High glucose-mediated effects on endothelial cell proliferation occur via p38 MAP kinase

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High glucose-mediated effects on endothelial cell proliferation occur via p38 MAP kinase. Am J Physiol Endocrinol Metab 285: E708–E717, 2003. First published June 5, 2003; 10.1152/ajpendo.00572.2002.—The mitogen-activated protein (MAP) kinases contribute to altered cell growth and function in a variety of disease states. However, their role in the endothelial complications of diabetes mellitus remains unclear. Human endothelial cells were exposed for 72 h to 5 mM (control) or 25 mM (high) glucose plus 20 mM mannitol (osmotic control). The roles of p38 and p42/44 MAP kinases in the high glucose-induced growth effects were determined by assessment of phosphorylated MAP kinases and their downstream activators by Western blot and by pharmacological inhibition of these MAP kinases. Results were expressed as a percentage (means ± SE) of control. High glucose increased the activity of total and phosphorylated p38 MAP kinase (P < 0.001) and p42/44 MAP kinase (P < 0.001). Coexposure of p38 MAP kinase blocker with high glucose reversed the antiproliferative but not the hypertrophic effects associated with high-glucose conditions. Transforming growth factor (TGF)-β1 increased the levels of phosphorylated p38 MAP kinase, and p38 MAP kinase blockade reversed the antiproliferative effects of this cytokine. The high glucose-induced increase in phosphorylated p38 MAP kinase was reversed in the presence of TGF-β1 neutralizing antibody. Although hyperosmolarity also induced antiproliferation (P < 0.0001) and cell hypertrophy (P < 0.05), there was no change in p38 activity, and therefore inhibition of p38 MAP kinase had no influence on these growth responses. Blockade of p42/44 MAP kinase had no effect on the changes in endothelial cell growth induced by either high glucose or hyperosmolarity. High glucose increased p42/44 and p38 MAP kinase activity in human endothelial cells, but only p38 MAP kinase mediated the antiproliferative growth response through the effects of autocrine TGF-β1. High glucose-induced endothelial cell hypertrophy was independent of activation of the MAP kinases studied. In addition, these effects were independent of any increase in osmolarity associated with high-glucose exposure.

High glucose; endothelial cell; growth; p38 mitogen-activated protein kinase; p42/44 mitogen-activated protein kinase; transforming growth factor-β1

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p38 or p42/44 MAP kinase in human umbilical vein endothelial cells (HUVECs) after exposure to high glucose but showed increased JNK expression with resultant downstream caspase activation (11). Other in vivo studies showed that p42/44 MAP kinase activity was increased in glomeruli harvested from diabetic animals (10, 14). These studies, however, focused either on intact glomeruli or the mesangial cells and failed to determine the relative contribution of the glomerular endothelial cells to the increased p42/44 MAPK activity (14).

p38 MAP kinase is preferentially activated by a variety of environmental stresses, including oxidative stress, osmotic shock, and inflammatory cytokines such as transforming growth factor (TGF)-β1 (8, 25, 30, 31). The growth effects of high glucose on a number of cell types have previously been attributed to all of these factors (2, 7, 25). Consequently, p38 MAP kinase is speculated to mediate a number of the pathogenic effects of high glucose. Recently, increased levels of p38 MAP kinase have been reported in diabetic neuropathy and diabetic nephropathy (14, 26). In addition, activation of p38 MAP kinase has been implicated in the increased apoptosis observed in endothelial cells exposed to high glucose (24). Furthermore, hypertonicity has been demonstrated to induce p38 MAP kinase activity in endothelial cells (8, 20). However, the relationship between the increased osmolarity in experimental models of hyperglycemia, as distinct from effects directly due to increased levels of glucose on MAP kinase activity, remain unclear.

The aim of this study was to correlate the direct effects of high glucose on cell growth in human endothelial cells with the levels of total and phosphorylated p42/44 and p38 MAP kinases and their functional activity. Furthermore, the role of increased osmolarity and TGF-β1 on levels of p38 MAP kinase activity observed with high glucose were investigated.

METHODS

Cell isolation and culture conditions. HUVECs were isolated from umbilical cords of normal pregnancies by the method of Jaffe et al. (13). Ethical approval for the study was obtained from the Royal North Shore Hospital Human Research Ethics Committee. These cells were cultured on flasks coated with 0.2% gelatin (BDH, Poole, UK) in medium M199 (ICN, Aurora, OH) supplemented with 10% fetal calf serum coated with 0.2% gelatin (BDH, Poole, UK) in medium M199.

Endothelial cell growth supplement. Endothelial cell growth supplement was obtained from bovine hypothalamus (19) and tested against a commercial preparation (Sigma). Cells were grown at 37°C in a humidified 5% CO₂ incubator and subcultured at confluence by trypsinization with 0.05% trypsin-0.02% EDTA (GIBCO-BRL, Gaithersburg, MD), penicillin (100 U/ml, ICN, Aurora, OH) supplemented with 10% fetal calf serum and tested against a commercial preparation (Sigma). Cells were grown on 6-well plates and exposed to the various experimental conditions, including both osmotic controls. After 72 h, Triton X-100-soluble fractions were prepared from cells by use of 50 mM Tris·HCl, 150 mM NaCl, 0.5% Triton X-100, 1 mM sodium orthovanadate, and protease inhibitors (Roche Diagnostics, Mannheim, Germany) and were centrifuged at 10,000 rpm at 4°C for 10 min. The Triton X-100-soluble fractions were then mixed with 6× Laemmli gel sample buffer containing mercaptoethanol and heated to 95°C for 10 min. Samples were then run on a 10% SDS-polyacrylamide gel and then transferred to a Hybond C nitrocellulose filter (Amersham Pharmacia Biotech, Buckinghamshire, UK) by Western blotting. The blot was blocked overnight with 5% casein in PBS and 0.1% Tween 20. After thorough washing, the blot was incubated for 2 h at room temperature with rabbit antihuman total MAPK (MAPK) expressed under the various experimental conditions. Results were determined as total protein content per treatments, the analysis of the band intensities was adjusted to the density of immobilized phospho-p42/44 MAPK monoclonal antibody were added and incubated for 2 h at room temperature. The blot was then developed using the Amersham enhanced chemiluminescence kit (Amersham Pharmacia Biotech) and visualized using X-ray film. The resultant films were then scanned and the relative band intensities measured using the NIH Image software. Equal volumes of sample were loaded onto each gel and, because of the differing growth responses observed between treatments, the analysis of the band intensities was adjusted according to the differences in cell number.

p38 and p42/44 MAPK assay. MAPK assays (Cell Signaling) were performed by following the manufacturer’s instructions to correlate the expression of the phosphorylated MAPKs with their functional activity. Briefly, endothelial cells were grown on 6-well plates and exposed to the various experimental conditions including both osmotic controls. Cell lysates were prepared as we have described for Western blotting; however, to 200 µl of cell lysate (containing ~200 µg of total protein) 15 µl of immobilized phospho-p42/44 MAPK monoclonal antibody were added and incubated at 4°C over-
night. The sample was then washed twice with lysis buffer and kinase buffer supplied by the manufacturer. The pellet was then suspended in 50 μl of kinase buffer supplemented with 200 μM ATP and 2 μg of Elk-1 fusion protein (for p42/44 MAPK) and 2 μg activating transcription factor (ATF)-2 fusion protein (for p38 MAPK) and incubated at 30°C for 30 min. The reaction was then terminated with 25 μl of 3× Laemmli gel sample buffer. The sample was then boiled for 5 min and microcentrifuged for 2 min. A 30-μl sample was then loaded onto a 10% SDS-PAGE gel and transferred to a Hybond nitrocellulose filter (Amersham Pharmacia Biotech) by Western blotting. The blot was blocked overnight with 5% casein in PBS and 0.1% Tween 20. The standard Western immunoblotting method was then used as above. The primary antibody concentrations were 1:1,000 for both phospho-Elk-1 and phospho-ATF-2 antibodies. The membranes were incubated with horseradish peroxidase (HRP)-conjugated anti-rabbit secondary antibody (1:2,000) and HRP-conjugated antibiotin antibody (1:1,000) for both p38 and p42/44 MAPK assays. The proteins were then detected by use of the LumiGLO system supplied by the manufacturer and were visualized using X-ray film. Analysis was performed as for Western blotting.

MAPK blockade and cell growth. Endothelial cells were seeded onto 96-well tissue culture plates (Costar). Cells were then exposed to control, high-glucose, and osmotic control treatment in the presence or absence of MAPK blockers. After 72 h of exposure to these conditions, the growth parameters of cell number and cell protein content were measured as we discussed. We used two different p42/44 MAPK blockers, PD-98059 (0.5 mM), both from Cell Signaling; these are noncompetitive inhibitors of mitogen-activated protein kinase kinase (MEK)-1 and MEK-2. To inhibit p38 MAPK, the inhibitor SB-203580 (Biomol, Plymouth Meeting, PA) was used at a concentration of 5 μM.

TGF-β1 and p38 MAPK. We (22) have previously demonstrated that autocrine TGF-β1 mediates high glucose-induced endothelial cell growth effects. We therefore measured the levels of phosphorylated p38 MAPK in cells exposed to either TGF-β (0.5 ng/ml) alone or TGF-β and high glucose for 72 h. To assess whether phosphorylated p38 MAPK was induced via a mechanism that involved endogenously produced TGF-β1, a panspecific antihuman TGF-β1-neutralizing antibody was added at a concentration of 30 μg/ml to cells exposed to high glucose for 72 h.

Statistical analysis. The experiments were performed on a minimum of four different endothelial cell isolates, unless otherwise stated. For each set of data the frequency distribution was assessed. In data sets that were normally distributed, the results were expressed as a percentage change from the control value (5 mM n-glucose) and were expressed as means ± SE. Statistical comparisons between the normally distributed groups were made by analysis of variance (ANOVA), with pairwise multiple comparisons made by Fisher’s protected least significant differences test. For the data that exhibited a nonparametric distribution, the results are expressed as the median and the interquartile range (IQR). The Mann Whitney U-test was then applied to determine significance. Analyses were performed using Statview version 5.0 (Abacus Concepts, Berkeley, CA). P values <0.05 were considered significant.

RESULTS

Growth studies. The endothelial cells exposed to high glucose showed the previously described antiproliferative and hypertrophic responses (17, 22). After 72-h exposure to high glucose, there was a significant reduction in cell number to 75.3 ± 2.1% (n = 4; P < 0.001) of control values, with an increased protein content per cell to 142.5 ± 5.8% of control values (n = 4; P < 0.001). The osmotic control induced a similar but less marked response with cell number at 83.1 ± 2.7% (n = 4; P < 0.0001) and cell protein content at 116.3 ± 2.5% (n = 4; P < 0.05) compared with control values.

Levels of total and phosphorylated p42/44 MAPK. Initially, we investigated whether continuous exposure to high glucose altered the total levels of p42/44 MAPK and the phosphorylated isoforms and whether these effects were attributable to an osmotic effect of high glucose. Treatment of endothelial cells with high glucose for 72 h significantly increased the levels of both total and phosphorylated p42/44 MAPK protein expression per cell (Fig. 1A). Western blotting with an antibody against total p42/44 MAPK showed a median increase of 178% (IQR = 38.2%; n = 3; P < 0.01) and against the phosphorylated form of p42/44 MAPK showed a median increase in the level of phosphorylation of p42/44 MAPK to 169.9% (IQR = 43.4%; n = 4; P < 0.0001) of control levels when adjusted for the decrease in cell number observed with high glucose (Fig. 1B). In contrast, exposure to mannitol had no effect on the levels of phosphorylated p42/44 MAPK, with a median level of 104.4% (IQR = 22.2%; n = 4) not significantly different from control levels (Fig. 1B).

p42/44 MAPK activity. We then used functional assays of p42/44 MAPK activity to confirm that the increased levels of phosphorylation of MAPK were accompanied by increased kinase activity. Phospho-MAPK was immunoprecipitated and then used to phosphorylate downstream effectors. The phosphorylation levels of the individual effectors were then assessed using Western blotting and phosphospecific antibodies. Elk-1 is a downstream target of p42/44 MAPK. A representative blot showing Elk-1 phosphorylated by p42/44 MAPK immunoprecipitated from cells treated with high glucose or mannitol for 72 h is shown in Fig. 2A. High glucose increased the levels of Elk-1 phosphorylation on a per cell basis to 182.2 ± 21.9% (n = 4; P < 0.001) of control levels (Fig. 1B), an increase that reflected the increased phospho-p42/44 levels. p42/44 MAPK immunoprecipitated from cells treated with mannitol had no effect on levels of Elk-1 phosphorylation (99.3 ± 9.1%; n = 4; Fig. 2B), a result that was also consistent with the lack of effect of mannitol on p42/44 MAPK phosphorylation levels.

Levels of total and phosphorylated p38 MAPK. Western blotting was then used to determine the effects of high glucose on the phosphorylation levels of p38 MAPK. Representative Western blots showing levels of total and phospho-p38 MAPK expression is shown in Fig. 3A. Exposure of endothelial cells to high glucose for 72 h was associated with an increase in levels of total and phosphorylated p38 MAPK protein expression per cell to 156 ± 10% (n = 3; P < 0.01) and 150.2 ± 9.8% (n = 4; P < 0.0001), respectively, compared with control levels (Fig. 3B). Exposure to the osmotic control did not alter the expression of the phosphorylated

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enzyme, with the level per cell measured at 93.8 ± 8.8% of control levels (Fig. 3B).

p38 MAPK activity. ATF-2 is a downstream target of p38 MAPK. A representative blot showing ATF-2 phosphorylated by p38 MAPK immunoprecipitated from cells treated with high glucose or mannitol for 72 h is shown in Fig. 4A. High glucose increased the levels of ATF-2 phosphorylation on a per cell basis to 227.3 ± 31.7% (n = 4; P < 0.001) of control levels (Fig. 4B), confirming that the increase in phospho-p38 MAPK levels was accompanied by a parallel increase in levels of downstream activity. Because the osmotic control had no effect on the levels of phospho-p38 MAPK, we did not measure the effects of the osmotic control on ATF-2 phosphorylation.

Effects of MAPK blockade on cell growth. We then investigated the effects of MAPK blockade on cell number and cell protein content. Under control conditions for 72 h, blockade of p42/44 MAPK was associated with a marked antiproliferative endothelial cell growth response, with cell number at 60.9 ± 4.5% (n = 4; P < 0.0001) for PD-98059 and 76.5 ± 7.8% (n = 4; P < 0.0001) for UO-126 compared with control levels (100%; Fig. 5A). Blockade of p42/44 MAPK did not reverse the growth effects observed after exposure of endothelial cells to either high glucose or the osmotic control. Inhibition of p42/44 MAPK in the presence of high glucose maintained the antiproliferative response observed with high glucose, with cell number at 56.9 ± 4.6% (n = 4; P < 0.0001) for PD-98059 and 75.8 ± 7.4% (n = 4; P < 0.0001) for UO-126 compared with control levels (Fig. 5A). The reduction in cell number induced by high glucose was not reversed by the osmotic control, with cell number at 67.5 ± 4.1% (n = 4; P < 0.0001) for PD-98059 and 77.8 ± 7.2% (n = 4; P < 0.0001) for UO-126 compared with control levels (100%; Fig. 5B).
by p42/44 MAPK blockade was also maintained during exposure to the osmotic control (Fig. 5A).

Blockade of p42/44 MAPK also had a profound effect on cell protein content, resulting in the induction of a hypertrophic growth response. Exposure of cells under control conditions to either PD-98059 or UO-126 for 72 h increased cell protein, respectively, to 136.4 ± 10.5% (n = 4; P < 0.01) and 140.4 ± 10.7% (n = 4; P < 0.0001) of control levels (100%; Fig. 5B). When cells were exposed to high glucose in the presence of MAPK blockers, there was a significant increase in the protein content per cell above the levels observed with the blockers alone. Cell protein content increased to 193.2 ± 14.8% (n = 4; P < 0.0001) with PD-98059 and 121.3 ± 7.7% (n = 4; P < 0.05) with UO-126 compared with control values (100%; Fig. 5B). Similarly, cells grown in the osmotic control and p42/44 MAPK blockers had increased protein content per cell above the levels observed with the blockers alone (Fig. 5B).

In contrast to the effects of p42/44 MAPK inhibition, blockade of p38 MAPK with SB-203580 (5 μM) under control conditions had no effect on endothelial cell number (Fig. 6A). However, when cells were grown in high glucose and SB-203580, the antiproliferative effect of high glucose was reversed to control levels (median cell number of 102.2% of control; IQR = 11.2%; n = 4; Fig. 6A). This effect was specific for glucose, since inhibition of p38 MAPK did not reverse the antiproliferative effect of the osmotic control (median cell number of 79.9% of control levels; IQR = 11.6%; n = 4; P < 0.0001; Fig. 6A). Despite having no effect on cell number, treatment of cells with SB-203580 alone for 72 h resulted in an increase in cell protein content to a median of 132.2% (IQR = 21.3%; n = 4; P < 0.0001) of control levels (Fig. 6B). However, in the presence of high glucose and SB-203580, there was no additive effect on cell protein content, with a median level of 151.0% (IQR = 50.7%; n = 4; P < 0.0001; Fig. 6B). Similarly, coexposure of endothelial cells to SB-203580 and the osmotic control did not alter the hypertrophic effect of SB-203580, resulting in a cellular protein content median of 144.5% (IQR = 45; n = 4; P < 0.01; Fig. 6B). To eliminate the possibility that the effects of PD-98059 and SB-203580 were due to nonspecific inhibition of cyclooxygenase (COX), we performed growth studies by using the COX-1 inhibitor resveratrol and the COX-2 inhibitor NS-398. COX inhibition did not reverse the antiproliferative effect of high glucose or the potentia-
We then determined whether the increase in phospho-p38 MAPK in high glucose was due to an autocrine effect of TGF-β1. No change in phosphorylated p38 MAPK was observed in cells concurrently exposed to high glucose and TGF-β1-neutralizing antibody (86 ± 8%; n = 3; P = NS) compared with control. Cells treated with high glucose and nonimmune IgG had levels similar to those observed in cells exposed to high glucose alone (145 ± 8% and 140 ± 4%, respectively; Fig. 8).

**DISCUSSION**

The current study determines the relative contribution of the p42/44 and p38 MAP kinases in mediating...
the high glucose-induced changes in human endothelial cell growth. p42/44 MAPK is classically associated with mitogenesis (6, 31); therefore, increased activity of this enzyme would be expected to occur with cell proliferation. We found, however, that there was an increase in total protein levels of p42/44 MAPK and an increase in downstream activity when the cells were undergoing proliferative arrest and hypertrophy after exposure to high-glucose conditions. Inhibition of p42/44 MAPK in cells under control conditions resulted in a significant decrease in the cell number, reflecting that endothelial cells have a significant level of basal p42/44 MAPK activity during normal growth. Furthermore, this study also shows that inhibition of constitutive p42/44 MAPK activity results in cellular hypertrophy. Initially, p42/44 MAPK inhibition appeared to mimic the growth effects of high glucose; however, inhibition of p42/44 MAPK in cells exposed to high glucose resulted in an additive potentiation of the hypertrophic response, indicating that this effect of high glucose was independent of the action of p42/44 MAPK.

**Fig. 6.** p38 MAPK blockade. A: endothelial cell number after 72-h exposure to control (5 mM glc), high glucose (25 mM glc), and mannitol (Mann) in the presence or absence of SB-23580 (SB). **P < 0.01 compared with control. B: endothelial protein content/cell after 72-h exposure to control (5 mM glc), high glucose (25 mM glc), and mannitol in the presence or absence of SB-23580. **P < 0.01 and *P < 0.05 vs. control.

A: 38 kDa

**Fig. 7.** A: a representative Western blot demonstrating levels of phospho-p38 MAPK before adjustment for differences in cell number observed between treatments: control (5 mM glc) or high glucose (25 mM glc) in the presence or absence of transforming growth factor (TGF)-β1 (0.5 ng/ml). Equal volumes were loaded onto the gel, so Western blot does not reflect differences in cell number observed between treatments. B: levels of phospho-p38 MAPK after 72-h exposure to experimental conditions. Adjustments for differences in cell number between treatments have been made. **P < 0.01 vs. control values; *P < 0.01 vs. control + TGF-β1. C: endothelial cell number after 72-h exposure to control (5 mM glc) or control and TGF-β1 (0.5 ng/ml) in the presence or absence of SB-23580. **P < 0.01 vs. control; *P < 0.001 vs. control + TGF-β1.
The increased p42/44 MAPK activity that we have observed in endothelial cells exposed to high glucose must therefore have other roles, such as the increased extracellular matrix deposition that is commonly observed in the vasculature of patients with diabetes mellitus. In keeping with this, mouse mesangial cells show increased p42/44 MAPK activity after exposure to high glucose, with increased TGF-β1 production and enhanced collagen and fibronectin production (12).

p38 MAPK was also activated by high-glucose exposure to endothelial cells. However, in contrast to p42/44 MAPK, inhibition of p38 MAPK under control conditions had no effect on cell proliferation but did result in cellular hypertrophy. The lack of effect on cell number is consistent with p38 MAPK not being involved in the normal proliferative response on the endothelial cell. However, the main finding of this study was that inhibition of p38 MAPK in cells exposed to high glucose reversed the antiproliferative effect of high glucose, with the cell number returning to control levels. The p38 MAPK pathway has been shown to play a negative regulatory role in cell cycle progression, with direct effects on the cyclin-dependent kinase inhibitors (23, 31). Furthermore, both in vivo and in vitro studies demonstrate that p38 MAPK blockade is associated with increased angiogenesis (1, 21, 27). However, increased p38 MAPK activity has not previously been linked to the antiproliferative effect of high glucose commonly observed with endothelial cells (17, 22).

We have previously shown that the antiproliferative effects of high glucose occur largely as a result of a TGF-β1-mediated delay in cell cycle progression and a concomitant increase in levels of apoptosis (22). TGF-β1 is known to mediate part of its effects through p38 MAPK (28), and the increased levels of apoptosis that occur after exposure to high glucose in human and bovine aortic endothelial cells are attenuated after blockade of p38 MAPK (24). Our study demonstrates that both high glucose and TGF-β1 increased levels of phospho-p38 MAPK in human endothelial cells and that this increased expression has effects beyond those on apoptosis. Importantly, coexposure of cells to TGF-β1 and high glucose did not have any synergistic effect on the levels of phospho-p38 MAPK, and the high glucose-induced increase in phospho-p38 MAPK was completely blocked in the presence of a TGF-β1-neutralizing antibody.
tralizing antibody. These data suggest that TGF-β1 may be the sole mediator of the effects of high glucose on p38 MAPK activity, an observation confirmed by the p38 MAPK-blocking experiments demonstrating that the antiproliferative effects of TGF-β1 occur via p38 MAPK. TGF-β1 is known to exert an antiproliferative effect on a number of cells, and Terada et al. (28) showed that, in LLC-PK1 cells, this cytokine’s growth inhibitory effects are at least partially mediated by TGF-β-activated kinase (TAK)/p38 MAPK pathway. This TAK/p38 MAPK pathway was shown to regulate cyclin D1 and A promoter activity negatively, with resultant delay in cell cycle progression. It is likely that, in human endothelial cells, high glucose may also act via this pathway by induction of autocrine TGF-β1.

In addition to autocrine effects of TGF-β1, some of the effects of high glucose are attributed to an increase in osmolarity (2). Moderate changes in osmolarity as observed with high glucose (15–30 mM) have been shown to reduce endothelial cell proliferation and induce cell hypertrophy (17, 22), whereas exposure to 50 mM glucose was found to cause a moderate increase in expression of p38 MAPK, suggestive of induction of a hyperosmotic stress pathway (8). In the current study with an osmotic load of 20 mM, blockade of p38 MAPK failed to reverse any effects of the osmotic control on endothelial cell growth. This suggests that the growth responses observed with this minor increase in osmolality occur independently of the p38 MAPK pathway but perhaps through other stress-activated pathways.

The current study demonstrated that the endothelial cell hypertrophy induced by high glucose occurred independently of the p38 and p42/44 MAPK pathways. Little is known regarding the role of the MAPK cascades in cell hypertrophy. Cardiac myocyte hypertrophy has been shown to be associated with the stress-activated JNK pathway and to be independent of the p38 MAPK cascade (5). We have previously shown that neutralization of TGF-β1 with blocking antibodies reverses both the antiproliferative and hypertrophic responses of high glucose. Therefore, autocrine TGF-β1 must also be acting via additional pathways, such as the JNK pathway, to effect the hypertrophic response.

The clinical relevance of high glucose-induced antiproliferative and hypertrophic effects on the endothelium is not clear. It is possible that the reduction in endothelial cell number is responsible for the acellular capillaries and microaneurysms observed in patients with diabetes mellitus and may limit reendothelialization after endothelial injury. However, the net effect on the vasculature requires additional concurrent studies on the vascular smooth muscle cells.

In conclusion, this study shows that the exposure of human endothelial cells to high glucose results in increased p42/44 and p38 MAPK activity, effects that are independent of the increase in osmolarity associated with high glucose. Significantly, the antiproliferative effect of high glucose is likely to be mediated by the autocrine action of TGF-β acting via p38 MAPK. The mechanisms underlying endothelial cell hypertrophy induced by high glucose require further delineation but occur independently of these two members of the MAPK family.

DISCLOSURES

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