Kidney International, Vol. 58, Suppl. 77 (2000), pp. S-13–S-18

Role of the hexosamine biosynthetic pathway in diabetic nephropathy

ERWIN D. SCHLEICHER and CORA WEIGERT

Department for Internal Medicine, Division of Endocrinology, Metabolism and Pathobiochemistry, University of Tübingen, Germany

The role of the hexosamine biosynthetic pathway in diabetic nephropathy. The hexosamine biosynthetic pathway has been hypothesized to be involved in the development of insulin resistance and diabetic vascular complications. In particular, it was demonstrated that hyperglycemia-induced production of transforming growth factor-β (TGF-β1), a prosclerotic cytokine causally involved in the development of diabetic nephropathy. Several lines of evidence indicate that TGF-β1 induction is mediated by the hexosamine pathway. In cultured mesangial cells, high glucose levels induce TGF-β1 production. This effect is eliminated by inhibition of glutamine : fructose-6-phosphate-amidotransferase (GFAT), the rate-limiting enzyme of this pathway. Furthermore, stable overexpression of GFAT increased levels of TGF-β1 protein, mRNA, and promoter activity. Inasmuch as stimulation or inhibition of GFAT increased or decreased high glucose-stimulated activity of protein kinase C (PKC), respectively, the observed effects appear to be transduced by PKC. In similar experiments, involvement of the hexosamine pathway in hyperglycemia-induced production of cytokines (TGF-α and basic fibroblast growth factor [bFGF]) was demonstrated in vascular smooth muscle cells. These studies also revealed a rapid increase in GFAT activity by treatment with agents that elevated levels of cyclic adenosine 3′,5′ monophosphate (cAMP), thus indicating that GFAT activity is tightly regulated by cAMP-dependent phosphorylation. Using immunohistochemistry and in situ hybridization, high expression of GFAT was found in human adipocytes, skeletal muscle, vascular smooth muscle cells, and renal tubular epithelial cells, whereas glomerular cells remained essentially unstained. However, significant staining occurred in glomerular cells of patients with diabetic nephropathy. Current data indicate that the flux through the hexosamine pathway, regulated by GFAT, may be causally involved in the development of diabetic vascular disease, particularly diabetic nephropathy.

During development of diabetic nephropathy, the renal arterioles, tubules, interstitium, and particularly the glomeruli undergo structural changes that include hyalinosis of arteriolar wall, thickening of the glomerular and tubular basement membranes, and extension of the mesangial matrix [1, 2]. Quantitative morphometric studies have shown that the changes most closely related to the decline in renal function in type 1 and type 2 diabetes derived from an increase in mesangial matrix [2–5]. Immunohistochemical and biochemical studies indicated an increased deposition of various collagen types, laminin, and fibronectin, particularly in glomeruli of patients with diabetes and in experimental animal models [6–11]. Numerous studies have shown that elevated glucose concentrations can cause an increased expression of these components of the extracellular matrix in cultured mesangial cells, which suggests that these cells are an useful model in studying the pathogenesis of diabetic nephropathy [10, 12, 13]. These in vitro studies provided evidence that the high glucose-induced stimulation of mesangial and tubular matrix production is mediated by transforming growth factor-β1 (TGF-β1) [14–17]. Early glomerular and tubular increase of TGF-β1 expression has been shown in experimental and human diabetic nephropathy [18–20]. A causal involvement of TGF-β was also demonstrated given that application of TGF-β-antibodies attenuated the effects in cultured mesangial cells [15] and in experimental animals [21]. These studies indicate that elevated glucose levels induce cytokine production, particularly that of TGF-β1, which in turn induces renal extracellular matrix synthesis and finally leads to renal scarring. Different pathobiochemical pathways are thought to be involved in these damaging events. These include: the sorbitol pathway, oxidative stress, the formation and action of AGE-products and activation of protein kinase C (PKC) [22] and other kinases. Investigation of the specificity of the high glucose effect revealed that D-glucosamine but not other related hexoses efficiently mimicked the effect in stimulating TGF-β1 production in mesangial cells [23] and production of TGF-α and basic fibroblast growth factor (bFGF) in vascular smooth muscle cells [24]. In combination, these studies have indicated that the hexosamine biosynthetic pathway may be involved in mediating the effects of high levels of glucose.

HEXOSAMINE BIOSYNTHETIC PATHWAY

Amino sugars are physiologically synthesized through the hexosamine biosynthetic pathway (Fig. 1). In this path-

Key words: transforming growth factor, hyperglycemia, glutamine : fructose-6-phosphate-amidotransferase, gene expression.

© 2000 by the International Society of Nephrology

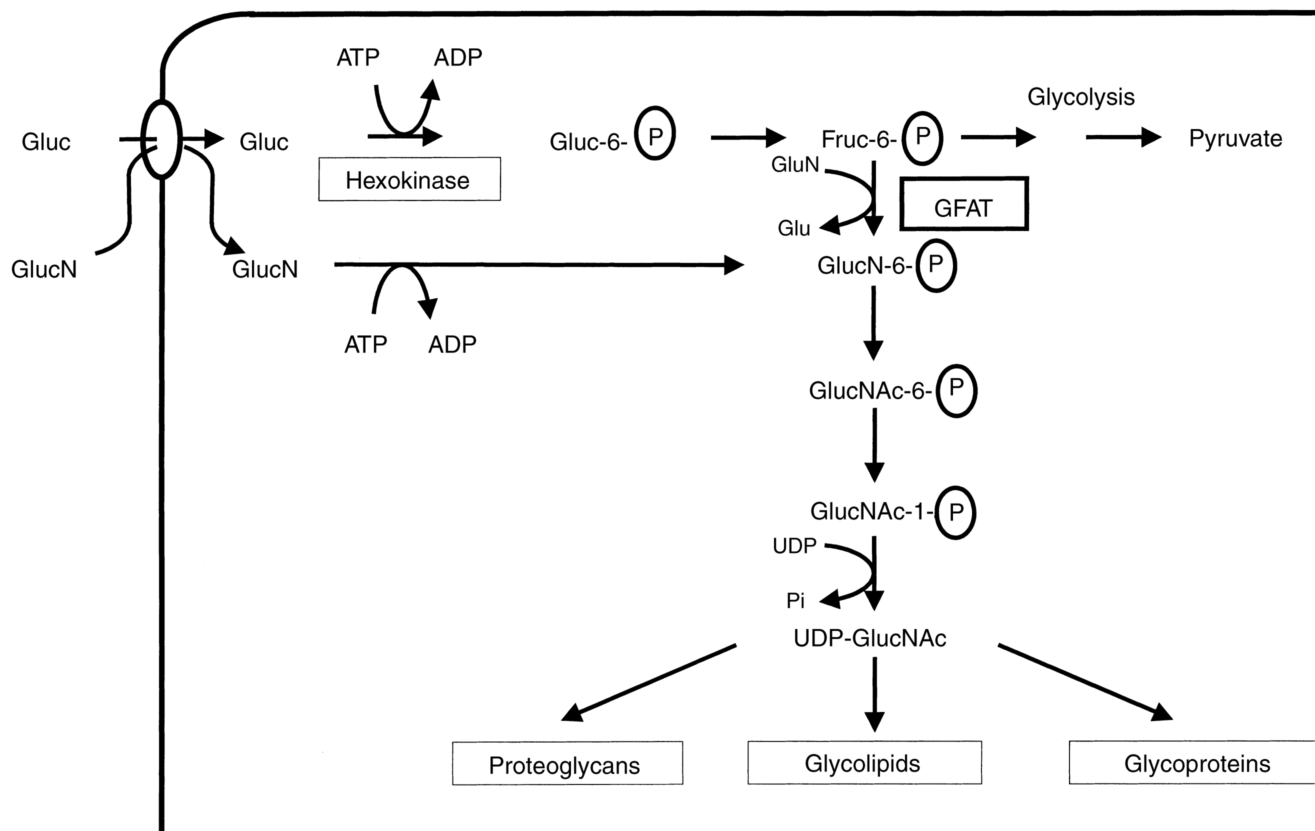

Fig. 1. The hexosamine biosynthetic pathway. Glucose (Gluc) enters the cell through the glucose transporter and is metabolized. The hexosamine biosynthetic pathway merges from glycolysis using fructose-6-phosphate (Fruc-6-P) to form glucosamine-6-P (GlucN-6-P). Glutamine (GluN) serves as donor of the aminogroup. The reaction is catalyzed by the rate-limiting enzyme glutamine : fructose-6-phosphate-amidotransferase (GFAT). GlucN-6-P is rapidly acetylated, isomerized to N-Acetylglucosamine-6-phosphate (GlucNAc-1-P) and activated to UDP-N-acetylglucosamine (UDP-GlucNAc) that serves as common precursor for all amino sugars used for the synthesis of glycoproteins, -lipids, and proteoglycans. Glucosamine (GlucN) can also enter the cell through the glucose transporter and is rapidly phosphorylated by hexokinase yielding GlucN-6-P, thereby bypassing the rate-limiting first step of the hexosamine biosynthetic pathway.

way, fructose-6-phosphate is converted to glucosamine-6-phosphate (GlucN-6-P) by the rate-limiting enzyme glutamine : fructose-6-phosphate-amidotransferase (GFAT), which uses glutamine as an amino donor [25, 26]. GlucN-6-P is rapidly converted further and activated to uridine-5-diphosphate-N-acetylglucosamine (UDP-GlucNAc). UDP-GlucNAc is the precursor for all other amino sugars that are necessary for the biosynthesis of glycoproteins, glycolipids, proteoglycans, and glycosaminoglycans. Because glucosamine levels of extracellular fluids are below the limit of detection (i.e., <0.02 mmol/L), cellular uptake of glucosamine may be neglected under physiologic conditions. However, if glucosamine is added to cells, it is avidly taken up by the glucose transporter and phosphorylated by hexokinase yielding GlucN-6-P, thereby bypassing the rate-limiting enzyme GFAT. Thus, use of glucosamine represents a method to mimic the high glucose effects that are mediated by the hexosamine pathway [23–28]. However, for unequivocal evidence that the effects of high glucose levels are mediated by the hexosamine pathway, inhibition of this pathway by either inhibition of GFAT synthesis using, for example, antisense technology or by application of glutamine analogs, such as azaserine or diazo-oxo-norleucin, may be applied [23, 26, 27]. Most glucose taken up by the cells is metabolized through glycolysis; only 2% to 5% enters the hexosamine pathway. It appears that the amount of glucose entering this pathway is regulated by GFAT activity. Therefore, it is not unexpected that GFAT is highly regulated. The major points of regulation are (*1*) the concentration of fructose-6-phosphate is important because the affinity for the GFAT substrate is low (i.e., in the 3 to 10 mmol/L range); (*2*) GFAT activity is feedback-inhibited by its downstream product UDP-GlucNAc through an allosteric mechanism [29]; (*3*) GFAT activity can be influenced by the quantity of GFAT protein in the cell [30]; and (*4*) GFAT activity is increased by 3′,5′ monophosphate (cAMP)-dependent phosphorylation [31]. Given that the latter regulation of GFAT activity is rapid and occurs within 10 minutes, it may be important for fast regulation

Table 1. Properties of glutamine : fructose-6-phosphate-amidotransferase (GFAT)

Oligomers in eukaryotes
Monomer 77 kD molecular weight
Feedback inhibition by UDP-GlucNAc
High Km for both substrates
High homology (yeast, mouse, man)
Regulated by cAMP-dependent phosphorylation
Two isoforms (GFAT 1 and 2)
Chromosomal location GFAT1: 2p13i; GFAT2: 5q

of the flux through the hexosamine pathway. The flux through the hexosamine pathway may also be regulated by GFAT gene expression. Little is known about the half-life of GFAT protein in vivo, but studies in isolated adipocytes suggest a half-life of less than 1 hour [32]. To elucidate regulation of gene expression, the promoter of mouse GFAT has been cloned and partially characterized [33]. Detailed analysis of the GC-boxes indicated that Sp1 binds to three elements and that this transcription factor plays an important role in the regulation of GFAT gene expression. Earlier studies have shown that GFAT gene transcription can be regulated by epidermal growth factor, glucose and glucosamine in human cells [34] and may be increased by α-pheromone in yeast [35]. The GFAT gene has been localized on chromosome 2p13 [36]. Recently, the existence of a second GFAT (GFAT2) was deduced from human cDNA showing 75.6% homology to GFAT1 [37]. The properties of GFAT are summarized in Table 1.

Involvement of the hexosamine pathway in high-glucose-level induced TGF-β1 production

Investigation of the specificity of the effect of high glucose on mesangial cells showed that the effect is specific for D-glucose, whereas L-glucose, sorbitol, and 3-O-methyl-D-glucose produced no effect [23]. However, 12 mmol/L D-glucosamine was much more potent than 30 mmol/L D-glucose itself in enhancing the production of TGF-β1 protein and subsequent mesangial production of matrix components. D-glucosamine also promoted conversion of latent TGF-β to the active form. These data indicated that the hexosamine biosynthetic pathway is involved in high glucose-level-induced mesangial TGF-β1 production. To prove the involvement of this pathway, GFAT activity was blocked by the substrate analog azaserine and by inhibition of GFAT protein synthesis with antisense-oligonucleotide. Both interventions prevented the high glucose-level-induced increase in cellular glucosamine metabolites, TGF-β1 mRNA, protein and bioactivity and the subsequent effects on enhanced matrix production. As expected, inhibition of GFAT activity did not abolish the glucosamine-induced effects because glucosamine bypasses the GFAT-catalyzed step. Similarly, glucosamine has been shown to be more potent than glucose in stimulating TGF-β1 transcription in cultured renal proximal tubular cells (abstract; Daniels MC, *J Am Soc Nephrol* 6:1040, 1995). These data indicate that the flux of glucose metabolism through the GFAT-catalyzed hexosamine biosynthetic pathway mediates the hyperglycemia-induced renal production of TGF-β leading to renal matrix expansion.

To elucidate further the involvement of the hexosamine pathway in TGF-β1 induction, human GFAT was stably overexpressed in NIH-3T3 fibroblasts, yielding a 2.5-fold increase in GFAT protein as determined by Western blot (Weigert and Schleicher, unpublished results). To verify that the hexosamine pathway was activated by GFAT overexpression, cellular concentrations of UDP-GlucNAc were determined by capillary electrophoresis as previously described [38]. A twofold increase in UDP-GlucNAc concentration indicated increased flux through the hexosamine pathway after 24 hours (Fig. 2A), whereas no difference could be found after 48 hours. Determination of TGF-β1 expression in these cells revealed that TGF-β1 mRNA increased approximately twofold after 24 hours (Fig. 2B). Although production of mesangial TGF-β1 protein was low in the first 24 hours, GFAT overexpressing cells showed a twofold production after 24 and 48 hours (Fig. 2C). The effect of GFAT overexpression on TGF-β1 gene expression was also studied by using the human TGF-β1 promoter [39] ligated to the reporter gene luciferase. With this sensitive assay, we were able to demonstrate that GFAT overexpressing cells exhibit approximately a 50% increase in TGF-β1 promoter activity (Weigert and Schleicher, unpublished results). These results further support the involvement of the hexosamine pathway in the gene regulation of TGF-β1.

Given that PKC has been implicated in the development of late complications of diabetes [12, 13, 40], the effect of the hexosamine pathway on mesangial PKC has been studied [41]. Exposure to 12 mmol/L glucosamine resulted in rapid and specific translocation of PKC isoenzymes PKC α, β, and ε whereas PKC ξ was unaffected. Glucosamine similarly induced the translocation of these PKC isoforms as rapidly and effectively as the widely used PKC stimulator phorbol myristate acetate. Treatment of mesangial cells with high glucose caused a translocation of PKC β whereas other isoforms were less affected. The presence of azaserine, an inhibitor of GFAT activity, ameliorated the effect of high glucose levels on PKC β translocation. These data also indicate that the high glucose level-induced activation that has been described by various researchers [12, 13, 40] may be mediated by the hexosamine pathway. In a report by Filippis et al [42], the effect of high glucose and glucosamine on PKC activity was studied in adipocytes. Both high glucose and glucosamine caused a threefold increase in PKC activity; the effect of high glucose, but not that of glucos-

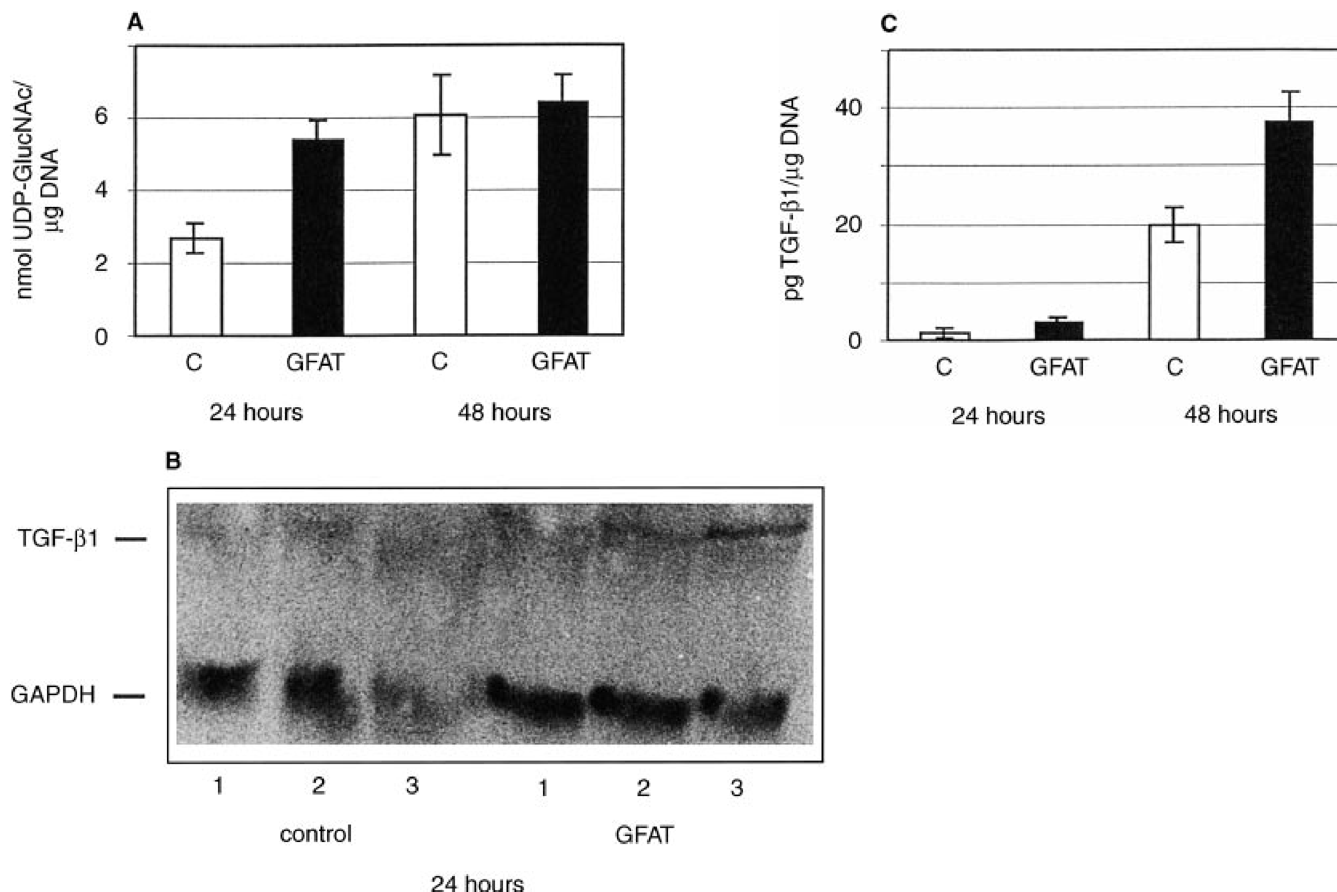

Fig. 2. Overexpression of GFAT in NIH-3T3 fibroblasts increases cellular UDP-GlucNAc concentration (*A*), TGF-β1 mRNA (*B*), and TGF-β1 protein levels (*C*). Fibroblasts were stably transfected with the expression vector pcDNA3 containing the human GFAT cDNA under control of the cytomegalovirus promoter or pcDNA3 alone as control. (*A*) Stably transfected fibroblasts were cultured for 24 or 48 hours in Dulbecco's modified Eagle's medium 2% Ultroser (Gibco, Eggenstein, Germany). Preparation of cell extracts and determination of UDP-GlucNAc with capillary electrophoresis were previously described [38]. DNA content of the cells was determined by fluorometry with bisbenzimidazol and used for normalization of cell number [23]. (*B*) Stably transfected fibroblasts were cultured for 24 hours as described in (*A*) and total RNA was prepared. Northern blotting was carried out with 25 μg RNA. For hybridization, digoxigenin-labeled RNA probes for TGF-β1 or glyceraldehyde 3-phosphate dehydrogenase were used [23]. Three different control and GFAT clones are shown. (*C*) Supernatants of stably transfected fibroblasts cultured in Dulbecco's modified Eagle's medium without fetal calf serum or Ultroser were collected after 24 and 48 hours, and TGF-β1 was determined by a commercially available enzyme-linked immunosorbent assay (R & D Systems, Wiesbaden, Germany).

Table 2. Expression of GFAT in human and rat tissues

Tissue	Protein (human)[a]	mRNA (human)[a]	mRNA (rat)[b]	Activity (rat)[c]
Kidney		ND	+	+
tubules	+ +			ND
glomeruli	−			ND
Muscle	+ + +	ND	+ +	+
Smooth muscle cells	+/++ +	+/++ +	+ + +	ND
Liver	+	ND	+	+ + +
Fat	+ + +	+ + +	+ + +	ND
Testis	ND	ND	+ + +	+ +
Colon	ND	ND	ND	+ + +

−, below detection limit; + to + + +, mild to strong expression; ND, not determined.
[a] GFAT protein was determined by immunohistochemistry or in situ hybridization in normal human subjects [44]
[b] Expression of rat GFAT mRNA was evaluated by Northern blotting [36]
[c] GFAT activity by measuring the enzyme activity in tissue extracts [45]. GFAT2 mRNA was preferentially expressed in nervous tissue (not shown) [37]

amine, was partially decreased by azaserine. In combination, these data indicate that an acute increase in the flux through the hexosamine biosynthesis pathway may activate PKC. However, experiments performed by Daniels et al [43] do not support a role of PKC in hexosamine pathway-mediated cytokine expression. They suggest a role for this pathway in leading to increased protein glycosylation, particularly the transcription factor Sp1, thereby effecting gene transcription [30, 43]. An alternative route of glucose-induced PKC activation, for instance, through diacylglycerol has been proposed by the groups led by King [22] and Ayo [40].

Localization of GFAT expression in human tissues

Investigation of the cellular expression of GFAT is important because it represents the rate-limiting enzyme of the hexosamine pathway. Expression of GFAT in

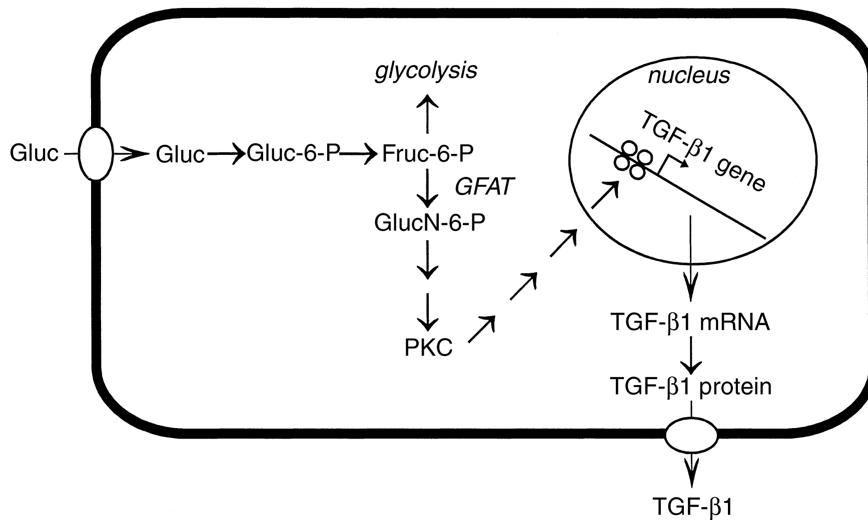

Fig. 3. Possible pathobiochemical link between hyperglycemia and mesangial TGF-β1 production. Elevated flux through the hexosamine pathway results in activation of PKC. Activated and translocated PKC in turn activates transcription factors (○) to form a complex that induces transcription of TGF-β1. Finally, TGF-β1 protein is secreted and acts autocrine/paracrine as prosclerotic cytokine.

various tissues has been studied in normal human subjects [44]. For this immunohistochemical study, a peptide-antibody raised against the C-terminus of GFAT was used. Given that GFAT1 and GFAT2 are identical at this location, the antibody recognizes both isoenzymes. This study revealed that GFAT protein expression is very different in different tissues. Strong staining was seen in all epithelia including renal tubuli or epithelial cells whereas endothelial and glomerular cells remained essentially unstained. Noteworthy, GFAT was significantly expressed in vascular smooth muscle cells. Furthermore, GFAT was strongly expressed in adipocytes and skeletal muscle cells, which supports the hypothesis that the hexosamine biosynthetic pathway may be involved in the pathogenesis of insulin resistance. The results obtained for GFAT protein expression could be confirmed on the mRNA level using in situ hybridization. Northern blot analysis essentially confirmed the findings; the use of specific probes for GFAT2 revealed that this subtype was preferentially expressed throughout the nervous system [37]. The data are summarized in Table 2. The unexpected finding that GFAT is not significantly expressed in normal glomeruli suggested that it may be regulated by diabetes. Analysis of GFAT expression in renal sections obtained from patients with diabetes showed significant expression in glomerular cells indicating that the enzyme is induced in diabetes. In line with this finding may be the report on increased GFAT activity in human skeletal muscle of patients with type 2 diabetes [46]. However, detailed analysis of GFAT regulation in diabetes or related diseases has not yet been performed.

CONCLUSIONS

Recent results provided evidence that the hexosamine pathway, previously solely regarded as biosynthetic pathway for amino sugars, exhibits regulatory functions. These include induction of gene expression (e.g., growth factors in smooth muscle cells or renal mesangial cells and of leptin gene expression in muscle and fat [47]) and the induction of insulin resistance [27]. Therefore, this pathway may be regarded as a glucose-sensing pathway [47]. Two possible molecular mechanisms have been elucidated to explain how increased flux through this pathway may transduce its effects by activating PKC [41, 42] alone or also by increasing glycosylation of transcription factors thereby increasing their activity [30]. The possible molecular mechanism of the hyperglycemia-induced production of the prosclerotic cytokine TGF-β1 through the hexosamine biosynthetic pathway is shown in Fig. 3. Although recent studies have increased our knowledge on the regulatory function of this pathway, the relevance for the patient with diabetes remains to be established.

ACKNOWLEDGMENTS

We thank Ms. K. Brodbeck for excellent technical assistance and Dr. U. Friess for critical discussion. The support by a grant of the Deutsche Forschungsgemeinschaft (Schl 239/6–2) is gratefully acknowledged.

Reprint requests to Erwin D. Schleicher, Medical Clinic and Policlinic IV, Otfried-Müller-Straße 10, D-72076 Tübingen, Germany.
E-mail: enschlei@med.uni-tuebingen.de

REFERENCES

1. Fioretto P, Steffes MW, Sutherland DER, Mauer M: Sequential renal biopsies in IDDM patients: Structural factors associated with clinical progression. *Kidney Int* 48:1929–1935, 1995
2. Mauer SM, Steffes MW, Ellis EN, Sutherland DER, Braun DM, Goetz FC: Structural functional relationships in diabetic nephropathy. *J Clin Invest* 74:1143–1153, 1984
3. Steffes MW, Bilous RW, Sutherland DE, Mauer SM: Cell and matrix components of the glomerular mesangium in type I diabetes. *Diabetes* 41:679–684, 1992
4. Osterby R, Gall MA, Schmitz A, Nielsen FS, Nyberg G, Parving HH: Glomerular structure and function in proteinuric type 2 (non-insulin-dependent) diabetic patients. *Diabetologia* 36:1064–1070, 1993
5. Osterby R: Glomerular structural changes in type 1 (insulin-depen-

dent) diabetes mellitus: causes, consequences, and prevention. *Diabetologia* 35:803–812, 1992
6. Nerlich A, Schleicher E: Immunohistochemical localization of extracellular matrix components in human diabetic glomerular lesions. *Am J Pathol* 139:889–899, 1991
7. Kim Y, Butkowski R, Burke B, Kleppel MM, Crosson J, Katz A, Michael AF: Differential expression of basement membrane collagen in membranous nephropathy. *Am J Pathol* 139:1381–1388, 1991
8. Mohan PS, Carter WG, Spiro RG: Occurrence of type VI collagen in extracellular matrix of renal glomeruli and its increase in diabetes. *Diabetes* 39:31–37, 1990
9. Ceol M, Nerlich A, Baggio B, Anglani F, Sauer U, Schleicher ED, Gambaro G: Increased glomerular α1 (IV) expression and deposition in long-term diabetic rats is prevented by chronic glycosaminoglycan treatment. *Lab Invest* 74:484–495, 1996
10. Ziyadeh FN: The extracellular matrix in diabetic nephropathy. *Am J Kidney Dis* 22:736–744, 1993
11. Fukui M, Nakamura T, Ebihara I, Shirato I, Tomino Y, Koide H: ECM gene expression and its modulation by insulin in diabetic rats. *Diabetes* 41:1520–1527, 1992
12. Studer RK, Craven PA, Derubertis FR: Role for protein kinase C in the mediation of increased fibronectin accumulation by mesangial cells grown in high-glucose medium. *Diabetes* 42:118–126, 1993
13. Schleicher ED, Nerlich A: The role of hyperglycemia in the development of diabetic complications. *Horm Metab Res* 28:367–373, 1996
14. Ziyadeh FN, Sharma K, Ericksen M, Wolf G: Stimulation of collagen gene expression and protein synthesis in murine mesangial cells by high glucose is mediated by autocrine activation of transforming growth factor-beta. *J Clin Invest* 93:536–542, 1994
15. Sharma K, Ziadeh FN: Hyperglycemia and diabetic kidney disease. The case for transforming growth factor-β as a key mediator. *Diabetes* 44:1139–1146, 1995
16. Kolm V, Sauer U, Olgemoller B, Schleicher ED: High glucose-induced TGF–β1 regulates mesangial production of heparan sulfate proteoglycan. *Am J Physiol* 270:F812–F821, 1996
17. Rocco MV, Chen Y, Goldfarb S, Ziyadeh FN: Elevated glucose stimulates TGF-beta gene expression and bioactivity in proximal tubule. *Kidney Int* 41:107–114, 1992
18. Nakamura T, Fukui M, Ebihara I, Osada S, Nagaoka I, Tomino Y, Koide H: mRNA expression of growth factors in glomeruli from diabetic rats. *Diabetes* 42:450–456, 1993
19. Yamamoto T, Nakamura T, Noble NA, Ruoslahti E, Border WA: Expression of transforming growth factor beta is elevated in human and experimental diabetic nephropathy. *Proc Natl Acad Sci USA* 90:1814–1818, 1993
20. Iwano M, Kubo A, Nishino T, Sato H, Nishioka H, Akai Y, Kurioka H, Fujii Y, Kanavachi M, Shiki S, Dohi K: Quantification of glomerular TGF-β1 mRNA in patients with diabetes mellitus. *Kidney Int* 49:1120–1126, 1996
21. Sharma K, Guo J, Ziyadeh FN: Neutralization of TGF-beta by anti-TGF-beta antibody attenuates kidney hypertrophy and enhanced extracellular matrix gene expression in streptozotocin diabetic mice. *Diabetes* 45:522–530, 1996
22. Inoguchi T, Battan R, Handler E, Sportsman JR, Heath W, King GL: Preferential elevation of protein kinase C isoform beta II and diacylglycerol levels in the aorta and heart of diabetic rats: differential reversibility to glycemic control by islet cell transplantation. *Proc Natl Acad Sci USA* 89:11059–11063, 1992
23. Kolm-Litty V, Sauer U, Nerlich A, Lehmann R, Schleicher ED: High glucose-induced transforming growth factor β1 production is mediated by the hexosamine pathway in porcine glomerular mesangial cells. *J Clin Invest* 101:160–169, 1998
24. McClain DA, Paterson AJ, Roos MD, Wie X, Kudlow JE: Glucose and glucosamine regulate growth factor gene expression in vascular smooth muscle cells. *Proc Natl Acad Sci USA* 89:8150–8154, 1992
25. Marshall S, Bacote V, Traxinger RR: Discovery of a metabolic pathway mediating glucose-induced desensitization of the glucose transport system. Role of hexosamine biosynthesis in the induction of insulin resistance. *J Biol Chem* 266:4706–4712, 1991
26. Marshall S, Garvey WT, Traxinger RR: New insights into the metabolic regulation of insulin action and insulin resistance: role of glucose and amino acids. *FASEB* J 5:3031–3036, 1991
27. McClain DA, Crook ED: Perspectives in diabetes. Hexosamines in insulin resistance. *Diabetes* 45:1003–1009, 1996
28. Rosetti L, Hawkins M, Chen W, Gindi J, Barzilai N: In vivo glucosamine infusion induces insulin resistance in normoglycemic but not in hyperglycemic conscious rats. *J Clin Invest* 96:132–140, 1995
29. McKnight GL, Mudri SL, Mathewes SL, Traxinger RR, Marshall S, Sheppard PO, O'Hara PJ: Molecular cloning, cDNA sequence, and bacterial expression of human glutamine: fructose-6-phosphate amidotransferase. *J Biol Chem* 267:25208–25212, 1992
30. Sayeski PP, Kudlow JE: Glucose metabolism to glucosamine is necessary for glucose stimulation of transforming growth factor-α gene transcription. *J Biol Chem* 271:15237–15243, 1996
31. Zhou J, Huynh QK, Hoffman T, Crook ED, Daniels MC, Gulve EA, McClain DA: Regulation of glutamine: fructose-6-phosphate amidotransferase by cAMP-dependent protein kinase. *Diabetes* 47:1836–1840, 1998
32. Marshall S, Bacote V: Complete inhibition of glucose-induced desensitization of the glucose transport system by inhibitors of mRNA synthesis. *J Biol Chem* 266:10115–10161, 1991
33. Sayeski PP, Wang D, Su K, Han IO, Kudlow JE: Cloning and partial characterization of the mouse glutamine: fructose-6-phosphate amidotransferase (GFAT) gene promoter. *Nucl Acids Res* 25:1458–1466, 1997
34. Peterson AJ, Kudlow JE: Regulation of glutamine: fructose-6-phosphate-amidotransferase gene transcription by epidermal growth factor and glucose. *Endocrinology* 136:2809–1816, 1995
35. Wätzele G, Tanner W: Cloning of the glutamine: fructose-6-phosphate-amidotransferase gene from yeast. *J Biol Chem* 264: 8753–8758, 1989
36. Zhou J, Neidigh JL, Espinosa R, Lebeau MM, McClain DA: Human glutamine: Fructose-6-phosphate amidotransferase: Characterization of mRNA and chromosomal assignment to 2p13. *Hum Genet* 96:99–101, 1995
37. Ok T, Yamazaki K, Kuromitsu J, Okada M, Tamak I: cDNA cloning and mapping of a novel subtype of glutamine: fructose-6-phosphate-amidotransferase (GFAT2) in humans and mouse. *Genomics* 57:227–234, 1999
38. Xu G, Lehmann R, Schleicher E, Häring HU, Liebich H: Advantages in the analysis of UDP-sugars by capillary electrophoresis—comparison of the conventional HPLC method with two new capillary electrophoretic microprocedures. *Biomed Chromatogr* 12: 113–115, 1998
39. Kim S-J, Glick A, Sporn MA, Roberts AB: Characterization of the promoter region of the human transforming growth factor-β1 gene. *J Biol Chem* 264:402–408, 1989
40. Ayo SH, Radnik R, Garoni JA, Troyer DA, Kreisberg JI: High glucose increases diacylglycerol mass and activates protein kinase C in mesangial cell cultures. *Am J Physiol* 261:F571–F577, 1991
41. Kolm-Litty V, Tippmer S, Häring HU, Schleicher ED: Glucosamine induces translocation of protein kinase C isoenzymes in mesangial cells. *Exp Clin Endocrinol Diab* 106:377–383, 1998
42. Filippis A, Clark S, Proietto J: Increased flux through the hexosamine biosynthesis pathway inhibits glucose transport acutely by activation of the protein kinase C. *Biochem* J 324:981–985, 1997
43. Daniels MC, Kansal P, Smith TM, Paterson AJ, Kudlow JE, McClain DA: Glucose regulation of transforming growth factor α expression is mediated by products of the hexosamine biosynthesis pathway. *Mol Endocrinol* 7:1041–1048, 1993
44. Nerlich AG, Sauer U, Kolm-Litty V, Wagner E, Koch M, Schleicher ED: Expression of glutamine: fructose-6-phosphate amidotransferase in human tissues: Evidence for high variability and distinct regulation in diabetes. *Diabetes* 47:170–178, 1998
45. Richards TC, Greengard O: Distribution of glutamine hexosephosphate aminotransferase in rat tissues; changes with state of differation. *Biochim Biophys Acta* 304:842–850, 1973
46. Daniels MC, Ciaraldi TP, Nikoulina S, Henry RR, McClain DA: Glutamine: fructose-6-phosphate amidotransferase activity in cultured human skeletal muscle cells. *J Clin Invest* 97:1235–1241, 1996
47. Wang J, Liu R, Hawkins M, Barzilai N, Rossetti L: A nutrient-sensing pathway regulates leptin gene expression in muscle and fat. *Nature* 393:684–688, 1998