P38 mitogen-activated protein kinase mediates hexosamine-induced TGF β 1 mRNA expression in human mesangial cells

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Abstract

Aims/hypothesis. The hexosamine pathway has been implicated in the induction of TGF β 1 expression and in the pathophysiology of diabetic glomerulopathy. Glucose-induced TGF β 1 expression is mediated by p38 mitogen-activated-protein-kinase (p38-MAPK) and this kinase is activated in the diabetic glomeruli. We examined whether the p38-MAPK is implicated in hexosamine-induced TGF β 1 mRNA expression in human mesangial cells.

Methods. The products of the hexosamine biosynthetic pathway were increased by the addition of glucosamine or by the overexpression of the rate-limiting enzyme of the hexosamine pathway, glutamine: fructose-6-phosphate amidotransferase (GFAT).

Results. Glucosamine addition resulted in cell death. UDP-N-Acetylglucosamine, one of the major hexosamine end-products, was increased in normal (7 mmol/l) and high (25 mmol/l) glucose conditions in GFAT-transfected cells compared to control transfected cells by twofold and 1.7-fold respectively ($p \leq 0.04$) and

this was accompanied by a 1.6- and 2.3-fold increase ($p \leq 0.02$) in TGF β 1 mRNA expression. Addition of the GFAT inhibitor azaserine (10 μ mol/l) prevented the induction of TGF β 1 in GFAT transfected cells. GFAT overexpression induced an increase in p38-MAPK activation after 6 and 12 h incubation in normal glucose, and this was prevented by the GFAT inhibitor azaserine. Furthermore, high glucose enhanced p38-MAPK activation in GFAT transfected cells ($p \leq 0.04$). P38-MAPK inhibition using SB202190 (1 μ mol/l) reduced hexosamine-induced TGF β 1 expression in normal and high glucose. The activation of the p38-MAPK was dependent on protein kinase-C.

Conclusion/interpretation. Overexpression of GFAT increases hexosamine accumulation which mediates TGF β 1 expression via a protein kinase-C and p38-MAPK dependent mechanism. Increased glucose concentrations magnify these effects. [Diabetologia (2003) 46:531–537]

Keywords Hexosamines, TGF β 1, p38-MAPK, protein kinase C, mesangial cells.

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Abbreviations: HBP, Hexosamine biosynthetic pathway; GFAT, glutamine: fructose-6 phosphate amido transferase; MAPK, mitogen activated protein kinase; ERK, extracellular signal related kinase; PKC, protein kinase C; HMC, human mesangial cells; UDP-NAG, uridine-5-diPhosphate-N-acetyl-Glucosamine; PMA, phorbol 12-myristate 13-acetate; PMSF, phenylmethylsulfonylfluoride.

Two to three percent of intracellular glucose is metabolized through the hexosamine biosynthetic pathway (HBP). This pathway converts fructose-6-phosphate to glucosamine-6-phosphate via the rate limiting enzyme glutamine: fructose-6-phosphate amidotransferase (GFAT) and is important in insulin resistance [1, 2], a condition closely associated with diabetic nephropathy [3]. The HBP has been implicated in other pathogenic processes such as the induction of growth factors in mesangial cells and of leptin in adipose tissue and skeletal muscle [4].

Of interest GFAT protein expression is increased in glomerular cells from patients with diabetic nephropa-

thy [5]. Furthermore, in porcine and rat mesangial cells the addition of glucosamine [6, 7], a precursor of the HBP, or over-expression of GFAT [8] increases TGF β 1 expression, a key factor in the pathogenesis of diabetic glomerulosclerosis [9, 10, 11, 12, 13]. However, there is currently no information on the role of the HBP in the induction of TGF β 1 expression in human renal cells or on the intracellular signalling pathways involved.

P38, a member of the mitogen-activated protein kinase (MAPK) family, is important in high glucose and stretch-induced TGF β 1 expression in human mesangial cells via a Protein Kinase C (PKC)-dependent pathway [14, 15] and is activated in the glomeruli from diabetic rats [16].

The aims of this study were to determine whether, in human mesangial cells, as in mesangial cells from other species, the HBP is involved in TGF β 1 expression and to examine the signalling pathway of this effect, in particular the role of MAPKs and PKC.

Materials and methods

Materials. General biochemicals, unless stated otherwise, were purchased from Sigma-Aldrich Company Ltd. (Dorset, UK). Fetal calf serum was purchased from Gibco BRL (Paisley, Scotland) and Streptomycin-Penicillin antibiotic solution from ICN (Hampshire, UK). The human embryonic kidney epithelial (293) cell line was from the European collection of cell cultures (Wiltshire, UK). The MBS calcium phosphate transfection kit was purchased from Stratagene (Amsterdam, The Netherlands). The QIA shredder and Rneasy kit were obtained from Qiagen (Crawley, UK). Oligonucleotide pd(T) 12–18, RNase inhibitor and deoxynucleotide mix were from Amersham Pharmacia Biotech (Buckinghamshire, UK) and PCR primers from Oswel DNA service (Southampton, UK). Amplitaq Taq polymerase was purchased from Perkin Elmer (Cheshire, UK). AMV reverse transcriptase and antibodies against activated p38-MAPK and ERK were obtained from Promega Corporation (Southampton, UK). The antibodies against total p38-MAPK and ERK were obtained from Insight Biotechnologies LTG (Middlesex, UK). The specific p38-MAPK inhibitor (SB202190) was from Calbiochem (Nottingham, UK).

Cell culture. Human mesangial cells (HMC) were obtained from glomeruli isolated from tumour-free portions of tumour nephrectomy specimens or from donor kidneys unsuitable for transplantation by serial sieving of cortex after collagenase digestion as described previously [17]. In brief, after digestion with collagenase (type IV, 750 U/ml), the isolated glomeruli were seeded in culture flasks. After the outgrowth of mesangial cells the glomeruli were removed by washing with PBS and the cells were cultured in RPMI-1640, supplemented with insulin-transferrin-selenium, L-glutamine and containing 20% fetal calf serum, 7 mmol/l glucose, 100 IU/ml penicillin and 100 μ g/ml streptomycin in a humidified 5% CO₂ incubator at 37°C. Mesangial cells were harvested using 0.25% trypsin and 0.5% EDTA. The cells were stellate or fusiform in appearance, grew in multilayer and formed hillocks in long-term culture and stained positive for α -smooth muscle actin by direct immunofluorescence. Cells did not stain for cytokeratin, factor

VIII, common leukocyte antigen or Thy-1, excluding contamination of epithelial cells, endothelial cells, lymphomonocytes and human fibroblasts respectively. Studies were done between passage 4 and 7 while the cells retain all the morphological and immunofluorescent characteristics as described above [17]. Ethics approval was obtained by the Guy's Hospital Research Ethics Committee (London, United Kingdom) and written consent obtained from the patient. Human embryonic kidney epithelial (293) cells used to generate the recombinant defective adenovirus expressing the GFAT cDNA were cultured in DMEM supplemented with 10% FCS, 20 mmol/l glucose and antibiotics in a humidified 5% CO₂ incubator at 37°C.

Generation of defective adenovirus expressing GFAT cDNA. The pACCMVpLpA-GFAT vector was prepared by cloning GFAT cDNA extracted from the plasmid PBS-GFAT (Gift of Dr McKnight, Seattle, Ore., USA) into the multiple cloning site of the vector pACCMVpLpA (Gift of Dr Newgard, Dallas, Texas, USA).

The recombinant adenovirus expressing GFAT cDNA was generated as described previously [18] by co-transfection of the plasmids pACCMVpLpA-GFAT and pJM17 in 293 cells cultured in DMEM with 7 mmol/l glucose using a calcium phosphate precipitation kit (MBS transfection kit, Stratagene, Heidelberg, Germany).

Experimental design. Sub-confluent HMC were transfected with β -galactosidase (control) or GFAT adenovirus vectors, at a concentration of 1×10^{12} plaque-forming units per 10^5 HMC for 4 h in each experiment. HMC transfection efficiency was 90 to 95% measured 24 h after viral infection with a control recombinant adenovirus expressing β -galactosidase (Gift of Dr Newgard, Dallas, Texas, USA). After transfection, complete RPMI media was added to the cells for 2 h before serum deprivation in 1% BSA overnight. In the experiments, cells were then cultured in either normal (7 mmol/l, made iso-osmolar by adding mannitol) or high glucose (25 mmol/l) serum-free media in the presence or absence of the specific inhibitors. More specifically after 24 h incubation in normal or high glucose concentrations: the effectiveness of the GFAT adenovirus was tested by measuring the intracellular concentration of uridine-5-diphosphate-N-acetylglucosamine (UDP-NAG), the major end product of the HBP; and the effect of GFAT overexpression in inducing TGF β 1 mRNA expression and p38-MAPK activation was measured in the presence or absence of azaserine (10 μ mol/l). The role of p38-MAPK on TGF β 1 mRNA expression was studied in cells overexpressing GFAT incubated for 24 h in normal or high glucose in the presence or absence of SB202190 (1 μ mol/l), a specific inhibitor of p38-MAPK activity. The role of PKC in hexosamine-induced p38-MAPK activation was investigated by measuring phosphorylated p38-MAPK after 6 h in normal and high glucose condition in β -galactosidase and GFAT transfected HMC after PKC down-regulation with PMA (0.5 μ mol/l) treatment for 24 h [19].

For the experiments where glucosamine was added, HMC were incubated with glucosamine at a final concentration of 2.5, 5, 7, and 12 mmol/l, in normal glucose media for 24 h.

Measurement of UDP-NAG cellular concentrations. UDP-NAG was measured by capillary zone electrophoresis (Hewlett Packard). Briefly, HMC were lysed with a solution of 60% acetonitrile, 0.5% Triton X-100 and the samples lyophilized and reconstituted in water. Cellular debris was removed by centrifugation and samples were pre-clared by centrifugation in a high molecular weight filter. Equal volumes of each sample were injected into a 50 μ mol/l 700 mm fused silica capillary column (Crawford Scientific) and electrophoresed in

20 mmol/l sodium borate buffer, pH 9.0 at 20 kV to separate UDP-NAG. UDP-NAG was detected at 262 nm and identity of the peak was confirmed by comparison to UDP-NAG standard peak migration time. UDP-NAG concentrations were calculated using an UDP-NAG standard curve (inter-assay and intra-assay CV was 14.8% and 3.4% respectively). All samples for each experiment were analysed in the same run. The concentrations of UDP-NAG were normalized to total cellular protein content measured by a Coomassie blue method.

TGF β 1 mRNA expression. TGF β 1 mRNA expression was assessed with competitive RT-PCR using the primers 5'-AGCGACTCGCCAGAGTGGTTATCTT-3' and 5'-TTATGCTGTTGTACAGGGCCAGGA-3' for TGF β 1 [20], and the primers 5'-CACCCACACTGTGCCCATCTA-3' and 5'-ACTCGTCATACTCCTGCTTGC-3' [20] for the housekeeping gene β -actin which respectively give a 473 bp and 617 bp product. Each amplified product was confirmed by restricting endonuclease digestion with BamHI for TGF β 1 and BanI for β -actin.

Primers for truncated (approximately 50 bp shorter than the native) competitive TGF β 1 and β -actin products were designed as described previously [21].

Total RNA was extracted using the Rneasy extraction kit and 2 μ g of RNA reverse transcribed using Avian Myeloblastosis Virus reverse transcriptase. The cDNA was amplified with a dilution series of either the TGF β 1 or β -actin competitive fragment for 21 or 17 cycles respectively (30 s at 95°C, 1 min at 65°C, and 1.5 min at 70°C). The native and competitor cDNAs had linear amplification kinetics within the chosen experimental conditions. Samples were resolved on a 2.5% high-resolution agarose gel with ethidium bromide and visualized using an UV spectrophotometer. The densities of each band were quantitated by scanning densitometry using a computer software program (QGEL, Stratagene) and TGF β 1 expression determinations were normalized to β -actin to control for the amount of cDNA loaded and efficiency of reverse transcription.

Cellular content of total and phosphorylated P38-MAPK and ERK. P38-MAPK and ERK phosphorylated and total cellular content were studied by immunoblotting. Total protein was extracted in a Tris HCl pH 7.6 lysis buffer containing 10 mmol/l EDTA, 1% Triton X, 1 mmol/l NaF, 1 mol/l NaVO₃, 2 mmol/l aprotinin, 2 mmol/l leupeptin, and 2 mmol/l PMSF. Equal amounts of protein were separated on a 10% polyacrylamide gel and transferred onto a nitrocellulose membrane. After blocking in 5% non-fat milk in TRIS buffered saline-tween 0.5% for 1 h at room temperature, the membrane was incubated with either anti-total p38-MAPK or ERK antiserum (1:5000 and 1:3000 dilution respectively) or anti-active p38-MAPK or ERK antiserum (1:2000 and 1:6000 dilution respectively) in 5% non-fat milk in TRIS-buffered saline-tween 0.5% overnight at 4°C. After extensive washing in TRIS-buffered saline-tween 0.5%, the membrane was incubated for 1 h with anti-rabbit IgG conjugated to horse radish peroxidase diluted 1:800–1:20 000 in 5% milk with TRIS-buffered saline-tween 0.5%. Bands were detected by enhanced chemiluminescence (ECL system, Amersham, London, UK) and quantification of the bands measured by scanning densitometry. Equal protein loading was confirmed by Ponceau-S staining. Activated p38-MAPK and ERK were expressed as a ratio of phosphorylated and total protein cellular content.

Statistical analysis. Differences among groups within each glucose concentration or comparisons between high and normal glucose conditions were tested by repeated ANOVA measurements with post hoc pairwise comparisons using LSD and

Dunnett's test (SPSS system software). Statistical significance was accepted at a p value of less than 0.05.

Results

Glucosamine reduces HMC viability. In HMC, the addition of varying glucosamine concentrations (2.5, 5, 7, and 12 mmol/l) in normal glucose media for 24 h resulted in a consistent 80% reduction in cell viability as measured by a trypan blue dye exclusion test, suggesting that glucosamine is cytotoxic for human mesangial cells. Therefore, GFAT overexpression was used in all subsequent experiments to increase intracellular hexosamine concentrations.

High Glucose and GFAT overexpression increase intracellular UDP-NAG concentration. Both high glucose and GFAT transfection increased UDP-NAG concentrations by approximately 100% when compared to control transfected cells in normal glucose ($p \leq 0.04$). High glucose potentiated the effect of GFAT transfection on UDP-NAG concentrations ($p = 0.005$) (Fig. 1).

GFAT overexpression increases TGF β 1 mRNA in both normal and high glucose. High glucose increased TGF β 1 mRNA expression in β -galactosidase transfected cells by 100% ($p = 0.001$). TGF β 1 mRNA expression was increased by 60% in GFAT-transfected cells in normal glucose ($p = 0.02$). In GFAT-overexpressing cells, high glucose enhanced TGF β 1 mRNA expression by approximately 200% ($p = 0.0001$) (Fig. 2B).

To ensure that transfection with the β -galactosidase adenovirus was not affecting the TGF β 1 response of HMC to high glucose, we studied non-transfected cells as control. As for β -galactosidase transfected cells, the addition of high glucose to non-transfected cells increased TGF β 1 mRNA expression by 100% ($p = 0.0001$) (Fig. 2A).

To confirm that TGF β 1 mRNA expression was mediated by an increase in intracellular hexosamine concentration, azaserine (10 μ mol/l), a GFAT inhibitor, was added to the cells in the culture media for 24 h.

Fig. 1. UDP-NAG content in β -galactosidase ($\square$) and GFAT ($\blacksquare$) transfected HMC in normal (NG) (7 mmol/l) and high (HG) (25 mmol/l) glucose. The results represent means $\pm$ SEM of three experiments per group. * $p \leq 0.04$ GFAT vs β -galactosidase in NG and HG; # $p \leq 0.03$ HG vs NG in β -galactosidase and GFAT-transfected cells

Fig. 2A, B. TGF β 1 mRNA expression in non-transfected (A), β -galactosidase (□) and GFAT- (■) transfected HMC cells (B) in normal (NG)(7 mmol/l) and high (HG)(25 mmol/l) glucose. The results represent means $\pm$ SEM of five–eight experiments per group. ^{*} $p=0.02$ GFAT vs β -galactosidase in NG. [#] $p\leq 0.001$ HG vs NG in non-transfected cells, GFAT and β -galactosidase-transfected cells

Fig. 3. TGF β 1 mRNA expression in β -galactosidase (□) and GFAT (■)-transfected HMC in normal (7 mmol/l) (upper panel) and high (25 mmol/l)(lower panel) glucose in the presence or absence of azaserine (10 μ mol/l). The results represent means $\pm$ SEM of five–eight experiments per group. [^] $p\leq 0.04$ GFAT-transfected cells in azaserine vs non-azaserine treated cells

High glucose-induced TGF β 1 was attenuated by azaserine (Az) but this did not reach statistical significance. The addition of azaserine to GFAT-transfected HMC-reduced TGF β 1 mRNA expression in normal ($p=0.04$) and in high glucose ($p=0.0001$) respectively by 30% and 70% (Fig. 3).

GFAT overexpression induces p38-MAPK, but not ERK activation. P38-MAPK phosphorylation was analysed in β -galactosidase and GFAT transfected cells after 6, 12, and 24 h incubation in normal or high glucose.

Fig. 4. p38-MAPK active expression in β -galactosidase (□) and GFAT (■)-transfected HMC in normal (7 mmol/l) and high glucose (25 mmol/l) in the presence or absence of azaserine (10 μ mol/l)(Az). Results represent fold change over β -galactosidase without Az in normal glucose condition. The results represent means $\pm$ SEM of four–eight experiments per group. [#] $p\leq 0.04$ HG vs NG in β -galactosidase and GFAT-transfected cells at 6 h. ^{*} $p\leq 0.005$ GFAT vs β -galactosidase-transfected cells in NG. [^] $p\leq 0.007$ GFAT-transfected cells, with azaserine vs without azaserine. A representative Western blot is shown for phosphorylated and total p38-MAPK after 6 h incubation in normal or high glucose in β -galactosidase (B) and GFAT (G)-transfected HMC in the absence and presence of azaserine (A)

P38-MAPK activated expression increased at 6 h by 100% ($p=0.04$) after high glucose was added to β -galactosidase transfected HMC, and returned to baseline by 12 h. In normal glucose, GFAT overexpression induced a 220% increase in activated p38-MAPK by 6 h ($p=0.0001$) and a 80% increase by 12 h ($p=0.005$) with a return to baseline by 24 h, when compared to β -galactosidase control cells. In GFAT-transfected cells high glucose further enhanced the content of phosphorylated p38-MAPK at 6 h by approximately 20% ($p=0.04$) with no difference at 12 h (Fig. 4). To examine whether the changes were due to intracellular hexosamine accumulation similar experiments were done in the presence of azaserine (10 μ mol/l).

Glucose-induced p38-MAPK activation was attenuated by azaserine but this was not statistically significant. Azaserine reduced the GFAT p38-MAPK activation in normal glucose after 6 h by 70% ($p=0.007$); in high glucose both after 6 ($p=0.0001$) and 12 h ($p=0.001$) azaserine reduced the GFAT-me-

Fig. 5. TGF β 1 mRNA expression in β -galactosidase (□) and GFAT (■)-transfected HMC in normal (upper panel) (7 mmol/l) and high (lower panel) (25 mmol/l) glucose conditions with or without SB202190 (SB) (1 μ mol/l). Results represent fold change over β -galactosidase without SB. The results represent means $\pm$ SEM of five–six experiments per group. $\sim p \leq 0.017$ SB+ vs SB– in β -galactosidase-transfected cells; $^{\wedge} p \leq 0.01$ SB+ vs SB– in GFAT-transfected cells

Fig. 6. p38-MAPK active expression in β -galactosidase (□) and GFAT (■)-transfected HMC in normal (7 mmol/l) and high glucose (25 mmol/l) in the presence or absence of PMA (0.5 μ mol/l) for 24 h. Results represent fold change over β -galactosidase without PMA treatment in normal glucose condition. The results represent means $\pm$ SEM of four–six experiments per group. $\# p \leq 0.007$ HG vs NG in β -galactosidase and GFAT-transfected cells. $* p \leq 0.002$ GFAT- vs β -galactosidase-transfected cells in NG. $p \leq 0.03$ PMA+ vs PMA– in β -galactosidase-transfected cells; $^{\wedge} p \leq 0.003$ PMA+ vs PMA– in GFAT-transfected cells

diated p38-MAPK activation respectively by 75% and 50% (Fig. 4).

There were no differences in ERK active content between GFAT and β -galactosidase transfected cells in either normal or high glucose conditions at any of the time points studied.

GFAT overexpression induces TGF β 1 mRNA expression via a p38-MAPK-dependent mechanism. In β -galactosidase transfected cells, high glucose-induced TGF β 1 expression was reduced by 33% after 24 h incubation with the p38-MAPK inhibitor SB202190 (1 μ mol/l) ($p = 0.017$).

Addition of the p38-MAPK inhibitor reduced the increase in TGF β 1 mRNA expression in GFAT-transfected cells in normal ($p = 0.01$) and high glucose ($p = 0.0001$) respectively by 34% and 43% (Fig. 5).

PKC down-regulation prevents GFAT overexpression mediated p38-MAPK activation. Down-regulation of PKC by PMA-reduced high glucose-induced p38-MAPK activation in β -galactosidase-transfected cells by 60% ($p = 0.03$) and reduced p38-MAPK activation in GFAT-transfected cells in both normal ($p = 0.003$) and high glucose ($p = 0.0001$) by approximately 70% and 80% respectively (Fig. 6).

Discussion

This study shows that in human mesangial cells, a glomerular cell type central to the process of diabetic glomerulosclerosis, intracellular hexosamine accumulation, obtained by overexpression of GFAT, induces TGF β 1 mRNA expression. This observation has also been made in other non-human cell types [22].

In previous studies glucosamine, a precursor of the HBP, was used to increase intracellular hexosamine concentrations to examine the effect on TGF β 1, extracellular matrix proteins expression, and glucose uptake [6, 19, 23]. However in human mesangial cells, adding glucosamine resulted in a loss of cell viability at concentrations as low as 2.5 mmol/l. This toxic effect has been reported in other cell types and the discrepancies observed in the literature are most likely due to cell species-specific response to glucosamine [19, 23].

The metabolic rate of the hexosamine pathway is intimately related to the extracellular glucose concentrations [1].

Of interest, the magnitude of the effect on TGF β 1 mRNA expression observed in GFAT-overexpressing cells cultured in normal glucose was similar to that obtained in the control cells cultured in high glucose. The addition of high glucose to GFAT-overexpressing cells magnified the induction of the TGF β 1 mRNA expression by almost threefold.

Our findings suggest that the intrinsic (constitutive) activity of GFAT could be important in determining the degree of TGF β 1 production and thus the resulting sclerosis when a high glucose condition occurs. The observation that GFAT expression is increased in the mesangium of biopsies from patient with diabetic nephropathy [5] provides further support to the notion that individual rates of GFAT activity could participate in the susceptibility to the development and/or progression of nephropathy in people with diabetes. It is possible that increased rates of intracellular hexosamine production and/or accumulation represent one of the mechanisms of glucose toxicity for the diabetic glomerulus [24, 25]. We found that activation of the

p38-MAPK pathway is one of the intracellular mechanisms which mediate the HBP-induced TGF β 1 mRNA expression.

Consistent with the data on TGF β 1 mRNA expression, the effect on p38-MAPK activation in GFAT-overexpressing cells both in normal and high glucose conditions was of a greater magnitude than that induced by high glucose in control transfected cells emphasizing once more the importance of the intrinsic activity of this enzyme in the sequence of cellular events translating the effect of glucose into extracellular matrix production.

P38-MAPK activation was increased in the glomeruli of diabetic rats [16, 26] and activated by both glucose [27] and mechanical stretch in human mesangial cells [15]. The time course of the activation of p38-MAPK in response to glucose is controversial, with some authors reporting early activation [14, 26], while others only detecting late (after several days) activation [27]. These discrepancies can be explained most probably by differences in the time points analysed in the various studies. Both glucose [14] and mechanical stretch [15] mediate early activation of p38-MAPK which induces active TGF β 1 secretion [28, 15]. The active TGF β 1 in turn maintains late activation of p38-MAPK [15, 27, 29], thus explaining the late effect of glucose and mechanical stretch on p38-MAPK as possibly the result of an autocrine TGF β 1-mediated phenomenon.

We did not find any effect of GFAT overexpression or of glucose per se on ERK phosphorylation excluding this molecular pathway in the hexosamine-mediated induction of TGF β 1. Previous studies only showed modest glucose-mediated ERK activation in rat mesangial cells [30].

The hexosamine-induced p38-MAPK activation was a PKC-dependent phenomenon. When intracellular PKC was down-regulated by PMA, p38-MAPK activation was impaired. These results complement previous observations suggesting a PKC-dependent mechanism in the regulation of hexosamine-induced TGF β 1 mRNA expression [24]. The cellular content of the transcription factor Sp1, involved in the regulation of TGF β 1 transcription [31], are up-regulated by PKC activation [32]. Furthermore hexosamines promote Sp1 O-linked N-acetylglucosamine glycosylation [33], which stabilizes this transcription factor, increasing its cellular concentrations [34]. Several other studies have proposed a role of the HBP in PKC activation in different cell types [19, 35, 36, 37], and PKC has been shown to mediate glucose-induced p38-MAPK activation [38], confirming our results in control transfected cells.

In conclusion, this report describes that hexosamine mediates TGF β 1 mRNA expression in human mesangial cells via a PKC- and p38-MAPK-dependent mechanism. High glucose enhances the effect of GFAT overexpression on UDP-NAG cellular concen-

trations, p38-MAPK activation, and TGF β 1 expression. Hexosamine accumulation could be one of the intracellular mechanisms for glucose toxicity and activation of the HBP is likely to play an important role in the pathophysiology of renal and possibly other vascular complications of diabetes [24, 25].

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