SURVEY REVIEW / SYNTHÈSE D'ENSEMBLE

Endothelins in chronic diabetic complications[1]

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Abstract: Endothelins are widely distributed in the body and perform several vascular and nonvascular functions. Experimental data indicate abnormalities of the endothelin system in several organs affected in chronic diabetic complications. In support of this notion, it has been shown that endothelin-receptor antagonists prevent structural and functional abnormalities in target organs of diabetic complications in animal models. Alterations of plasma endothelin levels have also been demonstrated in human diabetes. This review discusses the role of endothelins in the pathogenesis of chronic diabetic complications. The current experimental evidence suggests that endothelin-receptor antagonism may potentially be an adjuvant therapeutic tool in the treatment of chronic diabetic complications.

Key words: endothelins, diabetic complications, retinopathy, nephropathy, neuropathy, cardiomyopathy.

Résumé : Les endothélines sont réparties dans tout le corps et ont plusieurs fonctions vasculaires et non vasculaires. Des données expérimentales montrent des anomalies du système des endothélines dans plusieurs organes cibles des complications chroniques du diabète. À l'appui de cette notion, on a montré, dans des modèles animaux, que les antagonistes des récepteurs de l'endothéline préviennent les altérations structurelles et fonctionnelles dans les organes cibles des complications diabétiques. Des altérations des taux d'endothélines plasmatiques ont aussi été démontrées dans le diabète humain. La présente synthèse discute du rôle des endothélines dans la pathogenèse des complications chroniques du diabète. Les résultats expérimentaux actuels semblent indiquer que l'antagonisme des récepteurs de l'endothéline pourrait constituer un outil thérapeutique d'appoint dans le traitement des complications chroniques du diabète.

Mots clés : endothélines, complications diabétiques, rétinopathie, néphropathie, neuropathie, cardiomyopathie.

[Traduit par la Rédaction]

Introduction

A large body of experimental and clinical data generated over the last few years indicates that endothelins (ETs) are of importance in several human diseases (Benigni et al. 2000; King and Brownlee 1996; Inoue et al. 1989; Yanagisawa et al. 1988). Multiple functions along with predominant expression in vascular tissues, do indeed, suggest that alteration of ETs may be involved in diseases affecting both micro- and macrovasculature. In the context of diabetes, biochemical abnormalities occurring secondary to hyperglycemia may alter the ET system (King and Brownlee 1996). Alteration of ETs may be of significant importance in the development of structural and functional lesions characteristic of diabetic complications. Furthermore, ETs may also regulate the expression of other growth factors and cytokines which may contribute to the pathogenesis of chronic diabetic complications.

Patients with diabetes demonstrate wide array of abnormalities of plasma ET-1 levels. Type I diabetics have been reported to demonstrate both decreased (Malamitsi-Puchner et al. 1996; Smulders et al. 1994) and increased plasma ET-1 levels (Collier et al. 1992; Haak et al. 1992; Takahashi et al. 1990). In addition, patients with type II diabetes have been reported to exhibit elevated (Ak et al. 2001; Morise et al. 1995; Donatelli et al. 1994) as well as unchanged levels of plasma ET-1 (Guvener et al. 1997; Kanno et al. 1991). Variability in duration of diabetes and (or) the level of metabolic control may contribute to the inconsistency in these studies (Chakrabarti et al. 2000; Hopfner and Gopalakrishnan 1999). In addition, ETs act both in an autocrine and paracrine fashion. Therefore, plasma levels of these peptides may not provide an adequate assessment of their biological activity. In support of ET alteration in diabetes are in vitro studies conducted by several laboratories that have shown high glucose- and insulin-mediated increased ET-1 production in endothelial cells (Chen et al. 2000*a*; Hu et al. 1993; Yamauchi et al. 1990). In the following sections, we will discuss the physiology of ET system, the mechanisms that may lead to alter-

Received 31 October 2001. Published on the NRC Research Press Web site at http://cjpp.nrc.ca on 16 May 2003.

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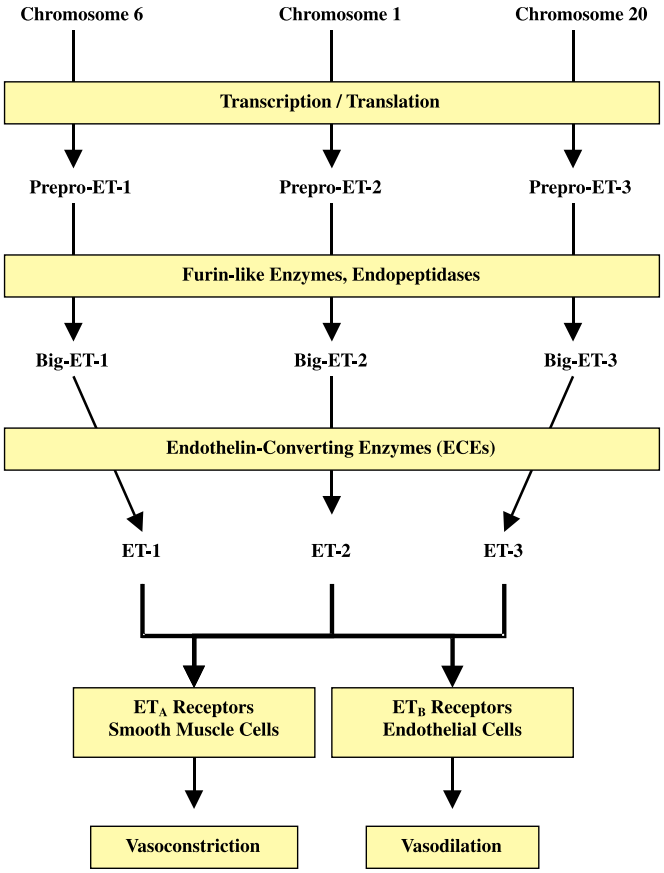
 doi: 10.1139/Y03-053

Fig. 1. An outline illustrating expression of endothelins. ETs are produced as precursors that are proteolytically processed to generate mature ET peptides. Two classes of proteins are implicated in the processing of prepro-ETs: furin-like endopeptidases and endothelin-converting enzymes. Mature ET peptides act in an autocrine and paracrine fashion to transduce signals via cell surface receptors, ET_A and ET_B. Generally, ET_A activation leads to vasoconstriction, and activation of the ET_B receptor mediates vasodilation. These receptors are also expressed by nonvascular cells and may mediate expression of various genes.



ation of ETs in diabetes, and salient features of chronic diabetic complications in selected organs in regards to ET alteration.

The endothelin system

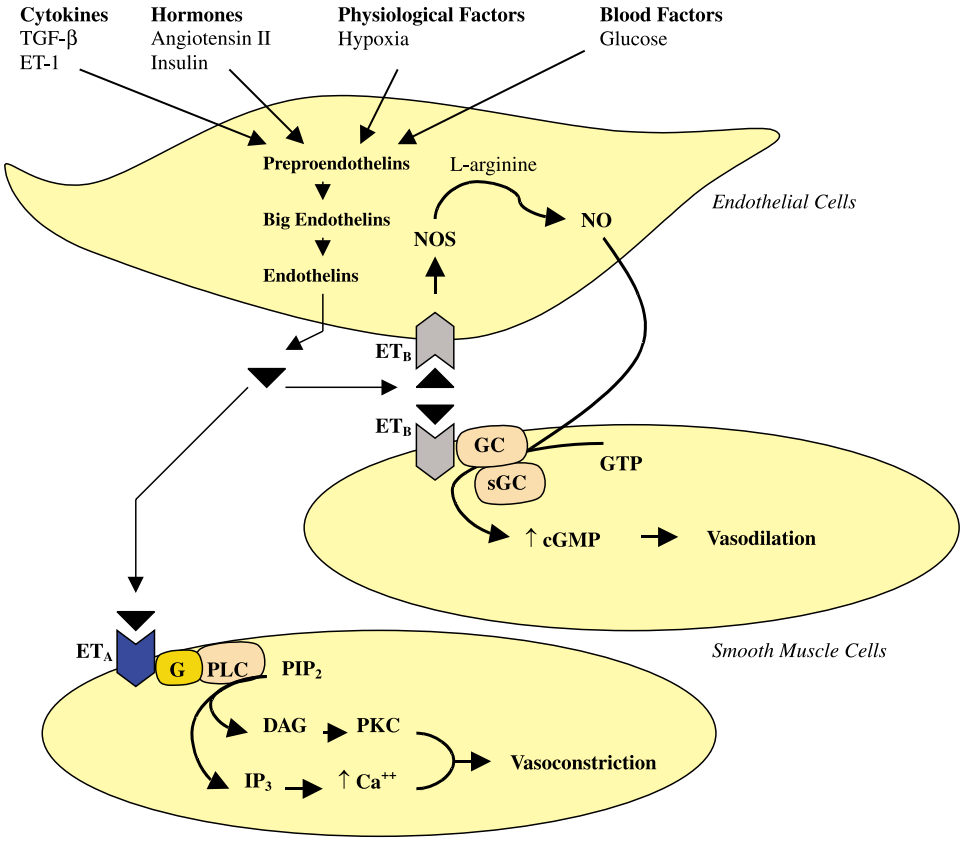
Endothelins are potent vasoactive factors which are produced by several cell types (Levin 1995;, Rubanyi and Polokoff 1994; Inoue et al. 1989; Yanagisawa et al. 1988). The ET family is comprised of three isoforms, ET-1, ET-2, and ET-3, which are encoded by distinct genes. Regulation of ET expression is achieved at the transcriptional level. Several cytokines, physiochemical factors, and blood factors have been shown to regulate expression of ETs (Malek et al. 1999; Benatti et al. 1994; Emori et al. 1992; Kohno et al. 1992; Kurihara et al. 1989). ETs are produced as precursors that are processed by two classes of proteolytic enzymes, furin-like endopeptidases and a group of proteins called endothelin-converting enzymes (ECEs) (Fig. 1). Two such ECEs have been cloned, ECE1 and ECE2. There are four isoforms of ECE1 (ECE1*a*–ECE1*d*) which, with the exception of ECE1*b*, are localized on cell surface (Valdenaire et al. 1999; Schweizer et al. 1997; Shimada et al. 1995; Valdenaire et al. 1995). ECE2 and ECE1*b* have been shown to be intracellular, being localized close to the Golgi complex (Schweizer et al. 1997).

ETs regulate signal transduction via specific cell surface receptors ET_A, ET_B, and ET_C (Sakurai et al. 1992; Sakamoto et al. 1991; Arai et al. 1990). Mammals have been shown to express only ET_A and ET_B receptor types. ET_A receptors bind ET-1 and ET-2 with higher affinity as compared with ET-3, whereas ET_B receptors bind all isoforms with similar affinity. ET_A receptors are primarily localized on vascular smooth muscle cells and regulate vasoconstriction (Hori et al. 1992; Sumner et al. 1992; Arai et al. 1990). Activation of ET_A receptors results in calcium influx via phospholipase C (PLC) mediated diacylglycerol (DAG) and inositol trisphosphate (IP_3) production (Fig. 2). DAG activates protein kinase C (PKC), which, alongside increased intracellular calcium, causes phosphorylation of myosin kinase and smooth muscle cell contraction. In addition, interaction of ETs with ET_A receptors can also lead to activation of phospholipase D (PLD), facilitating PKC activation and cellular contraction (Decker and Brock 1998).

ET_B receptors, predominantly expressed by vascular endothelial cells, are involved in vasodilation. Activation of ET_B receptors on endothelial cells leads to generation of nitric oxide (NO) (Molenaar et al. 1993; Takayanagi et al. 1991). This receptor type is also coupled to PLC via G-proteins. Interaction of ETs with ET_B receptors results in increased calcium influx similar to ET_A receptors. The resultant increased intracellular calcium modulates activity of calcium–calmodulin-dependent nitric oxide synthase (NOS) and generates NO. Following production, NO diffuses to smooth muscle cells and initiates the production of cyclic 3′,5′-guanosine monophosphate (cGMP) by activating soluble cytosolic guanylyl cyclase. Elevation of cGMP levels causes vasodilation by decreasing intracellular calcium in smooth muscle cells. ET_B are also expressed on smooth muscle cells to some extent (Davenport et al. 1993). In smooth muscle cells, activation of ET_B receptors can lead to dual vasoregulatory effects. Similar to activation of ET_A receptors, ET_B activation can also lead to increased intracellular calcium and cellular contraction. In addition, ET_B activation can result in conversion of guanosine triphosphate (GTP) to cGMP in a tissue-specific manner (Ding et al. 1999; Kohan and Padilla 1994) (Fig. 2). This ET-mediated cGMP increase is distinct from that mediated by endothelial-derived NO as it involves particulate (membrane-bound) guanylyl cyclase. This increase in cGMP can also be potentiated by endothelial-derived NO. The net reduction in intracellular calcium brought upon by elevated cGMP levels leads to vasodilation. Furthermore, in some cells, ET_B receptors have been shown to be coupled to inhibitory G-proteins that inhibit 3′,5′-cyclic adenosine monophosphate (cAMP) generation (Aramori and Nakanishi 1992). Thus, in vivo vasoregulatory action of ETs could be the outcome of a number of factors, including receptor affinity, number of receptors activated, types of receptors activated, and the tissue involved.

ETs also control functions such as morphogenesis and extracellular matrix (ECM) protein synthesis (Levin 1995;

Fig. 2. Possible mechanisms of vasoregulation by endothelins include various inducers and inhibitors that regulate the expression of precursor peptides. These precursor peptides undergo proteolytic processing to produce mature ETs. Interaction of ETs with ET_A receptors on smooth muscle cells leads to calcium influx via G-protein coupled phospholipase C (PLC) activation. Diacylglycerol (DAG) generated subsequent to PLC activation can lead to activation of PKC and facilitate cellular contraction. Activation of ET_B receptors on endothelial cells results in nitric oxide (NO) production. NO activates soluble guanylyl cyclase in smooth muscle cells and increases cGMP. The net reduction in intracellular calcium results in vasodilation. In some tissues, smooth muscle cells can also have ET_B receptors which are coupled to membrane-bound guanylyl cyclase which contribute to NO-mediated vasodilation. It should be noted that ET_B receptor possess dual function. ET_B activation in smooth muscle cells can also lead to calcium influx via PLC-mediated pathway. Therefore, ET_B receptor-mediated vasoregulation is tissue-dependent. NOS, nitric oxide synthase; GC, guanylate cyclase; sGC, soluble GC; G, G-protein; IP_3, inositol triphosphate; PKC, protein kinase C.



Rubanyi and Polokoff 1994; Simonson et al. 1992). These mitogenic signals could possibly be transduced by activation of PKC, phosphatidylinositol 3-kinase (PI_3 kinase), and extracellular-signal-regulated kinases 1/2 (ERK1/2) (Suzuki et al. 1999; Decker and Brock 1998; Foschi et al. 1997; Whelchel et al. 1997; Sugawara et al. 1996). These properties suggest that ETs may be involved in both early and late pathogenic changes in target organs of diabetic complications. Early events in the disease course include blood flow alteration and increased ECM protein synthesis leading to basement membrane thickening. Both of these changes could potentially be brought upon by ET upregulation. With disease progression, diabetes-induced biochemical anomalies lead to vascular cell dysfunction, eliciting a proliferative response of the endothelial cells, which could also be mediated by upregulated ET expression because of their potent mitogenic properties.

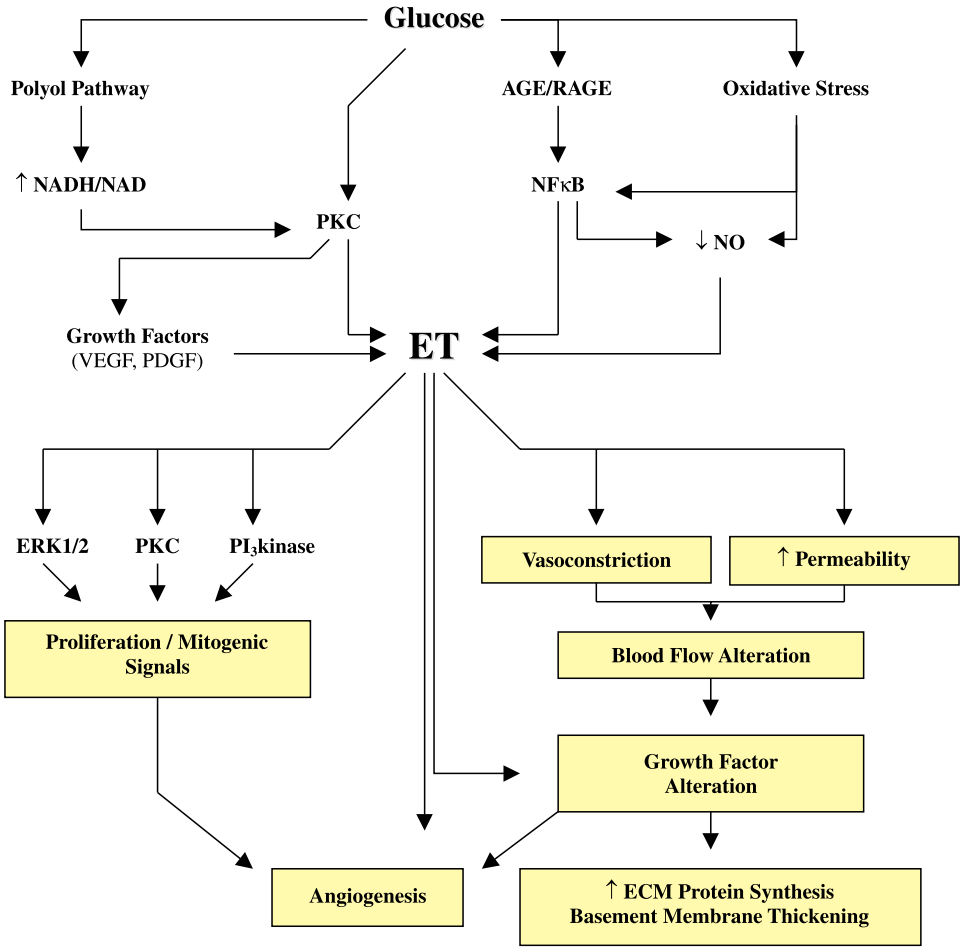
Biochemical mechanisms leading to ET alteration in diabetes

Several interactive factors may lead to alteration of ETs in diabetes. Most of these factors are initiated/activated secondary to hyperglycemia. We will briefly outline the potential mechanisms contributing to ET alteration in diabetes.

Polyol pathway

Augmented polyol pathway activity is an important mechanism in the pathogenesis of chronic diabetic complications. Elevated intracellular glucose is subsequently reduced to sorbitol by aldose reductase (AR). Sorbitol is further oxidized to fructose by sorbitol (polyol) dehydrogenase. Flux through the polyol pathway, secondary to hyperglycemia, increases both sorbitol and fructose (Greene and Stevens 1996; King and Brownlee 1996; Williamson et al. 1993). Accumulated sorbitol may lead to osmotic injury and cell death (Greene and Stevens 1996; Williamson et al. 1993). In addition, increased sorbitol may lead to depletion of *myo*-inositol, which may be of importance in diabetic neuropathy (Greene and Stevens 1996). Activity of AR is coupled to oxidation of NADPH, whereas the second enzymatic step, which converts sorbitol to fructose, requires reduction of NAD^+. The net result of augmented polyol pathway is altered NADPH–$NADP^+$ and NADH–NAD^+ ratios, causing a

Fig. 3. An outline of interactive mechanisms leading to endothelin (ET) activation and respective effects in the pathogenesis of diabetic complications. AGE, advanced glycation end product; RAGE, receptor of AGE; NFκB, nuclear factor-κ B; NAD, nicotinamide adenine dinucleotide; NADH, reduced NAD; VEGF, vascular endothelial growth factor; PDGF, platelet-derived growth factor; ERK1/2, extracellular signal-regulated kinase 1/2; PKB, protein kinase B; PI_3 kinase, phosphatidylinositol 3-kinase; ECM, extracellular matrix.



redox imbalance and a state of "pseudohypoxia" in target organs of diabetic complications (King and Brownlee 1996; Williamson et al. 1993). This change may favor increased free radical generation, inhibition of fatty acid oxidation, increased DAG synthesis, increased formation of reduced glutathione (Kowluru et al. 1997; Kern et al. 1994), increased prostaglandin synthesis, and defective DNA repair. However, controversies exist regarding the development of "pseudohypoxia" in some organs such as the retina (Obrosova et al. 1998; Winkler et al. 1997). In addition to changes mentioned above, augmented polyol pathway can lead to reduced NO production. The enzymatic reaction of NO synthesis requires NADPH, which is depleted by increased flux of glucose through the polyol pathway. One of the inhibitors of ET expression is NO; therefore, decreased NO could positively regulate ET upregulation in diabetes (Chen et al. 2000*a*; Cosentino and Luscher 1998; Levin 1995; Rubanyi and Polokoff 1994; Vanhoutte 1994). Increased DAG synthesis could also upregulate ET expression via PKC activation (Fig. 3).

Oxidative stress

Increased oxidative stress due to free radicals and NO generation have been suggested to play a significant role in the pathogenesis of diabetic complications (Nadler and Winer 1996). Hydroxyl radical produced by glucose autooxidation has been shown to damage proteins (Hunt et al. 1988). Hyperglycemia may also activate various lipoxygenase enzymes, promoting the interaction of NO with superoxide anions. This interaction yields peroxynitrite and hydroxyl radicals, which are toxic to vascular endothelial cells (Nadler and Winer 1996; Jiang et al. 1990). The exact mechanism of glucose-mediated endothelial dysfunction is still obscure. However, evidence suggests an imbalance between ET expression and NO activity (Cosenino and Luscher 2001, 1998; Cosentino et al. 1998; Stehouwer et al. 1997; King and Brownlee 1996; Nadler and Winer 1996). Sequestration of NO by superoxide anions could also contribute to reduced NO activity, leading to upregulation of ETs.

Antioxidant defense activities are also reduced in experimental galactosemic and diabetic animals. GSH levels, Ca^{++}/Mg^{++} ATPase activity, and Na^{+}/K^{+} ATPase activity have been demonstrated to be markedly diminished in both diabetic and galactose-fed animals (Kowluru et al. 1997; Kern et al. 1994; Tagami et al. 1992). Nonenzymatic glycation may also lead to direct oxidative stress (King and Brownlee 1996; Nadler and Winer 1996; Arai et al. 1987). It

has recently been demonstrated that hyperglycemia-induced increased production of superoxides may lead to PKC activation, increased nonenzymatic glycation, sorbitol accumulation, and NFκB activation in endothelial cells (Chen et al. 2001*b*; Nishikawa et al. 2000; Kowluru et al. 1996). All of these biochemical abnormalities can result in ET upregulation (Fig. 3).

PKC Activation

PKC activation has been demonstrated in chronic diabetic complications affecting both micro- and macrovasculature (Koya and King 1998; King and Brownlee 1996). High glucose levels increase DAG synthesis, a potent PKC activator (Koya and King 1998; King and Brownlee 1996). PKC may also be activated via the PI_3 kinase pathway (Le Good et al. 1998). It has been demonstrated that hyperhexosemia induces increased DAG levels and PKC activation in retinas and aortas of both diabetic and galactosemic animals (Le Good et al. 1998; Ishii et al. 1996; Kern et al. 1994; Xia et al. 1994; Shiba et al. 1991, 1993). Treatment of streptozotocin (STZ) induced diabetic rats with PKCβ-selective inhibitor prevents retinal PKC activation and decreased Na^+/K^+ ATPase activity (Ishii et al. 1996; Kowluru et al. 1996). Furthermore, PKCβ overexpressing mice demonstrate lesions in the heart that are reminiscent of diabetic cardiomyopathy (Wakasaki et al. 1997).

PKC influences several important vascular functions, such as regulation of blood flow and permeability (King and Brownlee 1996; Koya and King 1998; Lynch et al. 1990). PKC has been identified as a potential causal factor in producing characteristic retinal blood flow alterations in diabetic animals (Shiba et al. 1993, 1991). In addition, PKC activation, secondary to hyperglycemia, has been shown to regulate endothelial permeability (Chen et al. 2000*a*). PKC activation also regulates several factors that mediate cell survival and growth signals. These factors include ET-1, vascular endothelial growth factor (VEGF), platelet-derived growth factor (PDGF), epidermal growth factor (EGF), insulin-like growth factor (IGF), and fibroblast growth factor (FGF) (Koya and King 1998; Newton 1997; Greene and Stevens 1996; King and Brownlee 1996; Williamson et al. 1993). We and others have shown that inhibition of PKCβ activation can prevent glucose-induced ET-1 mRNA expression in endothelial cells (Chen et al. 2000; Park et al. 2000). Furthermore, PKC-mediated upregulation of above mentioned growth factors could also contribute to ET alteration in diabetes.

Nonenzymatic glycation

Glucose, glucose-6-phosphate, trioses, and fructose may all participate in nonenzymatic glycation of proteins (Vlassara 1997; King and Brownlee 1996; Takagi et al. 1995). Advanced glycation end products (AGEs) may also be produced from strong glycating dicarbonyl compounds such as 3-dioxyglucosone, methylglyoxal, and glyoxals (Glomb and Monnier 1995). AGEs accumulate in tissues over time during normal aging. However, diabetes leads to acceleration of this process (Stitt et al. 1997; Vlassara 1997; Blakesley and Lerother 1996; King and Brownlee 1996; Glomb and Monnier 1995; Takagi et al. 1995).

The mechanisms by which AGEs may cause pathologic changes include alteration of protein function, interference with extracellular matrix function, and elaboration of cytokines and free radicals (Stitt et al. 1997; Vlassara 1997; King and Brownlee 1996). Auto-oxidation of glucose and production of AGEs can generate reactive oxygen species (Stitt et al. 1997; Vlassara 1997; King and Brownlee 1996). AGE and AGE receptor interaction may also lead to oxidative stress and activation of NFκB (Mohamed et al. 1999). In vascular endothelial cells, AGE formation may affect gene expression of thrombomodulin and ET-1 and modify growth factors such as VEGF and basic fibroblast growth factor (bFGF) (Yamagishi et al. 1997; King and Brownlee 1996; Esposito et al. 1992). Recently, the mechanism of AGE-induced ET-1 expression has been illuminated to some extent. It has been shown that AGE formation in endothelial cells leads to upregulation of ET-1 via NFκB activation (Quehenberger et al. 2000). Furthermore, AGE formation has been causally related to defective vasodilator response to NO (Bucala et al. 1991). Reduced NO, possibly through NFκB activation, may further upregulate ET-1 expression.

Hyperinsulinemia

Hyperinsulinemia, a concurrent state in type II diabetes, may be another factor leading to ET alteration. Insulin upregulates ET expression in vascular endothelial and smooth muscle cells (Ferri et al. 1995; Anfossi et al. 1993; Oliver et al. 1991). Insulin has also been shown to increase plasma ET levels in both humans and animals (Hopfner et al. 1998*a*; Piatti et al. 1996; Pontiroli and Pozza 1996; Ferri et al. 1995; Frank et al. 1993). In addition to increasing ET peptide levels, insulin can regulate ET responsiveness by modulating ET receptor expression (Hopfner et al. 1998*b*; Juan et al. 1998; Frank et al. 1993). These findings suggest that hyperinsulinemia may amplify ET alteration and vasculopathy in diabetes. Indeed, hyperinsulinemia has been shown to accelerate macroangiopathy in diabetics (Hedblad et al. 2000).

Other factors

Several other mechanisms may also be of importance in augmented ET-1 expression in diabetes. ET-1 interacts with potent vasoactive factors such NO and VEGF (Matsuura et al. 1997; Pedram et al. 1997; Vanhoutte 1994). VEGF increases in diabetes, and in turn, may increase ET-1 expression (Chen et al. 2000*a*). We have demonstrated such an interaction in endothelial cells, as well as in diabetic animals (Cukiernik et al. 2000). ET-1 and ET-3 mediated VEGF production involves activation of PKC (Chen et al. 2000*a*; Pedram et al. 1997). We have shown that incubation of endothelial cells with high glucose levels, VEGF, or NOS inhibitor leads to ET-1 upregulation. Such glucose-induced ET-1 increases were prevented by VEGF-neutralizing antibodies and PKC inhibition (Chen et al. 2000*a*). We have recently demonstrated similar findings in retinas of diabetic animals (Cukiernik et al. 2000). These findings suggest multiple signaling pathways leading to alteration of ET-1 in chronic diabetes.

Pathophysiological role of ETs in chronic diabetic complications

Diabetes produces a variety of structural and functional lesions in several target organs, including retina, peripheral

nerves, kidneys, and the cardiovascular system. Although there are similarities in the pathogenesis of such lesions, variations due to tissue microenvironment are of great importance. In the following sections, we will discuss the role of ETs in specific complications.

Diabetic retinopathy

Diabetes is the most important systemic disease causing blindness (Mazze et al. 1985; Caird 1971). Diabetic retinopathy is classified in various progressive stages, namely nonproliferative (background) retinopathy, preproliferative (severe or advanced background) retinopathy, and proliferative retinopathy. Background retinopathy is characterized by basement membrane thickening, pericyte loss, endothelial loss, retinal hemorrhages, and exudate deposits (King and Brownlee 1996; Mizutani et al. 1996; Porta 1996, Blom et al. 1994; Kern and Engerman 1994; Kuwabara and Cogan 1963). Preproliferative retinopathy, on the other hand, is characterized by venous dilatation, beading, profuse retinal hemorrhages and exudates, widespread capillary nonperfusion, and intraretinal microvascular abnormalities. Patients with such lesions are prone to develop proliferative retinopathy. Neovascularization is the characteristic feature of the proliferative stage. Formation of new blood vessels may lead to hemorrhage and tractional retinal detachment (Blom et al. 1994; Mazze et al. 1985; Caird 1971).

ET-1 is present in several areas of the ocular tissue, including the optic nerve, retinal vascular and extravascular sites, and the uveal tract (Chakrabarti et al. 1998; Chakrabarti and Sima 1997; Chakravarthy et al. 1997). We and others have demonstrated increased ET-1 and ET-3 immunoreactivities in the retinas of STZ-induced diabetic rats and spontaneously diabetic BB/W rats (Chakrabarti et al. 1998; Chakrabarti and Sima 1997; Chakravarthy et al. 1997). Blockade of the ET_A-receptor signaling by specific antagonist, BQ 123, has been shown to increase retinal blood flow in STZ-diabetic rats (Takagi et al. 1996). Furthermore, blocking activity of ECE1 by phosphoramidon has been shown to normalize diabetes-induced reduced retinal blood flow (Takagi et al. 1996). Using laser Doppler sonography, we have recently demonstrated increased resistivity index (RI), a marker for vasoconstriction, in the retinal bed of both STZ-diabetic and galactose-fed rats after 1 month of follow-up. These changes were prevented by a dual ET_A–ET_B receptor antagonist, bosentan (Evans et al. 2000*a*, 2000*b*; Deng et al. 1999). Simultaneously, ET-1, ET-3, and ET_A mRNA levels were shown to be increased in hyperhexosemic rats. After 6 months of follow-up, ET_B receptor mRNA levels were also elevated in the retina of hyperhexosemic animals with no significant difference in RI values as compared with nondiabetic controls (Evans et al. 2000*a*, 2000*b*; Deng et al. 1999). These observations may be indicative of a duration-dependent, differential activation of various components of the ET-system, which may account for temporal alteration in retinal blood flow in diabetes.

Recently, we have demonstrated that hyperhexosemia-induced increased collagen a1(IV) and fibronectin mRNA levels can be prevented by bosentan treatment in both diabetic and galactose-fed rats (Evans et al. 2000*a*, 2000*b*). Bosentan also prevented retinal capillary basement membrane (BM) thickening in these animals. This suggests that ETs could play significant role early in the disease course leading to characteristic changes such as basement membrane thickening.

Alteration of ETs has been demonstrated in both type I and type II diabetes in humans (Ak et al. 2001; Guvener et al. 1997; Malamitsi-Puchner et al. 1996; Morise et al. 1995, Donatelli et al. 1994; Smulders et al. 1994; Collier et al. 1992; Haak et al. 1992; Kanno et al. 1991; Takahashi et al. 1990). Although increased plasma ET-1 levels have been demonstrated in diabetic patients with retinopathy (Kawamura et al. 1992), a number of studies provide contradictory reports of plasma ET levels in diabetic patients. Autocrine and paracrine activity of these peptides should be considered when interpreting these results. In a recent study, vitreous ET-1 levels were found to be significantly elevated in patients with proliferative diabetic retinopathy as compared with controls (Oku et al. 2001). This supports the notion that ETs may also be involved in later stages of diabetic retinopathy. Indeed, these peptides are angiogenic for vascular endothelial cells and may regulate endothelial proliferation during retinal neovascularization.

Diabetic nephropathy

Diabetes is the leading cause of renal failure in the western world, affecting approximately 30% type I diabetic patients (Held et al. 1990; Andersen et al. 1983). Microalbuminuria is the earliest clinical marker of renal affection in diabetes and is associated with glomerular hyperfiltration (Breyer et al. 1996). As renal damage progresses, patients develop heavy proteinuria and reduced glomerular filtration rate, which ultimately leads to renal failure.

Pathological features of diabetic nephropathy include thickening of glomerular capillary BM, mesangial matrix expansion, and tubulointerstitial fibrosis. Early in the disease course, the pathogenic changes are exhibited as renal enlargement due to cellular hypertrophy affecting both the glomeruli and tubules. With progression, patients develop arteriolosclerosis, continued mesangial matrix expansion, and glomerulosclerosis. In the later stages, patients develop characteristic Kimmelstiel–Wilson nodules (Cotran et al. 1999; Epstein 1998; Steffes et al. 1996; Mogensen et al. 1983). It has been demonstrated that, similar to other chronic complications, high blood glucose level is a key factor leading to the development of diabetic nephropathy (U.K. Prospective Diabetes Study (UKPDS) Group 1996; Diabetes Control and Complications Trial Research Group 1993). Systemic hypertension, which causes intraglomerular hypertension and hyperfiltration, is another risk factor in the progression of nephropathy (Epstein 1998; Mogensen et al. 1983).

In the kidneys, both ET-1 and ET-3 show widespread tissue distribution (Simonson 1993). From a physiological perspective, ETs regulate renal blood flow, glomerular filtration rate, and sodium and water reabsorption. ETs are expressed in the endothelium, epithelium, glomerular mesangium, tubular epithelium, collecting ducts, and vasa recta. ET-binding sites have also been localized in these cells. In addition, the ET system has been demonstrated to be altered in several disease processes affecting the kidneys (Rubanyi and Polokoff 1994; Simonson 1993).

In the kidneys of diabetic rats, increased ET-1 mRNA and renal ET-1 clearance has been demonstrated in association

with proteinuria (Turner et al. 1997). Upregulation of ET_A receptors has also been shown in the kidneys of diabetic rabbits (Khan et al. 1999). The long-term consequences of ET-peptides may involve cellular changes requiring differential gene expression (Levin 1995; Nakamura et al. 1995; Rubanyi and Polokoff 1994; Simonson et al. 1992). It has been demonstrated that diabetes-induced increased expression of glomerular collagen a1(I), a1(III), a1(IV), laminin B1 and B2, tumor necrosis factor-α, PDGF, transforming growth factor-β, and bFGF can be completely prevented by treatment with an ET_A receptor antagonist (Nakamura et al. 1995). In addition, we have recently shown that both diabetes and galactosemia lead to upregulation of ETs and ET-dependent ECM protein synthesis in the kidneys (Chen et al. 2001*a*). The dual ET_A–ET_B receptor antagonist bosentan prevents such changes, as well as diabetes-induced increased glomerular BM thickening and mesangial expansion (Chen et al. 2001*a*). Whether ETs regulate increased ECM protein synthesis directly or by induction of other fibrogenic factors still remains to be determined. However, current evidence does suggest that these peptides are involved in the progression of diabetic nephropathy.

Diabetic neuropathy

Diabetic neuropathy affects approximately 60–70% of all diabetics. At its extreme form, it is a major cause of lower extremity amputation (Boulton 1993; Thomas 1992). Both somatic and autonomic nerves can be involved in diabetic neuropathy, producing a variety of symptoms. Diabetic neuropathy, which may affect peripheral or cranial nerves, can broadly be classified as mononeuropathy and polyneuropathy. Mononeuropathies can involve isolated single nerves or may affect multiple nerves (mononeuritis multiplex) (Boulton 1993; Thomas 1992). On the other hand, polyneuropathies can affect sensory, motor, or autonomic nervous systems. Chronic sensorimotor polyneuropathy is the commonest type of neuropathy. It is manifested as impaired balance, propioception and vibration, progressive gloves and stocking anaesthesia, paresthesia, or hyperasthesia. Although motor weakness is not pronounced, wasting of small muscles and loss of reflex activity is also manifested. Foot ulceration and other neuropathic changes may subsequently develop. Impaired nerve conduction velocity is a key feature of this disease. Autonomic neuropathy may produce bladder, bowel, or gastric motility problems and postural hypotension (Boulton 1993; Thomas 1992).

Impaired phosphoinositide metabolism in the peripheral nerve may lead to impaired activity of PKC and Na^+/K^+ ATPase (Sima and Sugimoto 1999). This is in sharp contrast to the retinal findings, where activation of PKC has been established (Koya and King 1998; King and Brownlee 1996). Contrary to retinal findings, peripheral nerves from diabetic animals show reduced DAG levels (Sima and Sugimoto 1999; Zhu and Eichberg 1990). However, PKC inhibitors have been shown to prevent diabetes-induced reduced neuronal Na^+/K^+ ATPase activity (Hermenegildo et al. 1993). These data demonstrate that hyperglycemia-induced biochemical changes may vary in target organs of diabetic complications depending on the tissue microenvironment.

Treatment of STZ-diabetic rats with specific ET_A receptor antagonist and dual ET_A–ET_B receptor antagonist has been shown to prevent diabetes-induced nerve conduction velocity deficit and reduced endoneural blood flow (Stevens and Tomlinson 1995; Cameron et al. 1994). It is interesting to note that only the former treatment lead to a reduction in systemic blood pressure in these studies (Cameron et al. 1994). We have demonstrated that ET-1 and ET-3 immunoreactivity is increased in the peripheral nerves in diabetes (Deng et al. 1998). However, no data are yet available as to whether ET receptor antagonism is beneficial in preventing later changes in diabetic neuropathy such as nerve fibre loss.

Macrovasculopathy and cardiovascular complications

Diabetic heart disease comprises three major components: coronary artery disease, autonomic neuropathy, and diabetic cardiomyopathy. In addition, both atherosclerosis and hypertension occur at a much higher rate in the diabetic population as compared with nondiabetic counterparts (Epstein and Sowers 1992).

Cardiac complications are a major cause of morbidity and mortality in the diabetic population. Diabetic individuals are two to four times more likely to develop heart disease. In addition, 75% of diabetes-related deaths are due to heart disease (International diabetes federation triennial report 1994). Diabetic patients also develop congestive cardiac failure more readily and have significantly worse prognosis than their nondiabetic counterparts once they develop coronary disease (Lewinter 1996; Savage et al. 1988). Functional abnormalities affect both systolic and diastolic properties of the myocardium. These abnormalities include impaired relaxation, reduced compliance with elevated end-diastolic pressure, cardiac hypertrophy, and chamber dilatation (Lewinter 1996; Shehadeh and Regan 1995; Savage et al. 1988). Pathological findings include cardiomegaly and myocardial scaring. Myocardial hypertrophy, interstitial and perivascular fibrosis, myocyte necrosis, as well as thickening of the capillary BM are some other abnormal structural findings in the hearts of diabetics (Lewinter 1996; Shehadeh and Regan 1995; Savage et al. 1988).

ET-1 is produced by both myocytes and endothelial cells in the myocardium. High affinity ET-1 binding sites are also present in cardiac myocytes (Fukuchi and Giaid 1998). ET-1 has been shown to evoke positive inotropic and chronotropic effects and prolong action potentials (Hu et al. 1988; Ishikawa et al. 1988). Hypoxia and ischemia are two important upregulators of ET-1 expression in the heart (Karmazyn 1996). Several investigators have shown upregulated ET-1 mRNA and receptor binding in rat hearts as early as few weeks following induction of diabetes (Lin et al. 1996; Vesci et al. 1995). Furthermore, a duration-dependent alteration of chronotropic and inotropic responses to ET-1 has been demonstrated in isolated atria of diabetic rats (Lieu and Reid 1994). In keeping with these studies, we have demonstrated that increased expression of ET-1, ET_A, and ET_B mRNA levels parallel diabetes-induced myocardial cell death and focal scarring of the myocardium in diabetic rats (Chen et al. 2000*b*). In addition, we have demonstrated increased ET immunoreactivity and ET receptor density in association with the aforementioned changes. It is interesting to note that a similar increase in mRNA expression of two ECM proteins, collagen a1(IV) and fibronectin, was detectable. The increased ECM protein mRNA levels, myocardial scar-

ring, and myocardial apoptosis were completely prevented by treatment of diabetic animals with bosentan. This study provided direct evidence as to the importance of the ET system in the pathogenesis of diabetic heart disease (Chen et al. 2000*b*). Recently, we have further demonstrated that ET-1 may participate via sodium–hydrogen exchanger-1 in mediating diabetes-induced structural and functional changes (Hileeto et al. 2001). Sodium–hydrogen exchanger-1 activation may lead to calcium overload and subsequent cell death (Karmazyn et al. 1999).

As mentioned earlier, ETs also carry out mitogenic signals. Smooth muscle cells express both ET_A and ET_B receptors and proliferate in response to elevated ET levels. This could potentially play a role in the development and progression of atherosclerosis. Increased plasma ET-1 levels have been demonstrated in diabetic patients with atherosclerosis and hypertension (Perfetto et al. 1997; Fernandez et al. 1995). In addition, elevated levels of prepro-ET-1 have been demonstrated in aortas of diabetic rats (Makino et al. 2001). Interestingly, angiotensin-II-mediated vascular hypertrophy can be prevented by ET-receptor antagonism in a non-diabetic model (Makino et al. 1998). Taken together, these data suggest that ETs play important roles in macrovasculopathy by modulating structural and functional properties of vascular smooth muscle cells. It should be noted that insulin resistance, if present, may also accelerate atherosclerosis (Hedblad et al. 2000). As mentioned earlier, insulin can upregulate both ET peptides and ET receptors in vascular endothelial and smooth muscle cells. This suggests that hyperinsulinemia in type II diabetics may potentiate ET upregulation and may contribute to the progression of macroangiopathy.

Concluding remarks

Experimental evidence suggests that ETs, because of their multiple functional capabilities, may play significant roles as effector molecules in chronic diabetic complications. PKC activation, nonenzymatic glycation, oxidative stress, and augmented polyol pathway may contribute to alteration of ETs. ETs, in turn, may regulate other vasoactive factors and growth factors such as nitric oxide, VEGF, and PDGF. Evidences gathered from multiple animal experiments, indeed, indicate that ETs are of importance in the pathogenesis of chronic diabetic complications. Inhibition of ET-mediated signaling has been shown to prevent diabetes-induced structural and functional alterations in target organs of diabetic complications. This suggests a central role of these multifunctional peptides in diabetes-induced pathogenic changes in various organs. Based on the available data, ET antagonism may have a potential role in the treatment of these complications. However, longer term animal studies and well designed clinical trials are necessary to validate such notions.

Acknowledgments

The authors acknowledge grant supports from the Canadian Diabetes Association, in honor of Margaret Francis; the Canadian Institutes of Health Research; and the Lawson Health Research Institute.

References

Ak, G., Buyukberber, S., Sevinc, A., Turk, H. M., Ates, M., Sari, R., Savli, H., and Cigli, A. 2001. The relation between plasma endothelin-1 levels and metabolic control, risk factors, treatment modalities, and diabetic microangiopathy in patients with type 2 diabetes mellitus. J. Diabetes Complications, **15**: 150–157.

Andersen, A.R., Christiansen, J.S., Anderson, J.K., Kriener, S., and Dekert, T. 1983. Diabetic neuropathy in type I (insulin-dependent) diabetes: an epidemiological study. Diabetologia, **25**: 496–501.

Anfossi, G., Cavalot, F., Massucco, P., Mattiello, L., Mularoni, E., Hahn, A., and Trovati, M. 1993. Insulin influences immunoreactive endothelin release by human vascular smooth muscle cells. Metabolism, **42**: 1081–1083.

Arai, K., Maguchi, S., Fugii, S., Ishibashi, H., Oikawa, K., and Taniguchi, N. 1987. Glycation and inactivation of human Cu-Zn-superoxide dismutase. Identification of the in vitro glycated site. J. Biol. Chem. **262**: 16 969 – 16 972.

Arai, H., Hori, S., Aramori, I., Ohkubo, H., and Nakanishi, S. 1990. Cloning and expression of a cDNA encoding an endothelin receptor. Nature (London), **348**: 730–732.

Aramori, I., and Nakanishi, S. 1992. Coupling of two endothelin receptor subtypes to differing signal transduction in transfected Chinese hamster ovary cells. J. Biol. Chem. **267**: 12468–12474.

Benatti, L., Fabbrini, M.S., and Patrono, C. 1994. Regulation of ET-1 biosynthesis. Ann. N.Y. Acad. Sci. **714**: 109–121.

Benigni, A., Perico, N., and Remuzzi, G. 2000. Endothelin antagonists and renal protection. J. Cardiovasc. Pharmacol. **35**: S75–S78.

Blakesley, V.A., and Lerother, D. 1996. The role of growth factors in the pathogenesis of diabetic vascular complications. *In* Diabetes mellitus. *Edited by* D. LeRoith, S.I. Taylor, and M. Olefsky. Lippincott–Raven, Philadelphia. pp. 824–831.

Blom, M.L., Green, W.R., and Schalbat, A.P. 1994. Diabetic retinopathy: a review. Del. Med. J. **66**: 379–388.

Boulton, A.J.M. 1993. Pathogenesis of diabetic neuropathy. *In* The diabetes annual 11. *Edited by* S.M. Marshall, P.D. Home, K.G.M.M. Alberti, and L.P. Krall. Elsevier Science Publishers BV. Amersterdam, The Netherlands. pp. 192.

Breyer, J.A., Bain, R.P., Evans, J.K., Nahman, N.S. Jr., Lewis, E.J., Cooper, M., McGill, J., and Berl, T. 1996. Predictors of the progression of renal insufficiency in patients with insulin-dependent diabetes and overt diabetic nephropathy. Kidney Int. **50**: 1651–1658.

Bucala, R., Tracey, K.J., and Cerami, A. 1991. Advanced glycosylation products quench nitric oxide and mediate defective endothelium-dependent vasodilation in experimental diabetes. Diabetologia, **87**: 432.

Caird, F.I. 1971. The epidemiology of diabetic microangiopathy. Acta Diabetol. Lat. **8**: S240–S248.

Cameron, N.E., Dines, K.C., and Cotter, M.A. 1994. The potential contribution of endothelin-1 to neurovascular abnormalities in streptozotocin diabetic rats. Diabetologia, **37**: 1209–1215.

Chakrabarti, S., and Sima, A.A.F. 1997. Endothelin-1 and endothelin-3 like immunoreactivity in the eyes of diabetic and non-diabetic BB/W rats. Diab. Res. Clin. Prac. **37**: 109–120.

Chakrabarti, S., Gan, X.T., Merry, A., Karmazyn, M., and Sima, A.A.F. 1998. Augmented retinal endothelin-1, endothelin-3, $endothelin_A$ and $endothelin_B$ gene expression in chronic diabetes. Curr. Eye Res. **17**: 301–307.

Chakrabarti, S., Cukiernik, M., Hileeto, D., Evans, T., and Chen, S. 2000. Role of vasoactive factors in the pathogenesis of early

changes in diabetic retinopathy. Diabetes Metab. Rev. **16**: 393–407.

Chakravarthy, U., Douglas A.J., Bailie, R., McKibben, B., and Archer, D.B. 1997. Immunoreactive endothelin distribution in ocular tissue. Invest. Ophthalmol. Vis. Sci. **35**: 2448–2454.

Chen, S., Apostolova, M., Cherian, G., and Charkrabarti, S. 2000*a*. Interaction of endothelin-1 with vasoactive factors in mediating glucose-induced increased permeability in endothelial cells. Lab. Invest. **80**: 1311–1321.

Chen, S., Evans, T., Mukherjee, K., Karmazyn, M., and Chakrabarti, S. 2000*b*. Diabetes-induced myocardial structural changes: role of endothelin-1 and its receptors. J. Mol. Cell Cardiol. **32**: 1621–1629.

Chen, S., Evans, T., Deng, D., Cukiernik, M., and Chakrabarti, S. 2001*a*. Hyperhexosemia induced functional and structural changes in the kidneys: role of endothelins. Nephron, **90**: 86–94.

Chen, S., Mukherjee, S., and Chakrabarti, S. 2001*b*. Hyperglycemia induced, endothelin (ET) mediated increased extracellular matrix (ECM) protein synthesis is mediated via NF κB activation. Diabetes, **50**: A191.

Collier, A., Leach, J. P., McLellan, A., Jardine, A., Morton, J.J., and Small, M. 1992. Plasma endothelin-like immunoreactivity levels in IDDM patients with microalbuminuria. Diabetes Care, **15**: 1038–1040.

Cotran, R.S., Kumar, V., and Collin, S. 1999. The kidney. *In* Robbins pathologic basis of disease. 6th ed. *Edited by* R.S. Cotran, V. Kumar, T. WB Sanders, Toronto. pp. 966–968.

Cosentino, F., and Luscher, T.F. 1998. Endothelial dysfunction in diabetes mellitus. J. Cardiovasc. Pharmacol. **32**: S54–S61.

Cosentino, F., and Luscher, T.F. 2001. Effects of blood pressure and glucose on endothelial function. Curr. Hypertens. Rep. **3**: 79–88.

Cosentino, F., Hishikawa, K., Katusic, Z.S., and Luscher, T.F. 1997. High glucose increases nitric oxide synthase expression and superoxide anion generation in human aortic endothelial cells. Circulation, **96**: 25–28.

Cukiernik, M., Evans, T., Hileeto, D., Downey, D., and Chakrabarti, S. 2000. Interaction of endothelins and nitric oxide synthase in the retina of streptozotocin induced diabetic rats in mediating vasoconstriction. Can. J. Diabetes Care. **24**: 73.

Davenport, A.P., O'Reilly, G., Molenaar, P., Maguire, J.J., Kuc, R.E., Sharkey, A., Bacon, C.R., and Ferro, A. 1993. Human endothelin receptors characterized using reverse transcriptase-polymerase chain reaction, in situ hybridization, and subtype-selective ligands BQ123 and BQ3020: evidence for expression of ET_B receptors in human vascular smooth muscle. J. Cardiovasc. Pharmacol. **22**: S22–S25.

Decker, E.R., and Brock, T.A. 1998. Endothelin receptor-signalling mechanisms in vascular smooth muscle. *In* Endothelin molecular biology, physiology and pathology. *Edited by* R.F. Highsmith. Humana Press. Totowa, N.J. pp. 93–120.

Deng, D., Evans, T., Xu, G., and Sima, A.A.F. 1998. Endothelin-1 and its receptors in the pathogenesis of neuronal and retinal functional abnormalities in the diabetic rats. Diabetes, **47**: 559A.

Deng, D.X., Evans, T., Mukherjee, K., Downey, D., and Chakrabarti, S. 1999. Diabetes induced dysfunction in the retina: role of endothelins. Diabetologia, **42**: 1228–1234.

Diabetes Control and Complications Trial Research Group. 1993. The effect of intensive treatment of diabetes on the development of long-term complications in insulin-dependent diabetes mellitus. N. Engl. J. Med. **329**: 977–986.

Ding, K.H., Latimer, A.J., and Abdel-Latif, A.A. 1999. Activation of particulate guanylyl cyclase by endothelins in cultured SV-40 transformed cat iris sphincter smooth muscle cells. Life Sci. **64**: 161–174.

Donatelli, M., Colletti, I., Bucalo, M. L., Russo, V., and Verga, S. 1994. Plasma endothelin levels in NIDDM patients with macroangiopathy. Diabetes Res. **25**: 159–164.

Emori, T., Hirata, Y., Imai, T., Ohta, K., Kanno, K., Eguchi, S., and Marumo, F. 1992. Cellular mechanism of thrombin on endothelin-1 biosynthesis and release in bovine endothelial cell. Biochem. Pharmacol. **44**: 2409–2411.

Epstein, F.H. 1998. Pathophysiology of progressive nephropathies. N. Engl. J. Med. **339**: 1448–1456.

Epstein, M., and Sowers, J.R. 1992. Diabetes mellitus and hypertension. Hypertension, **19**: 403–418.

Esposito, C., Gerlach, H., and Brett, J. 1992. Endothelial receptor mediated binding of glucose modified albumin is associated with increased monolayer permeability and modulation of cell surface coagulant properties. J. Exp. Med. **170**: 1387–1397.

Evans, T., Deng, D.X., Chen, S., and Chakrabarti, S. 2000*a*. Endothelin receptor blockade prevents augmented extracellular matrix component mRNA expression and capillary basement membrane thickening in the retina of diabetic and galactose fed rats. Diabetes, **49**: 662–666.

Evans, T., Deng, D.X., Mukherjee, K., Downey, D., and Chakrabarti, S. 2000*b*. Endothelins, their receptors, and retinal vascular dysfunction in galactose-fed rats. Diabetes Res. Clin. Pract. **48**: 75–85.

Fernandez, A.C., Patino, R., Ibarra, J., Fernandez, L., Roldan, M., Moya, J., and Fernandez, D.R. 1995. Endothelin in hypertension and diabetes mellitus. *In* Endothelin in cardiovascular diseases. *Edited by* T.F. Luscher. Springer, New York. pp. 157–170.

Ferri, C., Pittoni, V., Piccoli, A., Laurenti, O., Cassone, M.R., Bellini, C., Properzi, G., Valesini, G., De Mattia, G., and Santucci, A. 1995. Insulin stimulates endothelin-1 secretion from human endothelial cells and modulates its circulating levels in vivo. J. Clin. Endocrinol. Metab. **80**: 829–835.

Foschi, M., Chari, S., Dunn, M.J., and Sorokin, A. 1997. Biphasic activation of p21ras by endothelin-1 sequentially activates the ERK cascade and phosphatidylinositol 3-kinase. Eur. Mol. Biol. Organ. J. **16**: 6439–6451.

Frank, H.J., Levin, E.R., Hu, R.M., and Pedram, A. 1993. Insulin stimulates endothelin binding and action on cultured vascular smooth muscle cells. Endocrinology, **33**: 1092–1097.

Fukuchi, M., and Giaid, A. 1998. Expression of endothelin-1 and endothelin-converting enzyme-1 mRNAs and proteins in failing human hearts. J. Cardiovasc. Pharmacol. **31**: S421–S423.

Glomb, M.A., and Monnier, V.M. 1995. Mechanism of protein modification by glyoxal and glycolaldehyde, reactive intermediates of the Maillard reaction. J. Biol. Chem. **270**: 10 017 – 10 026.

Greene, D.A., and Stevens, M.J. 1996. The sorbitol-osmotic and sorbitol-redox hypotheses. *In* Diabetes mellitus. Lippincott Raven Publishers, Philadelphia. pp. 801–809.

Guvener, N., Aytemir, K., Aksoyek, S., and Gedik, O. 1997. Plasma endothelin-1 levels in non-insulin dependent diabetes mellitus patients with macrovascular disease. Coron. Artery. Dis. **8**: 253–258.

Haak, T., Jungmann, E., Felber, A., Hillmann, U., and Usadel, K.H. 1992. Increased plasma levels of endothelin in diabetic patients with hypertension. Am. J. Hypertens. **5**: 161–166.

Hedblad, B., Nilsson, P., Janzon, L., and Berglund, G. 2000. Relation between insulin resistance and carotid intima-media thickness and stenosis in non-diabetic subjects. Results from a cross-sectional study in Malmo, Sweden. Diabet. Med. **17**: 299–307.

Held, P.J., Port, F.K., Blagg, C.R., and Agoda, L.Y.C. 1990. United Sates Renal Data System 1990 annual report. Am. J. Kidney Dis. Suppl. **2**: 1–106.

Hermenegildo, C., Felipo, V., Minana, M.D., Romero, F.J., and Grisolia, S. 1993. Sustained recovery of Na^+/K^+-ATPase activity in sciatic nerve of diabetic mice by administration of H7 or callphostin C, inhibitors of protein kinase C. Diabetes, **42**: 257.

Hileeto, D., Cukiernik, M., Evans, T., Chen, S., Mukherjee, S., Downey, D., Karmazyn, M., and Chakrabarti, S. 2001. Interaction of endothelin-1 with sodium/hydrogen exchanger-1 in the pathogenesis of diabetes induced in the heart. Diabetes, **50**: A155.

Hopfner, R.L., and Gopalakrishnan, V. 1999. Endothelin: emerging role in diabetic vascular complications. Diabetologia, **42**: 1383–1394.

Hopfner, R.L., Hasnadka, R.V., Wilson, T.W., McNeill, J.R., and Gopalakrishnan, V. 1998*a*. Insulin increases endothelin-1-evoked intracellular free calcium responses by increased ET_A receptor expression in rat aortic smooth muscle cells. Diabetes, **47**: 937–944.

Hopfner, R.L., Misurski, D., Wilson, T.W., McNeill, J.R., and Gopalakrishnan, V. 1998*b*. Insulin and vanadate restore decreased plasma endothelin concentrations and exaggerated vascular responses to normal in the streptozotocin diabetic rat. Diabetologia, **41**: 1233–1240.

Hori, S., Komatsu, Y., Shigemoto, R., Mizuno, N., and Nakanishi, S. 1992. Distinct tissue distribution and cellular localization of two messenger ribonucleic acids encoding different subtypes of rat endothelin receptors. Endocrinology, **130**: 1885–1895.

Hu, J.R., Von Harsdorf, R., and Lang, R.E. 1988. Endothelin has potent inotropic effects in rat atria. Eur. J. Pharmacol. **158**: 275–278.

Hu, R.M., Levin, E.R., and Frank, H.J.L. 1993. Insulin stimulates production and secretion of endothelin from bovine endothelial cells. Diabetes, **42**: 351–358.

Hunt, J.V., Dean, R.T., and Wolff, S.P. 1988. Hydroxyl radical production and autoxidative glycosylation. Glucose autoxidation as the cause of protein damage in the experimental glycation model of diabetes mellitus and ageing. Biochem. J. **256**: 205–212.

Inoue, A., Yanagisawa, M., Kimura, S., Kasuya, Y., Miyauchi, T., Goto, K., and Masaki, T. 1989. The human endothelin family: three structurally and pharmacologically distinct isopeptides predicted by three separate genes. Proc. Natl. Acad. Sci. U.S.A. **86**: 2863–2867.

International Diabetes Federation Triennial Report. 1991–1994. IDF Brussels.

Ishii, H., Jirousek, M.R., Koya, D., Takagi, C., Xia, P., Clermont, A., Bursell, S.E., Kern, T.S., Ballas, L.M., Heath, W.F., Stramm, L.E., Feener, E.P., and King, G.L. 1996. Amelioration of vascular dysfunctions in diabetic rats by an oral PKC beta inhibitor. Science (Washington, D.C.), **272**: 728–731.

Ishikawa, T., Yanagisawa, M., Kimura, S., Goto, K., and Masaki, T. 1988. Positive chronotropic effects of endothelin, a novel endothelium-derived vasoconstrictor peptide. Pflugers Arch. **413**: 108–110.

Jiang, Z.Y., Woollard, A.C., and Wolff, S.P. 1990. Hydrogen peroxide production during experimental protein glycation. Fed. Eur. Biochem. Soc. Lett. **268**: 69–71.

Juan, C.C., Fang, V.S., Hsu, Y.P., Huang, Y.J., Hsia, D.B., Yu, P.C., Kwok, C.F., and Ho, L.T. 1998. Overexpression of vascular endothelin-1 and endothelin-A receptors in a fructose-induced hypertensive rat model. J. Hypertens. **16**: 1775–1782.

Kanno, K., Hirata, Y., Shichiri, M., and Marumo, F. 1991. Plasma endothelin-1 levels in patients with diabetes mellitus with or without vascular complication. J. Cardiovasc. Pharmacol. **17**: S475-S476.

Karamazyn, M. 1996. The role of endothelins in cardiac function in health and disease. *In* Myocardial ischemia: mechanisms, reperfusion, protection. *Edited by* M. Karmazyn. Birkhauser, Basel, Switzerland. pp. 209–230.

Karmazyn, M., Gan, X.T., Humphreys, R.A., Yoshida, H., and Kusumoto, K. 1999. The myocardial Na^+–H^+ exchanger: structure, regulation and its role in heart disease. Cir. Res. **85**: 777–786.

Kawamura, M., Ohgawara, H., Naruse, M., Suzuki, N., Iwasaki, N., Naruse, K., Hori, S., Demura, H., Omori, Y. 1992. Increased plasma endothelin in NIDDM patients with retinopathy. Diabetes Care, **15**: 1396–1397.

Kern, T.S., and Engerman, R.L. 1994. Comparison of retinal lesions in alloxan-diabetic rats and galactose-fed rats. Curr. Eye Res. **13**: 863–867.

Kern, T.S., Kowluru, R.A., and Engerman, R.L. 1994. Abnormalities of retinal metabolism in diabetes or galactosemia: ATPases and glutathione. Invest. Ophthalmol. Vis. Sci. **35**: 2962–2967.

Khan, M.A., Dashwood, M.R., Thompson, C.S., Mumtaz, F.H., Mikhailidis, D.P., and Morgan, R.J. 1999. Time-dependent upregulation of endothelin-A receptors and down-regulation of endothelin-B receptors and nitric oxide synthase binding sites in the renal medulla of a rabbit model of partial bladder outlet obstruction: potential clinical relevance. BJU Int. **84**: 1073–1080.

King, G.L., and Brownlee, M. 1996. The cellular and molecular mechanisms of diabetic complications. Endocrinol. Metab. Clin. North Am. **25**: 255–270.

Kohan, D.E., and Padilla, E. 1994. Endothelin-1 production by rat inner medullary collecting duct: effect of nitric oxide, cGMP, and immune cytokines. Am. J. Physiol. **266**: F291–F297.

Kohno, M., Horio, T., Yokokawa, K., Kurihara, N., and Takeda, T. 1992. C-type natriuretic peptide inhibits thrombin- and angiotensin II-stimulated endothelin release via cyclic guanosine 3′,5′-monophosphate. Hypertension, **19**: 320–325.

Kowluru, R.A., Kern, T.S., Engerman, R.L., and Armstrong, D. 1996. Abnormalities of retinal metabolism in diabetes or experimental galactosemia. III. Effects of antioxidants. Diabetes, **45**: 1233–1237.

Kowoluru, R.A., Kern, T.S., and Engerman, R.L. 1997. Abnormalities of retinal metabolism in diabetes or experimental galactosemia. IV. Antioxidant defense system. Free Radic. Biol. Med. **22**: 587–592.

Koya, D., and King, G.L. 1998. Protein kinase C activation and the development of diabetic complications. Diabetes, **47**: 859–66.

Kurihara, H., Yoshizumi, M., Sugiyama, T., Takaku, F., Yanagisawa, M., Masaki, T., Hamaoki, M., Kato, H., and Yazaki, Y. 1989. Transforming growth factor-beta stimulates the expression of endothelin mRNA by vascular endothelial cells. Biochem. Biophys. Res. Commun. **159**: 1435–1440.

Kuwabara, T., and Cogan, D.G. 1963. Retinal vascular patterns. VI. Mural cells of the retinal capillaries. Arch. Ophthalmol. **69**: 492.

Le Good, J.A., Ziegler, W.H., Parekh, D.B., Alessi, D.R., Cohen, P., and Parker, P.J. 1998. Protein kinase C isotypes controlled by phosphoinositide 3-kinase through the protein kinase PDK1. Science (Washington, D.C.), **281**: 2042–2045.

Levin, E.R. 1995. Endothelins. N. Engl. J. Med. **333**: 356–363.

Lewinter, M.M. 1996. Diabetic cardiomyopathy: an overview. Coron. Artery Dis. **7**: 95–98.

Lieu, A.T., and Reid, J.J. 1994. Changes in the responsiveness to endothelin-1 in isolated atria from diabetic rats. Eur. J. Pharmacol. **261**: 33–42.

Lin, Y.W., Duh, E., and Jiang, Z. 1996. Expression of preproEndothelin-1 mRNA in streptozotocin-induced diabetic rats. Diabetes. **45**: 48A.

Lynch, J.J., Ferro, T.J., Blumenstock, F.A., Brockenauer, A.M., and Malik, A.B. 1990. Increased endothelial albumin permeability mediated by protein kinase C activation. J. Clin. Invest. **85**: 1991–1998.

Makino, A., and Kamata, K. 1998. Elevated plasma endothelin-1 levels in streptozotocin induced diabetic rats and responsiveness of the mesenteric arterial bed to endothelin-1. Br. J. Pharmacol. **123**: 1065–1072.

Makino, A., Oda, S., and Kamata, K. 2001. Mechanisms underlying increased release of endothelin-1 from aorta in diabetic rats. Peptides, **22**: 639–645.

Malamitsi-Puchner, A., Economou, E., Katsouyanni, K., Karachaliou, F., Delis, D., and Bartsocas, C.S. 1996. Endothelin 1–21 plasma concentrations in children and adolescents with insulin-dependent diabetes mellitus. J. Pediatr. Endocrinol. Metab. **9**: 463–468.

Malek, A.M., Zhang, J., Jiang, J., Alper, S.L., and Izumo, S. 1999. Endothelin-1 gene suppression by shear stress: pharmacological evaluation of the role of tyrosine kinase, intracellular calcium, cytoskeleton, and mechanosensitive channels. J. Mol. Cell Cardiol. **31**: 387–399.

Matsuura, A., Kawashima, S., Yamouchi, W., Hirata, K., Yamaguchi, T., Emoto, N., and Yokoyama, M. 1997. Vascular endothelial growth factor increases endothelin-converting enzyme expression in vascular endothelial cells. Biochem. Biophys. Res. Commun. **235**: 713–716.

Mazze, R.S., Sinnock, P., Deeb, L., and Brimberry, J.L. 1985. An epidemiological model for diabetes mellitus in the United States: five major complications. Diab. Res. Clin. Prac. **1**: 185–191.

Mizutani, M., Kern, T.S., and Lorenzi, M. 1996. Accelerated death of retinal microvascular pericytes in human and experimental diabetic retinopathy. J. Clin. Invest. **97**: 2883–2890.

Mogensen, C.E., Christensen, C.K., and Vittinghus, E. 1983. The stages in diabetic renal disease. With emphasis in the stage of incipient diabetic neuropathy. Diabetes, **32**: 64–78.

Mohamed, A.K., Bierhaus, A., Schiekofer, S., Tritschler, H., Ziegler, R., and Nawroth, P.P. 1999. The role of oxidative stress and NF-κB activation in late diabetic complications. Biofactors, **10**: 157–167.

Molenaar, P., O'Reilly, G., Sharkey, A., Kuc, R.E., Harding, D.P., Plumpton, C., Gresham, G.A., and Davenport, A.P. 1993. Characterization and localization of endothelin receptor subtypes in the human atrioventricular conducting system and myocardium. Circ. Res. **72**: 526–538.

Morise, T., Takeuchi, Y., Kawano, M., Koni, I., and Takeda, R. 1995. Increased plasma levels of immunoreactive endothelin and von Willebrand factor in NIDDM patients. Diabetes Care, **18**: 87–89.

Nadler, J., and Winer, L. 1996. Free radicals, nitric oxide and diabetic complications. *In* Diabetes mellitus. *Edited by* D. LeRoith, S.I. Taylor, and M. Olefsky. Lippincott-Raven. Philadelphia. pp. 840–848.

Nakamura, T., Ebihara, I., Fukui, M., Tomino, Y., and Koida, H. 1995. Effect of a specific endothelin-A receptor antagonist on mRNA levels for extracellular matrix components and growth factors in diabetic glomeruli. Diabetes, **44**: 895–899.

Newton, A.C. 1997. Regulation of protein kinase C. Curr. Opin. Cell Biol. **9**: 161–167.

Nishikawa, T., Edelstein, D., Du, X.L., Yamagishi, S., Matsumura, T., and Kaneda, Y. 2000. Normalizing mitochondrial superoxide production blocks three pathways of hyperglycaemic damage. Nature (London), **404**: 787–790.

Obrosova, I.G., Sone, H., and Masterson, J.A. 1998. Evaluation of α_1-adrenoceptor antagonist and antioxidant therapy on diabetes-induced changes in retinal NAD(P)-redox status: evidence against pseudohypoxia. Diabetes, **47**: A40.

Oku, H., Kida, T., Sugiyama, T., Hamada, J., Sato, B., and Ikeda, T. 2001. Possible involvement of endothelin-1 and nitric oxide in the pathogenesis of proliferative diabetic retinopathy. Retina, **21**: 674–651.

Oliver, F.J., de la Rubia, G., Feener, E.P., Lee, M.E., Loeken, M.R., Shiba, T., Quertermous, T., and King, G.L. 1991. Stimulation of endothelin-1 gene expression by insulin in endothelial cells. J. Biol. Chem. **266**: 23 251 – 23 256.

Park, J.Y., Takahara, N., Gabriele, A., Chou, E., Naruse, K., Suzuma, K., Yamauchi, T., Ha, S.W., Meier, M., Rhodes, C.J., and King, G.L. 2000. Induction of endothelin-1 expression by glucose: an effect of protein kinase C activation. Diabetes, **49**: 1239–1248.

Pedram, A., Razandi, M., Hu, R.M., and Levin, E.R. 1997. Vasoactive peptides modulate vascular endothelial cell growth factor production and endothelial cell proliferation and invasion. J. Biol. Chem. **27**: 17 097 – 17 103.

Perfetto, F., Tarquini, R., de Leonardis, V., Piluso, A., Becucci, A., Anselmi, B., Curradi, C., Tapparini, L., and Tarquini, B. 1997. Vascular damage and not hypertension per se influences endothelin-1 plasma levels in patients with non insulin dependent diabetes mellitus. Recenti. Prog. Med. **88**: 317–320.

Piatti, P.M., Monti, L.D., Conti, M., Baruffaldi, L., Galli, L., Phan, C.V., Guazzini, B., Pontiroli, A.E., and Pozza, G. 1996. Hypertriglyceridemia and hyperinsulinemia are potent inducers of endothelin-1 release in humans. Diabetes, **45**: 316–321.

Porta, M. 1996. Endothelium: the main actor in the remodelling of the retinal microvasculature in diabetes. Diabetologia, **39**: 739–44.

Quehenberger, P., Bierhaus, A., Fasching, P., Muellner, C., Klevesath, M., and Hong, M. 2000. Endothelin 1 transcription is controlled by nuclear factor-κB in AGE-stimulated cultured endothelial cells. Diabetes, **49**: 1561–1570.

Rubanyi, G.M., and Polokoff, M.A. 1994. Endothelins: Molecular biology, biochemistry, pharmacology, physiology and pathophysiology. Pharmacol. Rev. **46**: 325–414.

Sakamoto, A., Yanagisawa, M., Sakurai, T., Takuwa, Y., Yanagisawa, H., and Masaki, T. 1991. Cloning and functional expression of human cDNA for the ET_B endothelin receptor. Biochem. Biophys. Res. Commun. **178**: 656–663.

Sakurai, T., Yanagisawa, M., and Masaki, T. 1992. Molecular characterization of endothelin receptors. Trends Pharmacol. Sci. **13**: 103–108.

Santiago, J.V. 1993. Lessons from the diabetes control and complication trial. Diabetes, **42**: 1549–1554.

Savage, M.P., Krolewski, A.S., Kenien, G.G., Lebeis, M.P., Christlieb, A.R., and Lewis, S.M. 1988. Acute myocardial infarction in diabetes mellitus and significance of congestive heart failure as a prognostic factor. Am. J. Cardiol. **62**: 665–669.

Schweizer, A., Valdenaire, O., Nelbock, P., Deuschle, U., Dumas Milne Edwards, J.B., Stumpf, J.G., and Loffler, B.M. 1997. Human endothelin-converting enzyme (ECE–1): three isoforms with distinct subcellular localizations. Biochem. J. **328**: 871–877.

Shehadeh, A., and Regan, T.J. 1995. Cardiac consequences of diabetes mellitus. Clin. Cardiol. **18**: 301–305.

Shiba, T., Inoguchi, T., Sportsman, J.R., Heath, W.F., Bursell, S., and King, G.L. 1993. Correlation of diacylglycerol level and

protein kinase C activity in rat retina to retinal circulation. Am. J. Physiol. **265**: E783–E793.

Shiba, T., Bursell, S.E., Clermont, A., Sportsman, R., Heath, W., and King, G.L. 1991. Protein kinase C (PKC) activation is a causal factor for the alteration of retinal blood flow in diabetes of short duration. Invest. Ophthalmol. Vis. Sci. **32**: 785–789.

Shimada, K., Takahashi, M., Ikeda, M., and Tanzawa, K. 1995. Identification and characterization of two isoforms of an endothelin-converting enzyme-1. Fed. Eur. Biochem. Soc. Lett. **371**: 140–144.

Sima, A.A.F., and Sugimoto, K. 1999. Experimental diabetic neuropathy: an update. Diabetologia, **42**: 773–788.

Simonson, M.S. 1993. Endothelins: multifunctional renal peptides. Physiol. Rev. **73**: 375–411.

Simonson, M.S., Jones, J.M., and Dunn, M.J. 1992. Differential regulation of *fos* and *jun* gene expression and AP-1 cis element activity by endothelin isopeptides: possible implications for mitogenic signalling by endothelin. J. Biol. Chem. **267**: 8643–8649.

Smulders, R.A., Stehouwer, C.D., Olthof, C.G., van Kamp, G.J., Teerlink, T., de Vries, P.M., and Donker, A.J. 1994. Plasma endothelin levels and vascular effects of intravenous L-arginine infusion in subjects with uncomplicated insulin-dependent diabetes mellitus. Clin. Sci. **87**: 37–43.

Steffes, M., Pluth, R., Schmidt, D., McCrery, R., Bassen, J., and Mauer, J. 1996. Glomerular and mesangial cell number in insulin dependent diabetes mellitus (IDDM). Proceedings of the European Diabetic Nephropathy Study Group, Parma, Italy. pp. 27.

Stehouwer, C.D., Lambert, J., Donker, A.J., and van Hinsbergh, V.W. 1997. Endothelial dysfunction and pathogenesis of diabetic angiopathy. Cardiovasc. Res. **34**: 55–68.

Stevens, E.J., and Tomlinson, D.R. 1995. Effects of Endothelin receptor antagonism with bosentan on peripheral nerve function in experimental diabetes. Br. J. Pharmacol. **115**: 373–379.

Stitt, A.W., Li, Y.M., Gardiner, T.A., Bucala, R., Archer, D.B., and Vlassara, H. 1997. Advanced glycation end products (AGEs) co-localize with AGE receptors in the retinal vasculature of diabetic and of AGE-infused rats. Am. J. Pathol. **150**: 523–531.

Stitt, A.W., He, C., and Vlassara, H. 1999. Characterization of the advanced glycation end-product receptor complex in human vascular endothelial cells. Biochem. Biophys. Res. Commun. **256**: 549–556.

Sugawara, F., Ninomiya, H., Okamoto, Y., Miwa, S., Mazda, O., Katsura, Y., and Masaki, T. 1996. Endothelin-1-induced mitogenic responses of Chinese hamster ovary cells expressing human endothelinA: the role of a wortmannin-sensitive signaling pathway. Mol. Pharmacol. **49**: 447–457.

Sumner, M.J., Cannon, T.R., Mundin, J.W., White, D.G., and Watts, I.S. 1992. Endothelin ET_A and ET_B receptors mediate vascular smooth muscle contraction. Br. J. Pharmacol. **107**: 858–860.

Suzuki, E., Nagata, D., Kakoki, M., Hayakawa, H., Goto, A., Omata, M., and Hirata, Y. 1999. Molecular mechanisms of endothelin-1-induced cell-cycle progression: involvement of extracellular signal-regulated kinase, protein kinase C, and phosphatidylinositol 3-kinase at distinct points. Circ. Res. **84**: 611–619.

Takagi, Y., Kashiwagi, A., Tanaka, Y., Asahian, T., Kikkawa, R., and Shigeta, Y. 1995. Significance of fructose-induced protein oxidation and formation of advanced glycation end product. J. Diabetes Comp. **9**:89–91.

Takagi, C., Bursell, S.E., Lin, Y.W. Takagi, H., Duh, E., Jiang, Z., Clermont, A.C., and King, G.L. 1996. Regulation of retinal hemodynamics in diabetic rats by increased expression and action of endothelin-1. Invest. Ophthalmol. Vis. Sci. **37**: 2504–2518.

Tagami, S., Kondo, T., Yoshida, K., Hirokawa, J., Ohtsuka, Y., and Kawakami, Y. 1992. Effect of insulin on impaired antioxidant activities in aortic endothelial cells from diabetic rabbits. Metab. Clin. Exp. **41**: 1053–1058.

Takahashi, K., Ghatei, M.A., Lam, H.C., O'Halloran, D.J., and Bloom, S.R. 1990. Elevated plasma endothelin in patients with diabetes mellitus. Diabetologia, **33**: 306–310.

Takayanagi, R., Kitazumi, K., Takasaki, C., Ohnaka, K., Aimoto, S., Tasaka, K., Ohashi, M., and Nawata, H. 1991. Presence of non-selective type of endothelin receptor on vascular endothelium and its linkage to vasodilation. Fed. Eur. Biochem. Soc. Lett. **282**: 103–106.

Thomas, P.K. 1992. Diabetic neuropathy: Models, mechanisms and mayhem. Can. J. Neuro. Sci. **19**: 1.

Turner, N.C., Morgan, P.J., Haynes, A.C., Vidgeon-Hart, M., Toseland, N., and Clapham, J.C. 1997. Elevated renal endothelin-1 clearance and mRNA levels associated with albuminuria and nephropathy in non-insulin dependent diabetes mellitus studies in obese *fa*/*fa* Zucker rats. Clin. Sci. **93**: 565–571.

U.K. Prospective Diabetes Study (UKPDS) Group. 1996. Intensive blood-glucose control with sulphonylureas or insulin compared with conventional treatment and risk of complications in patients with type 2 diabetes (UKPDS 33). Lancet, **352**: 837–853.

Valdenaire, O., Rohrbacher, E., and Mattei, M.G. 1995. Organization of the gene encoding the human endothelin-converting enzyme (ECE–1). J. Biol. Chem. **270**: 29 794 – 29 798.

Valdenaire, O., Lepailleur-Enouf, D., Egidy, G., Thouard, A., Barret, A., Vranckx, R., Tougard, C., and Michel, J.B. 1999. A fourth isoform of endothelin-converting enzyme (ECE–1) is generated from an additional promoter molecular cloning and characterization. Eur. J. Biochem. **264**: 341–349.

Vanhoutte, P.M. 1994. Endothelin-1. A matter of life and breath. Nature (London), **368**: 693–694.

Vesci, L., Mattera, G.G., Tobia, P., Corsico, N., and Calvani, M. 1995. Cardiac and renal endothelin-1 binding sites in streptozotocin-induced diabetic rats. Pharmacol. Res. **32**: 363–367

Vlassara, H. 1997. Recent progress in advanced glycation end products and diabetic complications. Diabetes, **46**: S19–S25.

Wakasaki, H., Koya, D., Schoen, F.J., Jirousek, M.R., Ways, D.K., Hoit, B.D., Walsh, R.A., and King, G.L. 1997. Targeted overexpression of protein kinase C 2 isoform in myocardium causes cardiomyopathy. Proc. Natl. Acad. Sci. U.S.A. **94**: 9320–9325.

Whelchel, A., Evans, J., and Posada, J. 1997. Inhibition of ERK activation attenuates endothelin-stimulated airway smooth muscle cell proliferation. Am. J. Respir. Cell Mol. Biol. **16**: 589–596.

Williamson, J.R., Chang, K., Frangos, M., Hasan, K.S., Ido, Y., Kawamura, T., Nyengaard, J.R., van den Enden, M., Kilo, C., and Tilton, R.G. 1993. Perspectives in diabetes. Hyperglycemic pseudohypoxia and diabetic complications. Diabetes, **42**: 801–813.

Winkler, B.S., Arnold, M.J., Brasell, M.A., and Sliter, D.R. 1997. Glucose dependence of glycolysis, hexose monophosphate shunt activity, energy status, and the polyol pathway in retinas isolated from normal (non diabetic) rats. Invest. Ophthalmol. Vis. Sci. **38**: 62–71.

Xia, P. Inoguchi, T., Kern, T.S., Engerman, R.I., Oates, P.J., and King, G.L. 1994. Characterization of the mechanism for the chronic activation of DAG–PKC pathway in diabetes and hypergalactosemia. Diabetes, **43**: 1122–1129.

Yamagishi, S., Yonekura, H., Yamamoto, Y., Katsuno, K., Sato, F., Mita, I., Ooka, H., Satozawa, N., Kawakami, T., Nomura, M., and Yamamoto, H. 1997. Advanced glycation end products-driven angiogenesis in vitro. Induction of the growth and tube formation of human microvascular endothelial cells through autocrine vascular endothelial growth factor. J. Biol. Chem. **272**: 8723–8730.

Yamauchi, T., Ohnaka, K., Takayanagi, R., Umeda, F., and Nawata, H. 1990. Enhanced secretion of endothelin-1 by elevated glucose levels from cultured bovine aortic endothelial cells. Fed. Eur. Biochem. Soc. Lett. **267**: 16–18.

Yanagisawa, M., Kurihara, H., Kimura, S., Tomobe, Y., Kobayashi, M., Mitsui, Y., Yazaki, Y., Goto, K., and Masaki, T. 1988. A novel potent vasoconstrictor peptide produced by vascular endothelial cells. Nature (London), **332**: 411–415.

Zhu, X., and Eichberg, J. 1990. 1,2-diacylglycerol content and its arachidonyl-containing molecular species are reduced in sciatic nerve from streptozotocin-induced diabetic rats. J. Neurochem. **55**: 1087–1090.