Mechanisms of Diabetic Vasculopathy: An Overview

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Diabetes is commonly associated with both microvascular and macrovascular complications. These vascular complications are accelerated in the context of systemic hypertension. During the past few years the underlying molecular mechanisms responsible for diabetic vascular complications have begun to be clarified. It appears that both metabolic and hemodynamic factors interact to stimulate the expression of cytokines and growth factors in the various vascular trees. Overexpression of the prosclerotic cytokine transforming growth factor-β has been observed in glomeruli and tubules from the diabetic kidney. In the retina the angiogenic cytokine vascular endothelial growth factor and its receptor, vascular endothelial growth factor R-2 are increased in experimental diabetes. These changes in growth factors are viewed to be responsible for the extracellular matrix accumulation in the diabetic kidney and new vessel formation in the diabetic retina.

Changes in cytokines have also been observed at other vascular sites including the mesenteric vascular tree. Vasoactive hormones, such as angiotensin II and endothelin, are potent stimulators of cytokines with recent studies showing that inhibitors of these vasoactive hormone pathways may confer organ protection in diabetes by inhibition of growth factor expression. Glucose-dependent factors, such as the formation of advanced glycation end products that interact with specific receptors and lead to overexpression of a range of cytokines, may play an important role in diabetic vascular complications including atherosclerosis. It is likely that the effects of inhibitors of this pathway such as aminoguanidine on cytokine production may play a pivotal role in mediating the renal, retinal, and vasoprotective effects observed with this agent in experimental diabetes. It is anticipated that the advent of specific inhibitors of cytokine formation or action will provide new approaches for the prevention and treatment of diabetic vascular complications. Am J Hypertens 2001;14:475–486 © 2001 American Journal of Hypertension, Ltd.

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The mesenteric vascular tree has been used to explore underlying diabetes-related vascular abnormalities including biochemical and molecular biological changes, but one has to be cautious in extrapolating findings from this site to other sites of vascular injury such as the kidney and the retina.

**Hemodynamic Factors**

**Arterial Hypertension and Mechanical Strain**

Arterial hypertension is present in up to 40% of patients with type 1 and up to 70% of those with type 2 diabetes. Regardless of its cause, hypertension in diabetes may amplify or accelerate the development and progression of both the macrovascular and microvascular complications of diabetes. Indeed, arterial hypertension per se has been shown to cause endothelial dysfunction and vascular remodeling. These morphologic alterations include increased wall thickness and medial enlargement resulting from vascular smooth muscle cell hyperplasia and extracellular matrix accumulation in resistance arteries.

The increase in blood pressure (BP) and the subsequent increase in cellular shear stress has been demonstrated to induce various deleterious pathways within the arterial wall, including activation of ion channels, tyrosine kinases, and integrins that influence interaction between cells and matrix. In diabetes, there is altered distribution of cardiac output with a subsequent increase of blood flow to the small intestines. Increased blood flow through mesenteric arteries has been reported to stimulate media hypertrophy and vascular smooth muscle cell proliferation, a phenomenon that is referred to as vascular remodeling.

Mechanical forces are also capable of stimulating autocrine/paracrine hormonal responses within the arterial wall. Angiotensin II (Ang II) has been shown to be generated in the arterial wall in response to increased vascular wall tension. Furthermore, mechanical stress has been shown to increase both mRNA and protein expression of the angiotensin receptor type I receptor (AT1) in myocytes. Arterial hypertension per se may also stimulate the production of various cytokines, which may amplify vascular injury. In vitro, the application of shear stress to vascular smooth muscle cells results in increased production of transforming growth factor-β1 (TGF-β1). Furthermore, stretch has been shown to stimulate vascular endothelial growth factor (VEGF) gene expression in endothelial cells and this may play a contributory role in endothelial dysfunction.

**Neurohumoral Factors**

**Renin-Angiotensin System**

A large range of clinical trials has shown that angiotensin converting enzyme (ACE) inhibitors prevent or slow the
formation and the progression of diabetic micro- and macroangiopathy, suggesting that the renin-angiotensin system (RAS) may play a key role in the pathogenesis of diabetic vasculopathy. Experimental studies focusing on the mesenteric arterial tree have further confirmed and extended these data. Treatment of diabetic rats either with different ACE inhibitors or with an AT1 antagonist has been shown to consistently attenuate diabetes-associated mesenteric vascular hypertrophy. In this model, blockade of the RAS is associated with a significant reduction in extracellular matrix (ECM) accumulation and type IV collagen mRNA and protein levels. Administration of an ACE inhibitor at a low dose, in the absence of BP reduction, also prevented mesenteric vascular hypertrophy in diabetic rats with wall/lumen ratio measurements similar to the values observed with hypotensive doses, suggesting that the antiprotein effects of RAS inhibitors may be partially independent of the reduction in BP.

Beyond its hemodynamic effects, Ang II has been considered to be centrally involved in vascular remodeling in diabetes through its growth-promoting actions. In vitro, Ang II has been demonstrated to exert growth-promoting effects on cultured vascular smooth muscle cells and to stimulate ECM production by these cells. In vivo, Ang II has been shown to stimulate vascular smooth muscle cell proliferation in normal and injured vessels. Our group has previously demonstrated that mesenteric vessel ACE was increased in diabetic rats. A large body of experimental evidence has also suggested that tissue Ang II may play an important role in the formation of target organ damage in diabetes and have confirmed the presence of all components of the RAS in arteries. The RAS may play a role in the formation of reactive oxygen species in the arterial wall as Ang II has been shown both in vitro and in vivo to stimulate the production of superoxide anion in smooth muscle cells. This increased free radical formation may, in turn, result in decreased formation of nitric oxide, leading to endothelial dysfunction, increased BP, and atherosclerosis. Therefore, it has been suggested that the reduction of oxidative stress by interruption of the RAS may indirectly contribute to the antiatherosclerotic effects experimentally attributed to ACE inhibitors. Furthermore, clinical studies have demonstrated the ability of ACE inhibitors to restore endothelial function in diabetic patients, emphasizing the role of RAS in the pathogenesis of endothelial dysfunction, possibly through the modulation of reactive oxygen species production.

Angiotensin converting enzyme inhibitors act not only to decrease Ang II production but also to decrease the degradation of kinins and to subsequently favor the release of nitric oxide. Previous studies from our laboratory have shown that kinins may be involved in the short-term antitrophic effects conferred by ACE inhibitor in experimental diabetes. Nevertheless, the capacity of Ang II receptor antagonists to prevent mesenteric vascular hypertrophy as effectively as ACE inhibitors strongly implicates Ang II rather than kinins in the pathogenesis of long-term diabetic vasculopathy.

Endothelin

Although most studies in diabetes have focused on the RAS, another important vasoconstrictor that needs to be considered is endothelin (ET). ET-1 is released by endothelial cells and causes an initial vasodilatation by activation of ET receptors on endothelial cells. This is followed by a sustained vasoconstriction caused by the ET-1 effect on ET receptors on vascular smooth muscle cells. In addition to its vasoconstrictor properties, ET-1 is a potent mitogen and induces vascular hypertrophy.

Endothelin is a potent vasoconstrictor and changes in plasma ET-1 levels have been shown in experimental and human diabetes, hypertension, and atherosclerosis, all states associated with endothelial dysfunction. ET-1 is a marker of atherosclerotic macro- and microvascular disease in patients with type II diabetes. In a nondiabetic model of vascular injury both ET and ET inhibitors have been reported to prevent neointima formation in the balloon injury model. The importance of ET-1 in diabetes-associated vascular hypertrophy was initially suggested by studies reporting an increased release of this peptide from mesenteric vessels in diabetic rats. Recent studies by our group demonstrated increased ET expression in the endothelial and adventitial layer and de novo expression of ET-1 in the media of diabetic mesenteric vessels. These changes of ET expression were associated with vascular hypertrophy, increased ECM deposition, and an increased gene expression of epidermal growth factor (EGF) and TGF-β1. Treatment with the ET receptor antagonist bosentan attenuated mesenteric vascular hypertrophy.

Interactions Between Endothelin and the Renin-Angiotensin System

It is important to appreciate that there are a range of interactions between various neurohormonal pathways including the RAS and endothelin. For example, Ang II-induced vascular hypertrophy can be attenuated by ET receptor antagonism. Indeed, in vitro studies indicate that Ang II promotes transcription of prepro-ET and may enhance endothelin converting enzyme activity. It is predicted that during the next few years there will be further delineation of this relationship between the RAS and ET, potentially providing new approaches for the prevention of diabetes-associated vascular hypertrophy.

Reactivity to Vasoconstrictors and Vasodilators

It remains controversial as to whether there is increased sensitivity of mesenteric vessels in experimental diabetes to vasoconstrictors such as noradrenaline (NA) and serotonin. Whereas some investigators have shown an increased NA-mediated vasoconstriction in the aorta and mesenteric...
vessels of diabetic rats, others could not observe any differences between control and diabetic animals. One has to be cautious in extrapolating findings from one vessel type or vascular bed to another, partially because the proportional receptor distributions in different vascular beds may vary. For example, in the aortic wall one finds predominantly the ET$_A$ receptor, whereas the mesenteric vascular tree has both ET$_A$ and ET$_B$ receptors. There is evidence that the duration of diabetes has an effect on the vascular sensitivity to some vasoconstrictors. If this relates to changes at the vascular receptor level or concomitant changes in vascular structure has not been fully clarified.

The increased vasoconstrictor response to NA in mesenteric vessels of diabetic rats has been explained by a number of different mechanisms. Several investigators have suggested particular mechanisms that mediate the vascular response to vasoconstrictors, some of which appear to be glucose independent. These include an increased expression of thromboxane A within the vessel wall, an overexpression of calcium channels in diabetes, activation of protein kinase C, or increased expression of second messengers in response to NA such as P (1,4,5) P$^3$ as a result of an abnormal phosphoinositide metabolism in diabetic vessels. However, in most models of experimental diabetes the altered responses to NA and ET-1 could be restored to normal by correcting hyperglycemia, suggesting that metabolic factors are predominantly involved in regulating vascular reactivity to vasoconstrictors.

Most studies have shown an impaired endothelium-dependent vasodilatory response to acetylcholine (Ach) or bradykinin, whereas nonendothelium-dependent relaxation did not seem to be affected by diabetes. The degree of impairment of the vasodilatory response to different agents also seems to vary among the various vascular beds. The vasodilation caused by iloprost (PGI$_2$ analog) was affected in coronary and mesenteric vessels but not in the aorta of diabetic rats. A number of substrates have been linked to the impaired vasodilation observed in experimental diabetes. It has been speculated that nitric oxide (NO) release is impaired or that the smooth muscle cell responsiveness to NO is reduced in the diabetic state. Other researchers reported that the levels of cAMP and cGMP, a marker of NO production, were reduced in the effluent of diabetic mesenteric vessels. Further explanations for the reduced vasodilation in diabetes have included abnormal cyclooxygenase activity and increased phosphoinositide metabolism. Other investigators have postulated a role for a cyclooxygenase-derived vasoconstrictor that is different to thromboxane A2. As described previously, the increased formation of superoxide anions in the diabetic vessels might modulate vascular function by inactivating NO and blocking NO-mediated vasodilation.

The degree of endothelial dysfunction correlates with the duration of diabetes and metabolic control. Impaired vasodilation in the diabetic state could be restored to normal by insulin treatment. It is not clear whether insulin treatment improved vascular dysfunction by improving metabolic control or whether insulin itself, which has NO-mediated vasodilatory effects, added to the beneficial effects on vascular function.

**Metabolic Factors**

Hyperglycemia is well established as an independent risk factor for accelerated atherosclerosis and microvascular disease. Glucose-induced damage occurs through at least three apparently disparate pathways: advanced glycation, activation of protein kinase C, and sorbitol accumulation by way of the polyol pathway. Recent studies suggest that seemingly unrelated separate pathways may be linked at the level of the mitochondria. For example, it has been shown that increased production of superoxide by the mitochondrial electron transport chain is common to all three pathways. Using inhibitors at various points in the chain it was possible to normalize levels of reactive oxygen species in the mitochondria. This resulted in prevention of glucose-induced activation of protein kinase C, intracellular advanced glycation end product (AGE) formation, sorbitol accumulation, and nuclear factor -κB (NF-κB) activation.

**Advanced Glycation End Products**

The accelerated formation of AGE, through a complex series of rearrangements between reducing sugars and free amino groups, is now increasingly linked to many complications of diabetes. The ability of AGE to form intermolecular cross-links in tissue, thereby trapping solubile proteins such as lipoproteins, quenching NO, and interacting with specific binding proteins to induce vascular permeability, ECM accumulation, oxidative stress, and a procoagulant state, suggest a role for these AGES in diabetic vascular dysfunction. This observation has been emphasized and strengthened by studies using specific glycation inhibitors such as aminoguanidine, that in addition to preventing AGE accumulation ameliorate diabetic complications.

Advanced glycation is a ubiquitous metabolic pathway that occurs during normal aging and at an accelerated rate in diabetes. The nonenzymatic and spontaneous reaction between reducing sugars and free amino groups on proteins, lipids and nucleic acids progresses proportionate to time, the concentration of the sugar and the prooxidative nature of the microenvironment. Spontaneous reaction between these sugars and free amino-terminal groups initially results in the formation of Schiff bases as reported almost a century ago. Dehydration and rearrangement of these adducts generates Amadori products, of which the best known is glycated hemoglobin (HbA$_1c$). A complex set of rearrangements through highly reactive intermediates leads, ultimately to a heterogeneous set of irreversible products termed AGE. Oxidation accompanying the reaction allows the formation of more permanent glycoxida-
tion products such as carboxymethyllysine (CML) and pentosidine. Other pathways such as lipid peroxidation and glucose autooxidation also generate additional AGE.

**Advanced Glycation End Product Receptors** One mechanism for AGE-mediated effects is through an interaction with specific binding proteins that act as receptors, allowing a wide range of cell activation. These include the receptor for advanced glycation end products (RAGE), p60, p90, galectin-3, and the macrophage scavenger receptor (MSRA). The best characterized receptor, RAGE, has been shown to mediate activation of NF-κB and MAP kinases. RAGE has been localized to endothelial cells where it promotes vascular permeability and is also found on other cells including pericytes, neurones, macrophages, and smooth muscle cells. Our group has localized RAGE by immunohistochemistry in the retina, kidney, and large arteries where it colocalizes with AGE deposition. RAGE expression is upregulated both in diabetes and conditions of inflammation, including atherosclerosis, where monocytes in plaques display intense RAGE staining.

The accumulation of AGE on long-lived matrix proteins increases the cross-linking of these components, resulting in their resistance to degradation, as well as increasing vessel rigidity. This ECM accumulation occurs at an increased rate, at least in part due to induction of growth factors and cytokines such as TGF-β77 and platelet derived growth factor (PDGF). The AGE receptor interaction biases cells toward an inflammatory state, inducing the elaboration of growth factors, cytokines, and procoagulant molecules. The presence of RAGE on the endothelial cell, the guardian of barrier function led to the discovery that AGE–RAGE interactions resulted in hyperpermeability, and that receptor blockade restores dysfunctional barrier function.

**Advanced Glycation End Products and Atherosclerosis** A number of studies have implicated AGE in the pathogenesis of atherosclerosis. In areas of atherosclerosis, the presence of AGE is correlated with the size and complexity of the lesion. Indeed, AGE are detectable in the atherosclerotic plaques of patients even without diabetes. AGE have even been detected in the fatty streaks of young nondiabetic animals. AGE interact with the receptor RAGE to induce VCAM-1 expression, which is considered a hallmark of early atherosclerosis. Under normal circumstances the vascular endothelium expresses little RAGE. However, RAGE staining is increased in atherosclerotic plaques and suppression of the AGE–RAGE interaction using a truncated version of the RAGE receptor has been shown to attenuate accelerated atherosclerosis in a mouse model of diabetes-associated macrovascular disease. Direct evidence linking AGE to atherosclerosis has been suggested from studies where the administration of AGE-modified albumin to euglycemic rabbits resulted in increased atherosclerotic lesions. Furthermore, it has been shown that inhibition of AGE formation with aminoguanidine reduces plaque formation in cholesterol-fed rabbits.

Intermolecular cross-link formation decreases collagen susceptibility to degradation and increases mechanical stiffness of collagen fibers. At the same time the AGE receptor interaction causes ECM accumulation through elaboration of cytokines such as TGF-β and PDGF, with consequent increases in thickness and rigidity. Agents that specifically block the formation of AGE prevent accelerated cardiovascular stiffening associated with diabetes. In a model of diabetes-associated vascular hypertrophy in the mesenteric vascular tree, our group demonstrated that inhibition of AGE formation resulted in a significant amelioration of various pathologic changes including a reduction in ECM accumulation, possibly through inhibition of TGF-β expression.

Nitric oxide is an important mediator of vascular tone and has vasodilatory in addition to antiproliferative properties. It appears that AGE interfere with NO action. This is relevant to the diabetic context, as diabetic rats develop a time-dependent impairment of NO-dependent vasodilatation, which is not prevented by insulin therapy, but is slowed by aminoguanidine administration.

**Inhibitors of Glycation** The role of AGE in micro- and macrovascular diseases has been further supported by studies using aminoguanidine, a nucleophilic hydrazine derivative that reacts with the glucose-derived intermediates of early glycation. Aminoguanidine has been shown to prevent AGE formation in rat tissues, including the vasculature, kidney, nerve, and retina. The vascular hypertrophy induced by diabetes in the mesenteric vascular tree could be ameliorated by treatment with aminoguanidine. Similar results are seen in the kidney and the retina. Additional benefit may accrue from the ability of aminoguanidine to scavenge reactive oxygen species (ROS) at therapeutic doses.

The formation of cross-links by AGE had previously been considered irreversible. Cross-link breakers such as phenacylthiazolium bromide (PTB) and newer compounds such as ALT 711 now offer the possibility of reducing diabetes-related tissue dysfunction after it has become established. Daily administration of PTB to streptozotocin diabetic rats reduced tail collagen cross-linking, as well as vascular AGE accumulation. Administration of a more stable derivative of PTB, ALT 711 (4,5-dimethyl thiazolium bromide) has been shown to improve vascular compliance in STZ diabetic rats. Furthermore, daily intraperitoneal injection of this drug improved myocardial compliance in aged dogs. These findings provide a potential role for reducing AGE accumulation and in particular cross-linked AGE in diabetes-related vascular hypertrophy, but also in attenuating the increased vascular and cardiac stiffness that occurs with aging and that may contribute to diastolic dysfunction and systolic hypertension.
Protein Kinase C

The intracellular second messenger, protein kinase C (PKC), appears to be activated in a range of diabetic tissues including the retina, kidney, heart, and aorta. In particular, the β2 isoform has been reported to be increased in diabetic vascular tissues. This potentially of major therapeutic significance as an inhibitor, LY 333531, has been reported to attenuate various diabetes-related abnormalities in the retina and kidney. A transgenic mouse with targeted overexpression of the PKC-β2 isoform in the myocardium has been reported to exhibit left ventricular hypertrophy and increased myocardial necrosis and fibrosis, which could be attenuated by the PKC-β2-specific inhibitor. Increases in myocardial PKC-β isoforms content and activity have recently been described in patients with heart failure, providing further evidence of a role for this enzyme in cardiac disease. Recent studies have reported in the kidney that various renoprotective treatments such as ACE inhibitors and aminoguanidine may reduce renal PKC activity. These findings imply a central role for PKC in mediating vascular injury, particularly in diabetes and provide a rationale for exploring the role of specific inhibitors of this enzyme in the treatment of diabetic vascular complications.

Other Glucose-Dependent Factors

Other metabolic pathways that may reduce vascular injury include activation of the transcription factor NF-κB. In vitro studies in endothelial and vascular smooth muscle cells have shown that hyperglycemia induces activation of NF-κB. This phenomenon has been reported to be PKC dependent as well as able to be inhibited by antioxidants. Another metabolic pathway that has been investigated in detail in the genesis of diabetic complications is the polyol pathway. In this pathway glucose is reduced to sorbitol by the enzyme aldose reductase. Sorbitol accumulation is associated with depletion of myoinositol and changes in the cellular redox potential. The advent of inhibitors of aldose reductase has allowed investigators to explore in vivo the role of the polyol pathway. Results in experimental diabetic complications such as nephropathy have been conflicting and the role of this pathway in vascular complications per se is not well understood. Endothelial dysfunction in the mesenteric vascular tree in diabetic animals has been reported to be reversed by the aldose reductase inhibitor ponalrestat, although the studies in this area have not been consistent.

Cytokines

Diabetes is associated with increased expression and action of various cytokines and growth factors. This appears to be as a consequence of hyperglycemia-induced events including PKC activation, upregulation of vasoactive hormones such as ET and Ang II, hypoxia, and oxidative and possibly glycoxidative stress (Fig. 1). It appears that certain cytokines may play a particular role in various diabetes-induced vascular injuries. For example, TGF-β plays a pivotal role in mediating ECM accumulation in the kidney. The role of cytokines in mediating micro- and macrovascular injury at other sites remains to be determined but may involve not only TGF-β and VEGF but also proliferative cytokines such as EGF and PDGF.

Transforming Growth Factor-β

A major role for TGF-β has been suggested in a range of progressive renal diseases including diabetes mellitus. However, TGF-β may also be a very important mediator of vascular injury at other sites in diabetes. TGF-β has been demonstrated in vitro to cause both hypertrophy and hyperplasia of vascular smooth muscle cells and to favor vascular ECM production and accumulation. Our group has demonstrated that ACE inhibition attenuates vascular hypertrophy and prevents the upregulation of TGF-β gene and protein expression in mesenteric vessels both short term and long term in diabetic rats, providing in vivo evidence for a link between Ang II and TGF-β in blood vessels in the presence of diabetes. TGF-β appears to be an important mediator of different pathways implicated in the genesis of diabetic vascular complications. Indeed, several pathogenic stimuli appear to be involved in the diabetes-associated upregulation of TGF-β, including glucose-induced PKC activation, advanced glycation products, Ang II, and cell stretch associated with hyperperfusion.

Vascular Endothelial Growth Factor

Vascular endothelial growth factor (VEGF) is one of the most potent inducers of vascular permeability and a powerful mitogen for vascular endothelial cells. Recent evidence suggests that VEGF plays a role in the pathogenesis of neovascularization and the increased vascular permeability that characterizes diabetic microangiopathy. This cytokine has been considered to play a pivotal role in mediating proliferative diabetic retinopathy, but its role in other vascular sites in diabetes is not well characterized. Low levels of constitutive VEGF mRNA in medium-to-large arteries have been observed in vivo in humans and this expression is restricted to vascular smooth muscle cells. Similarly, various stimuli relevant to the diabetic context have been reported to increase the vascular expression of VEGF including hypoxia, elevated glucose concentrations, AGEs, Ang II, and TGF-β. The intracellular signaling pathway responsible for hyperglycemia-induced VEGF upregulation appears to involve PKC activation. Recent studies have also detected increased expression of VEGF receptors in the diabetic retina. A link between Ang II and VEGF is suggested by in vitro studies where blockade of the AT1 receptor with losartan abolishes the upregulation of VEGF mRNA in cultured smooth muscle cells.
suggest that the ACE inhibitor perindopril reduces retinal VEGF expression in experimental diabetes.\textsuperscript{137}

**Epidermal Growth Factor**

Our group has recently reported increased expression of EGF in the endothelial layer of mesenteric vessels.\textsuperscript{136} Interestingly, EGF mRNA was also expressed in mast cells infiltrating the adventitia of mesenteric arteries undergoing hypertrophy in experimental diabetes. Mast cell infiltration and activation have been previously demonstrated in mesenteric vessels in diabetes.\textsuperscript{138,139} These mast cells have to be considered as an important source of cytokines and chemotactants because they are active and secreting as early as day 7 of diabetes. Mast cell-mediated proliferation could be demonstrated by quantification of specific DNA synthesis and mitotic indices in mesenteric vessels by day 28 of experimental diabetes.\textsuperscript{138,139} Mast cell infiltration and activation are also followed by an augmented angiogenesis in mesenteric vessels of diabetic rats.\textsuperscript{140} In our study, the endothelin receptor blocker bosentan reduced diabetes-associated vascular hypertrophy in association with a reduction of EGF gene expression in endothelial cells as well as in mast cells. TGF-\(\beta\) gene expression was not reduced by bosentan treatment, suggesting a probable independent role of EGF in the genesis of diabetic vasculopathy.\textsuperscript{46}

**Platelet-Derived Growth Factor**

Platelet-derived growth factor induces both cell proliferation and ECM synthesis in vascular smooth muscle cells and has been implicated in the development of atherosclerosis.\textsuperscript{127} Increased production of PDGF by the vascular endothelium in response to high glucose and Ang II has been reported in vitro.\textsuperscript{141} PDGF-\(\beta\) receptor expression has also been demonstrated to be stimulated by hyperglycemia through activation of PKC in vascular smooth muscle cells, suggesting the possible involvement of PDGF in the development of diabetic vasculopathy.\textsuperscript{142}

**SPARC**

The antiadhesive ECM protein SPARC (secreted protein and rich in cysteine, osteonectin, or BM-40) has been implicated in the regulation of matrix turnover, cell migration, and proliferation.\textsuperscript{143,144} As an inhibitor of cell cycle progression it directly counteracts PDGF-mediated proliferation.\textsuperscript{145} Furthermore, SPARC expression is stimulated by PDGF and TGF-\(\beta\) and recently it has been reported that SPARC may itself stimulate TGF-\(\beta\) expression.\textsuperscript{146} It has antiadhesive properties mediating cell detachment from the underlying substratum as a prelude to migration, proliferation, or phenotypic alteration.\textsuperscript{147} Our own studies showed that SPARC gene and protein expression were increased in hypertrophied vessels of diabetic rats compared to controls after 1, 3, and 32 weeks of experimental diabetes and preceded mesenteric vascular hypertrophy (Fig. 2).\textsuperscript{148} Aminoguanidine treatment reduced mesenteric vascular hypertrophy and SPARC gene and protein expression without influencing metabolic control or food intake.\textsuperscript{148} These studies suggest modulation of the expression of SPARC in diabetes might be of pathogenetic significance in the development of vascular remodeling in diabetes possibly by modulating matrix protein synthesis through effects on prosclerotic cytokines\textsuperscript{146,149} and on matrix-degrading metalloproteinases.\textsuperscript{144} The relationship between TGF-\(\beta\) and SPARC remains to be elucidated particularly in the context of diabetes as SPARC and TGF-\(\beta\) appear to stimulate each other, the ultimate functional significance of this phenomenon as yet unclarified.

**Summary**

The structural changes in diabetic vessels reflect complex interactions among various cell populations, growth factors, and matrix proteins that occur in response to the modulation of vasoactive hormone systems and glucose-dependent pathways that characterize the diabetic milieu. This review has focused on the mesenteric vascular tree, a vascular bed that consistently shows diabetes-associated vascular hypertrophy. Various metabolic pathways are involved in the development of diabetes-associated vascular injury and include AGEs, activation of PKC isoforms, and sorbitol accumulation through the polyol pathway. All three metabolic pathways lead to an increase of superoxides by the mitochondria.

In addition, hemodynamic pathways are activated in diabetes and are possibly amplified by concomitant systemic hypertension. These hemodynamic and metabolic pathways interact at various levels including activation of second messengers such as PKC, transcription factors such as NF-\(\kappa\)B, and expression of cytokines and their receptors to induce a range of pathologic changes in diabetic vessels. These changes include vascular proliferation, angiogenesis, and ECM accumulation. These pathologic changes lead to many of the functional and structural abnormalities seen in diabetic vessels such as endothelial dysfunction, reduced vascular compliance, and increased atherosclerosis. The advent of more sophisticated biochemical, histologic, and molecular biological techniques has allowed investigators to explore in more detail the underlying pathophysiologic mechanisms responsible for vascular injury and in particular to identify the role of both resident and infiltrating cells involved in this disease process. Novel inhibitors of these pathways are now available and appropriate interventional studies at both the experimental and clinical level will lead not only to improved understanding of the pathogenesis of diabetes-associated vascu-
lar injury, but also provide new therapeutic approaches to retard, reverse, and prevent diabetic vascular complications.

References


FIG. 2. SPARC protein by immunofluorescence in control (A) and diabetic (B) mesenteric vessels after 1 week of diabetes. Original magnification, ×500.


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