Conferences and Reviews

Role of Nitric Oxide in Insulin-Dependent Diabetes Mellitus–Related Vascular Complications

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Patients with insulin-dependent diabetes mellitus are at high risk for vascular disorders such as hypertension, nephropathy, and retinopathy. The most common cause of morbidity and mortality in patients with insulin-dependent diabetes is vascular disease. Despite ongoing research, the pathogenesis of vascular disease in diabetes remains unclear. In recent years, numerous investigators have examined the role of the endothelium-derived relaxing factor, nitric oxide, in the disease state of hypertension and its complications. We review the role of nitric oxide in the development of diabetes-related vascular disease and discuss findings suggesting that nitric oxide metabolism and vascular responsiveness to nitric oxide are altered in diabetes. Patients with diabetes may benefit from therapy that addresses this pathogenic deficiency.

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Over the past decade, considerable research has provided insight into the identity, function, and pharmacology of the endothelium-derived relaxing factor (EDRF). The consensus is that the vasoactive substance is the short-lived reactive compound, nitric oxide. More recently, two classes of nitric oxide synthase enzymes have been characterized. The first is a constitutive, calcium ion (Ca^{2+}) and calmodulin-dependent isoenzyme that, in healthy vessels, releases small amounts of nitric oxide from endothelial cells in response to agonist stimulation or shear stress. Agents such as acetylcholine, bradykinin, and serotonin require an intact endothelium to elicit part or all of their vasodilation. It appears that each shares a common mechanism beginning with a membrane receptor-mediated increase in intracellular calcium in vascular endothelial cells. This rise in intracellular Ca^{2+} results in the Ca^{2+}-calmodulin–dependent activation of the enzyme complex, nitric oxide synthase. Concurrent with the oxidation of the reduced form of nicotinamide-adenine dinucleotide phosphate, this complex converts L-arginine to L-citrulline and releases a molecule of nitric oxide. This short-lived compound diffuses directly into the vascular smooth muscle cell and activates guanylate cyclase through reaction with its heme group, in turn causing an increase in intracellular cyclic guanosine monophosphate (cyclic GMP) levels in the smooth muscle cell. Cyclic GMP acts on sarcoplasmic reticulum Ca^{2+}-adenosine triphosphatase (ATPase) mechanisms to promote the resequestration of intracellular Ca^{2+} into internal stores and hence causes relaxation of smooth muscle (Figure 1; see review[1]).

The other important class of nitric oxide synthase involves a cytokine-inducible, Ca^{2+}-calmodulin–independent isoform that releases large amounts of nitric oxide for extended periods.[2] The likely biologic effect is the elimination of tumor cells, bacteria, and parasites primarily by binding to and destroying iron-containing enzymes.[2,3] This inducible isoform was originally described in macrophages, but subsequently has been found in most vascular cells as well.

Certain vascular disorders have been suggested to occur as a result of a pathologic alteration in either major isoform. For example, an interruption in the constitutive-release pathway has been implicated in hypertension, whereas an overproduction of nitric oxide from the inducible-release pathway has been implicated in the severe hypotension of septic shock. The constitutive nitric oxide synthase has also been found in platelet cytosol, where it may act as part of a negative-feedback mechanism to regulate platelet aggregation through cyclic GMP. Nitric oxide production has also been detected in the nervous system, hepatocytes, neutrophils, and other cells, whereas its full biologic role remains to be elucidated.[2]

The incidence of vascular diseases such as hypertension, atherosclerosis, nephropathy, and retinopathy is higher among patients with diabetes than in the general population.[4-7] Thus, the risk of myocardial infarction,

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ABBREVIATIONS USED IN TEXT

ATPase = adenosine triphosphatase
cyclic GMP = cyclic guanosine monophosphate
EDRF = endothelium-derived relaxation factor
IDDM = insulin-dependent diabetes mellitus

cerebrovascular accidents, renal failure, and blindness is increased. Other common complications seen in persons with diabetes with peripheral vascular disease include impotence and abnormalities in clotting factors that may increase the risk of infarct and ischemia-related damage.[8,9] More advanced cases of peripheral vascular disease can result in amputation of the lower extremities due to vascular insufficiency (Table 1).[4-10] Altered blood vessel function is the common factor among these vascular complications. Blood vessels from persons with diabetes show many of the structural and functional alterations characteristic of the vessels of persons with hypertension, including vascular hypertrophy and an increased sensitivity to normal stimuli.[11] Because a lack of endothelium-derived nitric oxide has already been implicated in hypertension,[12] it is reasonable to suspect that alterations in nitric oxide metabolism may contribute to vascular complications associated with diabetes.

In this review, we discuss alterations in nitric oxide metabolism and efficacy that are associated with diabetic or hyperglycemic conditions. Abnormalities in the endothelium, intercellular diffusion, and smooth muscle cells are considered. It has generally been thought that the disorders stem from problems with the constitutive nitric oxide release pathway, and research has been directed with this in mind. It may be presumptuous, however, to assume that alterations in the inducible nitric oxide release pathway do not contribute to the vas-

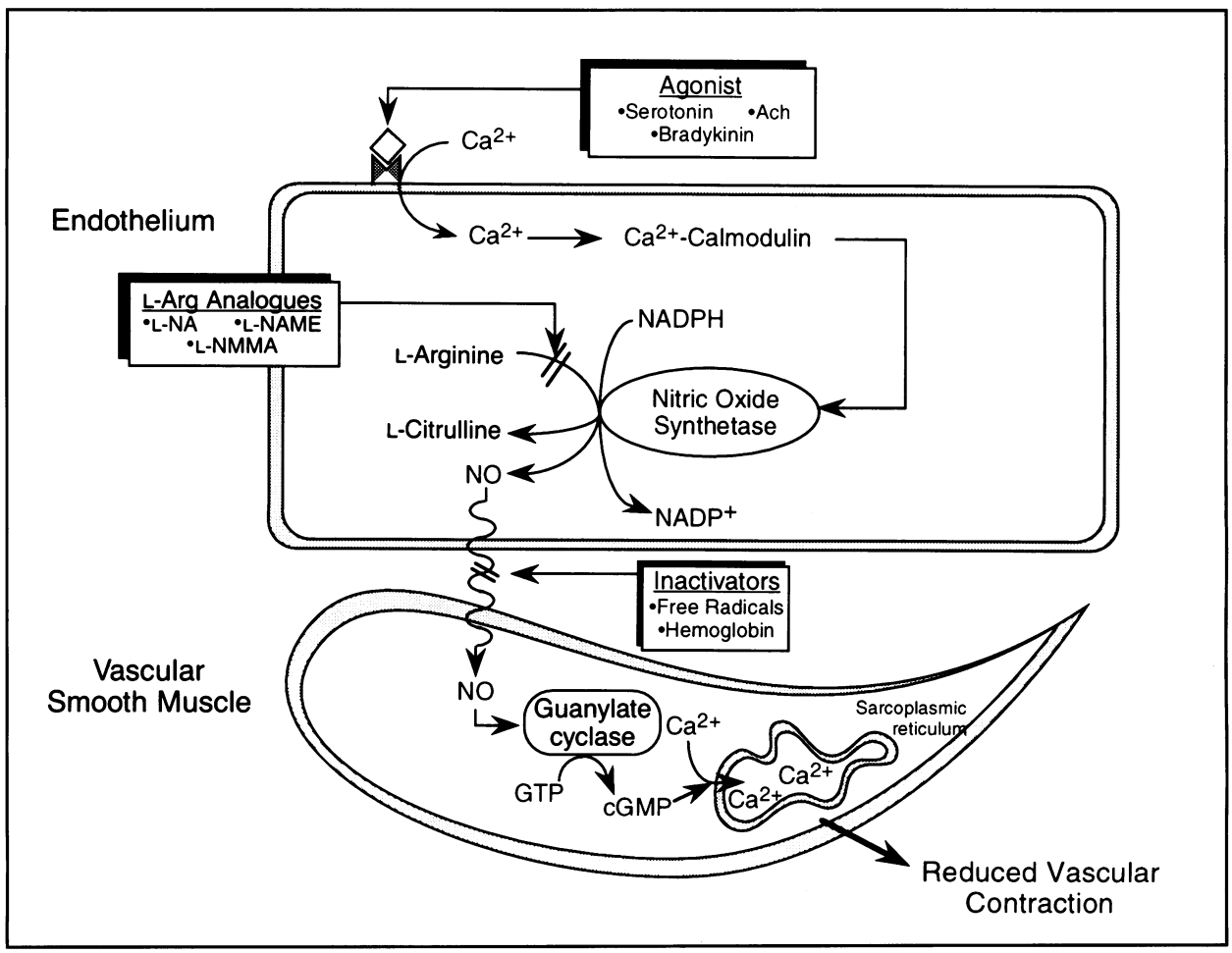

Figure 1.—The metabolic pathways of the endothelium-derived relaxing factor, nitric oxide **(NO)** are shown. Agonists that cause a rise in intracellular calcium **(Ca^{2+})** activate the NO synthetase enzyme through Ca^{2+}-calmodulin dependent activation. NO diffuses into smooth muscle cells, causing a rise in cyclic guanosine monophosphate **(cGMP)**, which leads to the resequestration of Ca^{2+} into internal stores and results in vasodilation. Nonmetabolizable analogues of arginine—L-nitroarginine **(L-NA)**, L-nitromonomethyl arginine **(L-NMMA)**, and L-nitroarginine methyl ester **(L-NAME)**—inhibit NO production. Reactive compounds (heme, free radicals) may act to degrade NO. Ach = acetylcholine, GTP = guanosine triphosphate, L-Arg = L-arginine, $NADP^+$ = the oxidized form of nicotinamide-adenine dinucleotide phosphate, NADPH = the reduced form of NADP

TABLE 1.—*Insulin-Dependent Diabetes Mellitus–Associated Vascular Complications*

Vascular Complication	*Consequences*	*Review or Reference*
Hypertension.....	Myocardial infarction, cerebrovascular accident	Anderson and Rocchini, 1993[4]
Hyperlipidemia or cholesterolemia..	Atherosclerosis	Walker, 1993[5]
Nephropathy.....	Renal failure	Breyer, 1992[6]
Retinopathy.....	Blindness	Rosenbloom, 1983[7]
Clotting abnormalities....	Infarct or ischemia	Maiello et al, 1988[9]
Other peripheral vascular disease..	Impotence, amputation of extremities	Maatman et al, 1987[8]; Falkenberg et al, 1990[10]

cular complications associated with insulin-dependent diabetes mellitus (IDDM).

Nitric Oxide and Insulin-Dependent Diabetes Mellitus

Gross Alteration in the Endothelium

Several studies report a change in endothelial integrity in diabetes. Independent investigators have shown that endothelial injury is associated with factors related to poor serum glucose regulation in patients with diabetes. Substantially higher levels of endothelial vascular desquamation have been reported in patients with IDDM, and it has been suggested that hyperglycemic conditions may be responsible.[13] Increased levels of nonenzymatic glycosylation occur in vivo for endothelial cells from rats with streptozocin (formerly streptozotocin)-induced diabetes,[14] a factor that may contribute to altered endothelial integrity.

Other studies report that the growth and migration of endothelial cells are inhibited with hyperglycemia. Free radicals generated by the auto-oxidation of glucose may delay endothelial cell replication time in vitro.[15] It remains to be seen whether this phenomenon occurs in vivo, although in one study, it was suggested that hyperglycemic conditions facilitate the sulfhydryl oxidation of serum proteins and contribute to the inhibition of endothelial cell proliferation in bovine carotid arteries.[16] The hypothesis that the depletion of antioxidants observed in diabetes[17] acts to complicate this factor is supported by studies in which the antihypertensive effect of exogenous antioxidant agents has been reported in patients with diabetes[18] and by a study that reported that EDRF was restored in diabetic rat aorta by superoxide dismutase.[19]

Another study showed inhibited migration of endothelial cells in hyperglycemic conditions.[20] This effect persisted even when cells were reincubated in physiologic concentrations of glucose, suggesting that irreversible damage may occur even when serum glucose levels are tightly regulated in a diabetic subject. The presence of L-fucose, a monosaccharide observed to be present at higher levels in diabetes, has also been associated with an inhibited proliferation of cultured endothelial cells.[21]

The general scheme portrayed in these studies is that endothelial damage in diabetic subjects is further complicated by hyperglycemia-induced impairment of endothelial proliferation and migration. This defect in endothelial repair may ultimately affect nitric oxide production and release from these cells.

Changes in Endothelial Metabolism

Both persons with diabetes and experimental models of diabetes show an impairment in endothelial-dependent relaxation of smooth muscle. In one study acute and chronic hyperglycemia almost completely suppressed the acetylcholine-induced vasodilation in arteries from rats with streptozocin-induced diabetes, but caused no impairment in the relaxation due to sodium nitroprusside—an endothelium-independent vasodilator.[22] These data suggest a defect in either the production or release of nitric oxide from endothelial cells.

The increased level of the monosaccharide, L-fucose, in diabetes has also been implicated in altered *myo*-inositol transport. L-Fucose has been found to competitively inhibit the uptake of *myo*-inositol into endothelial cells.[21] A reduction in the level of *myo*-inositol would be expected to reduce phosphoinositide-mediated calcium release from intracellular stores, hence interfering with the Ca^{2+}-dependent nitric oxide generation.

Impaired EDRF-mediated vasodilation, which has particular clinical relevance to diabetes, has been characterized in several articles. One study showed considerable impairment of acetylcholine-induced vasodilation in the corpus cavernosum of diabetic rabbits when compared with control groups.[23] The corpus cavernosum of hypercholesterolemic rabbits also showed reduced dilation in response to acetylcholine in a separate experiment by the same laboratory.[24] Alteration in lipid metabolism and subsequent or independent deficiency in EDRF-mediated vasodilation may therefore be contributing factors to the pathogenesis of impotence often noted in persons with diabetes.

Researchers have demonstrated that advanced glycosylation end products inactivate nitric oxide activity and that their reactivity was present at high levels in atherosclerotic plaques in vessels from patients with diabetes.[25] Other studies showed that EDRF-mediated vasodilation in the hindquarters of streptozocin-treated rats was particularly dependent on nitric oxide and that there was considerable impairment of nitric oxide synthesis and, therefore, blood flow in vessels from the hindquarters of these rats.[26] This observation may be relevant to the clinical finding that advanced vascular disease in the lower extremities often results in amputation of a foot or both feet of many diabetic patients.[10] Other studies suggest that nitric oxide dysfunction in the eyes[27] and brain[28] may be responsible for the pathogenesis of ophthalmic complications and the incidence of stroke commonly seen in diabetes.

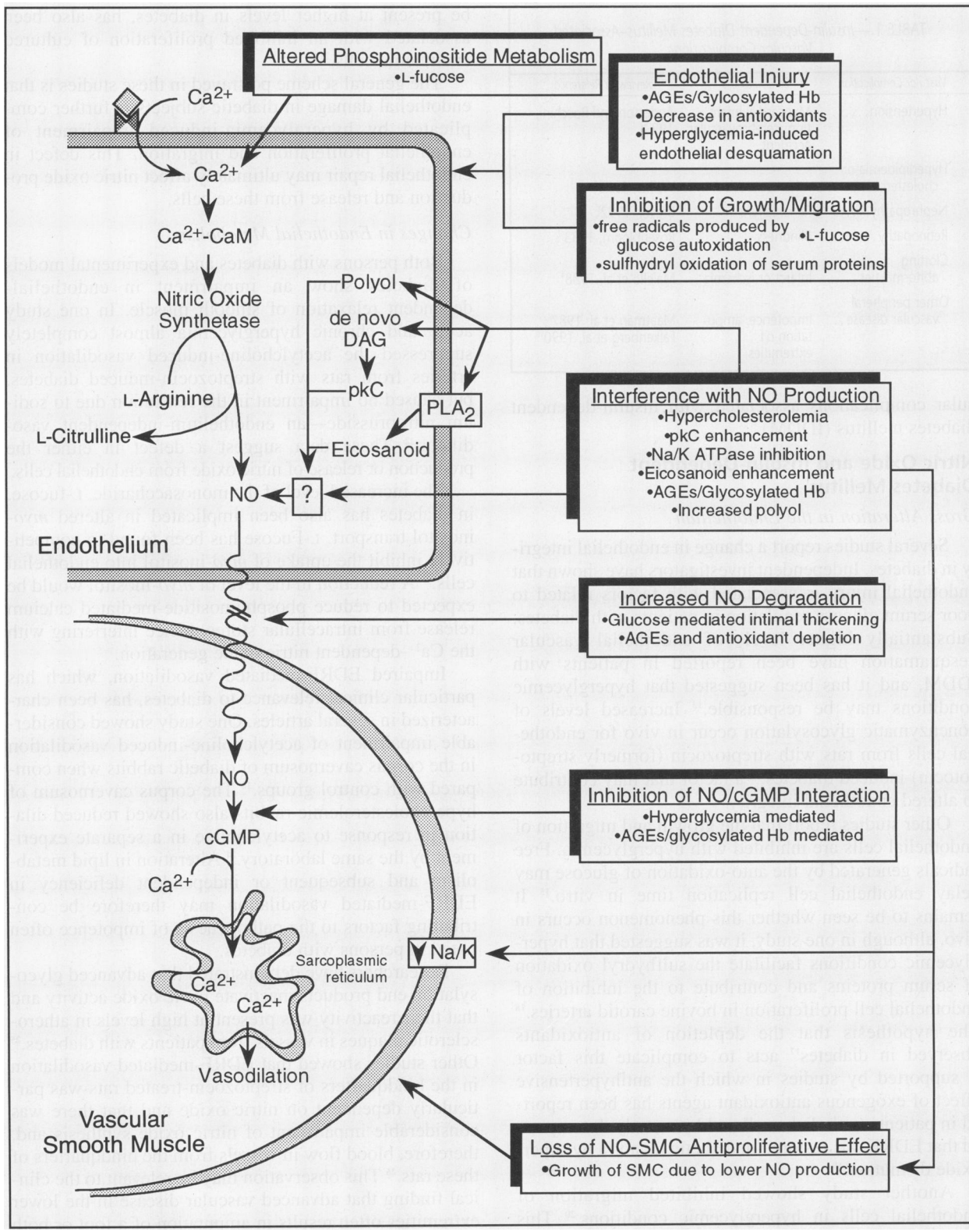

Figure 2.—A multifactorial model of nitric oxide **(NO)**-mediated vasodilation deficiency in insulin-dependent diabetes mellitus is shown. Various sites of alteration and reported causes of these alterations are illustrated. AGEs = advanced glycosylation end products, ATPase = adenosine triphosphatase, Ca^{2+} = calcium ion, CaM = calmodulin, cGMP = cyclic guanosine monophosphate, DAG = diacylglycerol, Hb = hemoglobin, Na/K = sodium-potassium pump, pkC = protein kinase C, PLA_2 = phospholipase A_2, SMC = smooth muscle cell

Experiments with arginine analogues, which act to inhibit nitric oxide production, are also useful in exploring the contribution of impaired nitric oxide production in IDDM-related vascular complications. In one study, administering *N*-ω-nitro-L-arginine methyl ester caused hypertension in controls and, to a lesser extent, in rats with streptozocin-induced diabetes.[29] Further, several studies show that the capacity of arginine analogues to reduce blood flow is reduced in persons with diabetes.[30-32] These data may be interpreted as evidence for lower basal production of nitric oxide in the endothelial cells of diabetic patients.

Other studies suggest that alterations in endothelial membrane components are caused by hyperglycemic conditions. In one report, investigators using rabbit aorta showed that the activation of protein kinase C through a hyperglycemia-mediated increase in de novo synthesis of diacylglycerol may interfere with endothelial-mediated relaxation by increasing vasoconstrictor prostanoid generation.[33] Even though the increased prostanoid release may directly affect vascular smooth muscle, the same prostanoids through the formation of oxygen radicals may also inhibit the activity of EDRF before it reaches smooth muscle,[34] thus compounding the impairment in relaxation. It has also been suggested that increased polyol pathway activity (often associated with diabetes, hyperglycemia, or both) contributes to the impairment in endothelial-dependent relaxation. Inhibited acetylcholine-mediated relaxation has been shown in aorta subsequent to increased polyol activity in galactosemic rats and diabetic rabbits, respectively.[35,36] The abnormalities in relaxation were prevented by the administration of an aldose reductase inhibitor that prevents the increase in polyol activity. The authors concluded that an abnormality in nitric oxide metabolism brought about by the increased polyol activity causes the impaired vasodilation.[35]

Although hyperglycemia may be a key factor in the alteration of nitric oxide metabolism, a critical study has shown nonspecific impairment in endothelium-dependent relaxation before serum glucose level elevation in genetically diabetic rats.[37] This suggests that the endothelium may play a primary role in the pathogenesis of vascular disease. Hyperglycemia-mediated alterations in nitric oxide metabolism may occur due to or in parallel to this defect.

Another line of research is exemplified in a study that demonstrated that EDRF release is actually increased from endothelial cells incubated in hyperglycemic media.[38] The authors proposed that this increase in nitric oxide release may be responsible for the pathologic vasodilation sometimes seen in the early stages of uncomplicated diabetes mellitus. Supporting this hypothesis is a study that suggests that inhibiting the inducible isoform of nitric oxide synthase in diabetic rats may help reduce pathologic vasodilation and increased vascular permeability at sites of diabetic complications.[39]

Changes in Nitric Oxide Diffusion

Because of its relatively short half-life,[1] nitric oxide is particularly vulnerable to factors that interfere with its migration from endothelium into the vascular smooth muscle cell. Hyperglycemia-mediated intimal thickening found in blood vessels from diabetic subjects may increase the diffusion time. It was reported that hyperglycemia increased the expression of the fibronectin gene in diabetes, which results in intimal thickening.[40] This alteration is not readily reversible. Another study showed increased synthesis of basement membrane components in endothelial cells cultured in hyperglycemic conditions.[41]

An increased amount of advanced glycosylation end products and depleted numbers of antioxidants are associated with an increase in nitric oxide degradation at the endothelial cell smooth muscle junction. One study showed that advanced glycosylation end products accumulate on basement membrane collagen and chemically inactivate nitric oxide.[42] Other studies demonstrated that advanced glycosylation end products quench nitric oxide in vivo and in vitro and that their inhibition with amino guanidine prevents nitric oxide quenching and restores endothelium-dependent relaxation in experimental models of diabetes.[43]

Changes in Cyclic Guanosine Monophosphate-Vascular Smooth Muscle Metabolism

Several studies have documented hyperglycemia-induced changes in smooth muscle responses to agonist-mediated vasodilation and to exogenous sources of nitric oxide that affect nitric oxide-guanylate cyclase coupling, cyclic-GMP generation, or both. In one, cyclic-GMP levels were shown to be reduced in pig aortic endothelial cell monolayers exposed to hyperglycemic conditions.[44] In an experimental paradigm that looked at rats with streptozocin-induced diabetes, an impairment in nitric oxide-dependent cyclic-GMP generation was found in the glomeruli of the diabetic animals.[45] Although the lower cyclic-GMP levels reported in these preparations may be the direct result of lower nitric oxide production, a study suggests that there can be a primary deficiency in the cyclic GMP of smooth muscle cells.[46] The investigators showed that low concentrations of glycosylated hemoglobin inhibited acetylcholine-induced and exogenous nitric oxide-induced relaxation in rat aorta,[46] suggesting that the cyclic-GMP activity is altered in this experimental model of diabetes. The lower levels of cyclic GMP generated in diabetic subjects would lead to reduced Ca^{2+} sequestration and hence an impaired vasodilation.

Hyperglycemic conditions have also been shown to affect the vascular sodium-potassium ATPase. It was shown that hyperglycemia-mediated inhibition of the vascular sodium-potassium ATPase in the rabbit aorta is mediated through endothelium-derived nitric oxide.[47] This inhibition of sodium-potassium ATPase was similarly evoked by exposure to an arginine analogue, L-nitromonomethyl arginine, in normal glucose,

confirming that the primary alteration is a decrease in endothelial nitric oxide generation. An inhibited sodium-potassium ATPase in the smooth muscle would be expected to depolarize those cells and antagonize the efficacy of vasodilatory stimuli.

Finally, we must consider the nonspecific effects of lowered basal nitric oxide production on vascular smooth muscle cells. Nitric oxide exerts an antiproliferative effect in the smooth muscle through a cyclic-GMP–mediated process.[48] A loss or decrease of nitric oxide production is therefore associated with hypertrophy and hyperplasia of vascular smooth muscle that is characteristic of vascular disease. This increase in smooth muscle growth and proliferation results in a higher vascular smooth muscle "load" for each endothelial cell. Nitric oxide-mediated vasodilation may be compromised because of lower responsiveness of the smooth muscle cells. Consistent with this hypothesis, it was reported that advanced glycosylation product-mediated destruction of nitric oxide inactivated the antiproliferative influence of nitric oxide on aortic smooth muscle.[42] These data support the idea that vascular smooth muscle is less sensitive to nitric oxide in patients with diabetes. (See Figure 2 and Table 2 for comprehensive illustration and references.)

Summary and Conclusions

Evidence indicates that many of the vascular complications associated with IDDM may result from alterations in endothelial-derived nitric oxide production and action. Several changes in the metabolism of nitric oxide were considered, as were changes in the structure of endothelial and smooth muscle cells. Factors that compromise nitric oxide diffusion and signal transduction in the smooth muscle cell were also discussed. The alteration in nitric oxide function is an important factor associated with vascular disease in patients with diabetes, and such a deficiency could contribute substantially to the development of vascular complications in diabetes. Persons with diabetes may benefit from therapeutic agents that target nitric oxide pathways, such as nitroglycerin and other nitric oxide-donating compounds. Complications such as increased vascular permeability may not be corrected by nitric oxide supplementation therapy, however. Further investigation should be undertaken to evaluate these compounds in the control or perhaps prevention of the vascular complications that are major factors in the morbidity and mortality of diabetes mellitus.

Other studies not discussed in this article suggest that nitric oxide is also altered in its capacity as a neurotransmitter in diabetes[49] and that it may be responsible for the destruction of pancreatic β-islets in the autoimmune development of IDDM.[50] These observations shed further light on alterations of nitric oxide in not only the course of vascular complications but in the pathogenesis of diabetes itself and warrant further investigation.

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TABLE 2.—*Alterations of Insulin-Dependent Diabetes Mellitus Affecting Endothelium-Derived Relaxation Factor–Mediated Vasodilation*

Alteration	*Reference*
Endothelial injury or desquamation	Popova et al, 1988[13]; Gilcrease and Hoover, 1991[14]
Inhibition of endothelial growth	Curcio and Ceriello, 1992[15]; Nakao-Hayashi et al, 1992[16]; Stefani et al, 1992[21]
Inhibition of endothelial migration	Mascardo, 1988[20]
Change in endothelial phosphoinositide metabolism	Stefani et al, 1992[21]
Change in nitric oxide production	Lash and Bohlen, 1991[22]; Azadzoi and Saenz de Tejada, 1991, 1992[23,24]; Nakamura et al, 1993[25]; Kiff et al, 1991[26]; Haefliger et al, 1992[27]; Pelligrino et al, 1992[28]; Abiru et al, 1993[29]; Taylor et al, 1992[30]; Lawrence and Brain, 1992[31]; Elliott et al, 1993[32]; Tesfamariam et al, 1991, 1993[33,36]; Bohlen and Lash, 1993[34]; Cameron and Cotter, 1993[35]; Miyata et al, 1992[37]
Intimal thickening	Roy et al, 1990[40]; Cagliero et al, 1988[41]
Increased nitric oxide degradation	Hogan et al, 1992[42]; Bucala et al, 1991[43]
Change in cyclic-GMP generation or nitric oxide-guanylate cyclase coupling	Weisbrod et al, 1993[44]; Craven et al, 1994[45]; Rodríguez-Mañas et al, 1993[46]
Smooth muscle hypertrophy or hyperplasia	Hogan et al, 1992[42]

cyclic GMP = cyclic guanosine monophosphate

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