX-1 protein levels were normalized to the levels of β-actin protein. Data illustrated on the graph bar represent the means ± SE of four different experiments. **P < 0.01, ***P < 0.001 vs. 5.6 mmol/l glucose.

as internal control, was observed (Fig. 4*A*). LOX-1 mRNA levels, normalized to the levels of GAPDH mRNA, are presented in Fig. 4*A*. A similar suppressive effect was observed when these cells were incubated with BAY 11-7085 (40 μmol/l), an inhibitor of the oxidative stress–sensitive transcription factor NF-κB (Fig. 4*A*).

Role of oxidative stress in high glucose-stimulated endothelial cell LOX-1 gene expression. Because vascular cells respond to high glucose by altering the intracellular redox state, we next determined the role of oxidative stress in glucose-induced LOX-1 mRNA expression. HAECs were pretreated for 1 h in the presence or absence of NAC (10 mmol/l), DMSO (0.5%), vitamin C (10 μmol/l), or vitamin E (50 μmol/l) and then incubated for 48 h with 30 mmol/l glucose. As shown in Fig. 4*B*, these antioxidants prevented the stimulatory effect of high glucose on HAECs LOX-1 gene expression. LOX-1 mRNA levels, normalized to the levels of GAPDH mRNA, are presented in Fig. 4*B*.

Effect of high glucose concentrations on the binding of nuclear proteins to the regulatory NF-κB sequences of the LOX-1 gene promoter. We next determined whether incubation of HAECs in the presence of high glucose concentrations might induce changes at the level of LOX-1 gene promoter binding proteins. We found that a 24-h exposure of HAECs to a high-glucose environment resulted in a dramatic increase in the binding of nuclear proteins to the NF-κB consensus sequence of the human LOX-1 promoter. (Fig. 5). This binding was specifically conducted in the presence of a 1,000-fold molar excess of the unlabeled NF-κB oligonucleotide and was completely abrogated by BAY 11-7085 and antibodies against p50 and/or p65 (Fig. 5). Inhibition of high-glucose–induced NF-kB binding was further observed after treatment of the cells with NAC, PKC, and MAPK inhibitors (Fig. 5).

Role for LOX-1 as mediator of the stimulatory effect of high glucose on monocyte binding to endothelial cells. Treatment of HAECs for 72 h with 30 mmol/l glucose significantly increased monocyte adhesion to these cells (Fig. 6). This effect was totally suppressed by preincubating HAECs with anti–LOX-1 antibody (monocyte adhesion [% of control values]: 30 mmol/l glucose 180 ± 16, $P < 0.05$; anti–LOX-1 83 ± 10, $P < 0.001$; anti-IgG 191 ± 24) (Fig. 6). To test the involvement of selectins and integrins in glucose-induced monocyte adhesion to endothelium, HAECs were also preincubated with antibodies to ICAM-1, VCAM-1, and E-selectin before exposure to glucose. Our results demonstrate that under our experimental conditions, exposure of the cells to these antibodies did not inhibit glucose-induced monocyte adhesion to endothelial cells (monocyte adhesion [% of control values]: 30 mmol/l

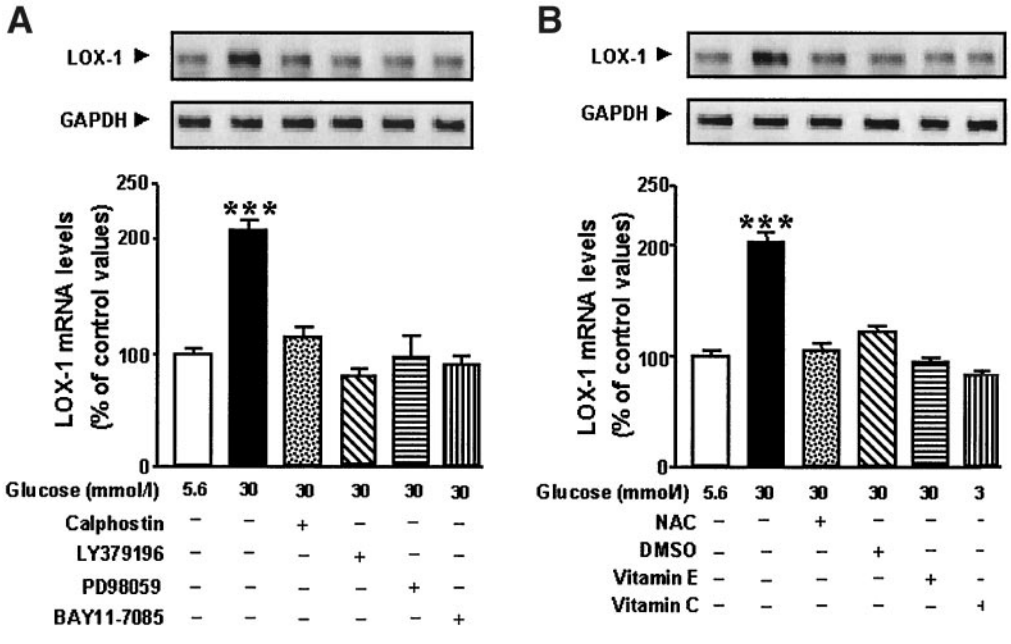

FIG. 4. Effect of PKC, MAPK, and NF-κB inhibitors (*A*) and the effect of antioxidants (*B*) on glucose-induced LOX-1 mRNA levels. HAECs were pretreated for 1 h with the pan-specific PKC inhibitor calphostin C (0.1 μg/ml), the PKCβ inhibitor LY379196 (30 nmol/l), the MAPK inhibitor PD98059 (50 μmol/l), antioxidants (NAC [10 mmol/l], DMSO [0.5%], vitamin E [50 μmol/l], and vitamin C [10 μmol/l]), or the NF-κB inhibitor BAY 11-7085 (40 μmol/l) and then exposed to 30 mmol/l glucose for 48 h. At the end of the incubation period, cells were lysed and LOX-1 mRNA was analyzed by RT-PCR. LOX-1 mRNA levels were normalized to the levels of GAPDH mRNA. Data illustrated on the graph bar represent the mean ± SE of seven different experiments. ***P < 0.001 vs. 5.6 mmol/l glucose.

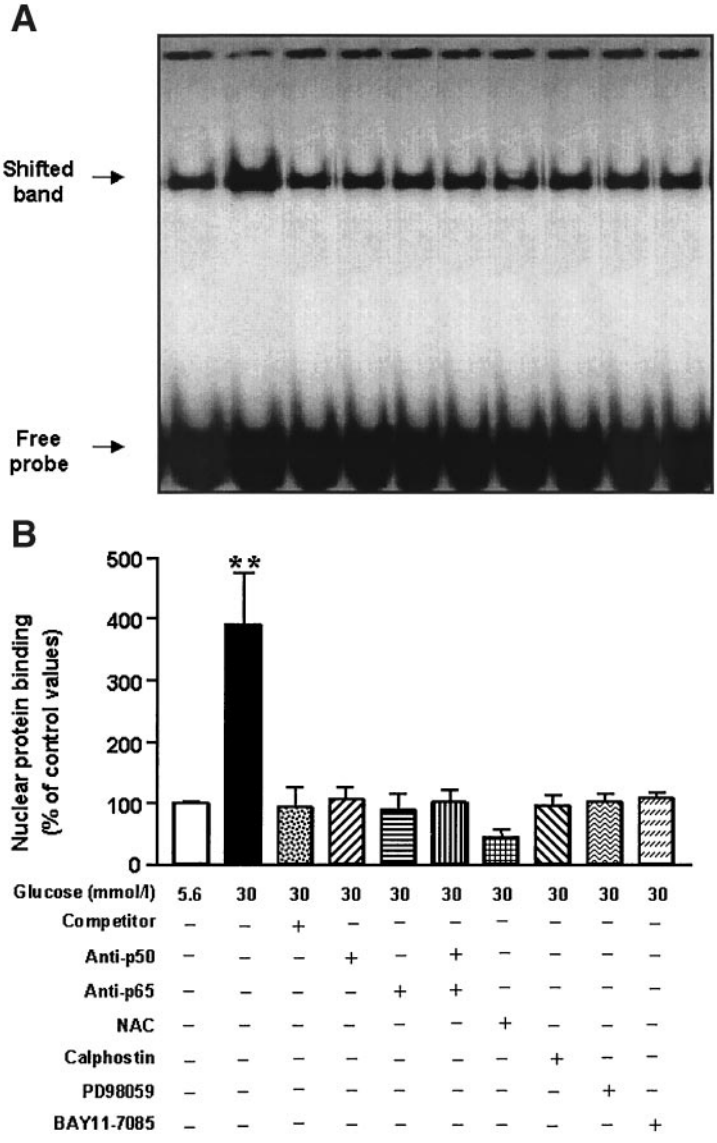

FIG. 5. Effect of high glucose on the binding of nuclear proteins extracted from HAECs to the NF-κB sequence of the LOX-1 gene promoter. HAECs were or were not pretreated for 1 h with NAC (10 mmol/l), calphostin C (0.1 μg/ml), PD98059 (50 μmol/l), or BAY 11-7085 (40 μmol/l) and then exposed for 24 h to 5.6 or 30 mmol/l glucose. Nuclear proteins isolated from these cells were incubated with end-labeled double-stranded oligonucleotide containing the NF-κB sequence of the LOX-1 promoter in the presence or absence of 1,000-fold molar excess of unlabeled DNA probe (competitor). In some experiments, nuclear proteins were incubated in the presence of anti-p50 and/or anti-p65 antibodies. Retardation was assessed by gel electrophoresis. *A*: Data represent the results of one of four representative experiments. *B*: Graph bar showing the results of four independent experiments. **$P < 0.01$ vs. 5.6 mmol/l glucose.

glucose 180 ± 16, $P < 0.05$; anti–ICAM-1 167 ± 33; anti–VCAM-1 163 ± 16; anti–E-selectin 156 ± 23).

DISCUSSION

Evidence that LOX-1 expression is increased in the vascular endothelium of diabetic rats (21) supports a role for this receptor in endothelial dysfunction associated with diabetes. The factors responsible for vascular LOX-1 upregulation in diabetes are unknown but may include atherogenic lipoproteins and AGEs (21). In the present study, we demonstrated that high glucose enhances LOX-1 expression, both at the gene and protein levels, in cultured HAECs and that this effect requires glucose metabolism by endothelial cells since the nonmetabolized glucose isomer failed to produce similar effects. These results suggest that hyperglycemia per se may contribute to LOX-1 induction in diabetes. In contrast to our data, Chen et al. (21) recently reported that control rat serum, along with high glucose concentrations, did not enhance LOX-1 mRNA expression in cultured bovine aortic endothelial cells. Because both studies differ in many experimental aspects, including the cell type tested and the stimulatory conditions used, these conflicting results may only be apparent. Our findings that glucose-induced LOX-1 expression is comparable to that elicited by TNF-α and that glucose and TNF-α do not synergize for LOX-1 induction support the possibility that these factors may regulate LOX-1 expression through one major and possibly identical pathway. Glucose and TNF-α are both well-known activators of NF-κB (35–39), and the 5′ flanking region of the LOX-1 gene contains a consensus NF-κB binding site–like sequence (33). While evidence has been provided that TNF-α activates the transcription of the LOX-1 gene (23), we found that high glucose increases endothelial LOX-1 mRNA levels and enhances nuclear protein binding to the NF-κB regulatory sequence of the LOX-1 promoter. In agreement with previous studies showing that high glucose and TNF-α stimulate the p50 and p65 subunits of NF-κB in monocytic cells (39), we found that the p50 and p65 are critical components of the transacting NF-κB complex stimulated by high glucose in endothelial cells. Taken together, these results suggest that transcriptional regulation of the LOX-1 gene mediated by p50 and p65 may be involved in LOX-1 induction by glucose and TNF-α.

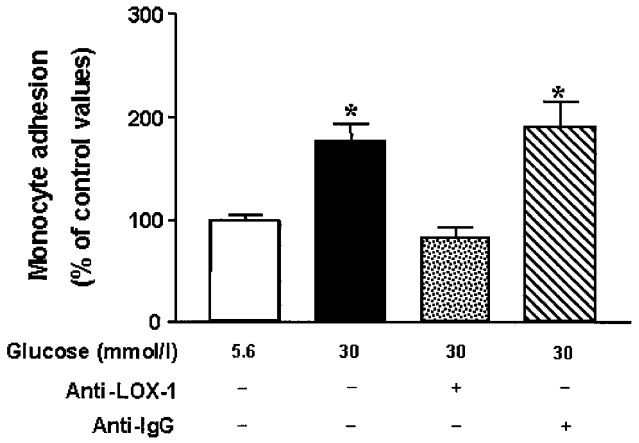

FIG. 6. Effect of high glucose on human monocyte adhesion to endothelial cells. Confluent HAECs were exposed for 72 h to 5.6 or 30 mmol/l glucose in the presence of anti–LOX-1 or anti-IgG_1 antibodies. At the end of this incubation period, cells were washed and monocytes were added to HAECs to determine monocyte adhesion. Data are expressed as percentage of adherent monocytes and represent the means ± SE of five different experiments. *P < 0.05 vs. 5.6 mmol/l glucose.

Another interesting finding of this study is that culturing endothelial cells in high glucose medium reduces the responsiveness of these cells to further TNF-α stimulation. One possible explanation for this effect is that high glucose may stimulate the release of TNF-α by endothelial cells and that accumulation of this cytokine into the culture medium may contribute, at least in part, in decreasing the TNF-α responsiveness of these cells. Although the effect of high glucose on TNF-α secretion by endothelial cells has not been studied, evidence exists that these cells secrete TNF-α in response to endotoxin and hydrogen peroxide (40,41). The possibility that glucose may stimulate TNF-α release by endothelial cells is currently being examined.

It is well demonstrated that vascular cells, including endothelial cells, respond to high glucose by altering the intracellular redox state (37,39,42,43) and increasing PKC and MAPK activation (44). The clear link established between oxidative stress evoked by glucose and PKC/MAPK activation (37,39,45,46) and the documented ability of these kinases to regulate NF-κB activity (47,48) suggests that glucose-stimulated LOX-1 gene expression in HAECs could involve increased oxidative stress and activation of PKC- and MAPK-dependent pathways. Our results, which demonstrate that antioxidants and inhibitors of PKC and MAPK prevented the activation of NF-κB and the induction of endothelial LOX-1 mRNA expression by glucose, confirm this possibility. Evidence that the PKCβ isoform–specific inhibitor, LY379196, effectively inhibits glucose-induced LOX-1 mRNA levels further indicates a role for PKCβ in this effect. On the basis of these results, we elaborate a tentative model in which reactive oxygen species (ROS) generated by glucose metabolism induces the coordinate activation of PKC and MAPK as upstream kinases and NF-κB as downstream transcription factor, thereby leading to the induction of LOX-1 gene expression.

One major adverse vascular consequence associated with endothelial dysfunction in diabetes is increased monocyte adhesion to endothelium. Evidence that endothelial LOX-1 supports adhesion of monocytes and is upregulated in diabetes (19–21) suggests a role for this receptor in the enhanced binding of monocytes documented in patients with diabetes (28,49,50). Previous studies have shown that glucose may contribute to the increased monocyte adherence to endothelium in diabetes (25–28). It has been proposed that the effect of glucose on monocyte adhesion may occur via mechanisms involving the upregulation of endothelial and/or leukocyte adhesion molecules (25,26,28,51,52), the activation of NF-κB, and the generation of ROS (27). Our results showing that blockade of LOX-1 completely abolished glucose-induced monocyte adhesion to endothelial cells demonstrate a new role for LOX-1—mediator of the stimulatory effect of glucose on monocyte adhesion. In contrast, our findings that blocking antibodies to endothelial leukocyte adhesion molecule-1, ICAM-1, and VCAM-1 did not significantly affect glucose-induced monocyte adhesion to HAECs clearly indicate that, under our experimental conditions, these adhesion molecules are not key determinants of glucose-induced monocyte binding to endothelium. Besides glucose, hypertriglyceridemia has been documented to enhance monocyte binding to endothelial cells in type 2 diabetes (49). Because VLDL fractions of diabetic serum induce endothelial LOX-1 expression (21), a possible role for this receptor as mediator of hypertriglyceridemia-induced monocyte-endothelial adhesion may be proposed. Experiments aimed at testing this possibility are under way.

One limitation of this study is the use of high glucose concentrations. While plasma glucose concentrations of ~20 mmol/l are not chronically seen in patients with diabetes, 30 mmol/l glucose is virtually incompatible with human life in chronic diabetes. In contrast, hyperglycemic excursions are frequent in patients with diabetes and peak glucose concentrations reaching 15–20 mmol/l are documented in poorly controlled diabetic patients. Whether in vitro exposure of vascular cells to high glucose concentrations may replicate, to some extent, the conditions experienced by the cells of patients with diabetes during short-lived hyperglycemic excursions is unknown.

In conclusion, our data indicate that high glucose concentrations enhance LOX-1 expression in endothelial cells. They also demonstrate a new role for LOX-1 as a mediator of glucose-induced monocyte adhesion. A better understanding of the mechanisms of hyperglycemia-induced endothelial dysfunction may unmask new strategies to reduce diabetic vascular complications.

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