Proatherosclerotic Mechanisms Involving Protein Kinase C in Diabetes and Insulin Resistance

Christian Rask-Madsen, George L. King

Abstract—In diabetes and insulin resistance, activation of protein kinase C (PKC) in vascular cells may be a key link between elevated plasma and tissue concentrations of glucose and nonesterified fatty acids and abnormal vascular cell signaling. Initial studies of PKC activation in diabetes focused on microvascular complications, but increasing evidence supports that PKC plays a role in several mechanisms promoting atherosclerosis. This review explains how PKC is thought to be activated in diabetes and insulin resistance through de novo synthesis of diacylglycerol. Furthermore, the review summarizes studies that implicate PKC in promoting proatherogenic mechanisms or inhibiting antiatherogenic mechanisms, including studies of endothelial dysfunction; gene induction and activation of vascular NAD(P)H oxidase; endothelial nitric oxide synthase expression and function; endothelin-1 expression; growth, migration, and apoptosis of vascular smooth muscle cells; induction of adhesion molecules; and oxidized low-density lipoprotein uptake by monocyte-derived macrophages. (*Arterioscler Thromb Vasc Biol. 2005;25:1-10.*)

Key Words:...
vascular smooth muscle cells (VSMCs).8 Radioactive labeling studies have established that increased DAG mass occurs in such conditions, not through hydrolysis of PIP2, but partly by de novo synthesis from glucose,8 probably via glyceraldehyde 3-phosphate and phosphatidic acid or from nonesterified fatty acids.1 De novo synthesis of DAG and increased DAG mass are manifest 2 to 3 days after exposure of vascular cells to high-glucose concentrations. In the aorta of diabetic dogs, increased DAG levels are sustained for several years.9 High-glucose concentrations can likely cause increased DAG concentrations by de novo synthesis through several different metabolic pathways. One proposed mechanism is that increased synthesis of DAG is caused by inhibition of the glycolytic enzyme GAPDH, perhaps through poly(ADP-ribosyl)ation during high-glucose concentrations.10 This will cause build-up of the glycolytic intermediate dihydroxyacetone phosphate, which can be used for DAG synthesis after reduction to glycerol-3-phosphate.11

PKCβ activation has been demonstrated in aortic VSMCs grown in high-glucose concentrations,13 whereas other isoforms have been observed in the retina (PKCα, β1, β2, and ε)14 and renal glomeruli of diabetic rats (PKCα and β1).15 The introduction of ruboxistaurin (LY333531), a PKC inhibitor specific for the β isoforms, showed improvement of glomerular filtration rate, albumin excretion rate, and retinal circulation in diabetic rats,16 supporting that activation of PKCβ1 or β2 is important for vascular dysfunction in several organs in diabetes. Even though PKCβ isoforms are expressed at low levels in vascular cells, activation of PKCβ in diabetes leads to an increase in membrane-associated general PKC activity.12

Interpreting Studies of Isoform-Specific PKC Function

Isoform-specific characteristics of PKC are important for drawing conclusions from studies of PKC-mediated effects. Phorbol esters are DAG analogues that only activate conventional and novel PKC, not atypical PKC that lack a DAG binding domain. The most widely used phorbol ester is

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cPKC indicates conventional PKC; nPKC, novel PKC; ROS, reactive oxygen species.
12-myristate 13-acetate (PMA) (synonymous with 12-O-tetradecanoylphorbol 13-acetate). Prolonged activation (>≈12 hours) by phorbol ester leads to downregulation of the expression of conventional and novel PKC,5 albeit differently for individual isoforms and in different cell types.

Phosphorylation of PKC in its activation loop is critical for kinase activity but is not sufficient for activation.6 Generally, PKC translocation (detected as protein content or as activity) from the cytosol to the plasma membrane or to internal membranes is the only reliable measure of PKC activation. Only few PKC inhibitors possess useful specificity toward a single isoform. Ruboxistaurin inhibits splice variants of PKCβ, β1 and β2, with an IC50 ≈50-times lower than that for other PKC isoforms.16 Its general specificity, for PKC as opposed to other kinases, is favorable. Even though it was recently reported that ruboxistaurin inhibits 3-phosphoinositide-dependent kinase-1,17 it does so with an IC50 of 750 nM in kinase assays using purified enzyme,17 whereas PKC inhibition in vivo is observed at plasma concentrations of 19 nM or lower.16 As another example, rottlerin inhibits PKCβ with an IC50 ≈10-times lower than for PKCα and β in vitro.18 Other inhibitors mentioned repeatedly in this review are GF109203X and calphostin C, both of which show selectivity toward conventional and novel PKC.

In general, a more specific approach than pharmacological inhibitors is targeting an individual PKC isoform with dominant-negative techniques (eg, expression of kinase-dead mutants or treatment with oligonucleotides), RNA interference, or targeted gene deletion in animals; reports have been published of mice with deletion of the genes for PKCα, β, γ, and δ, and they are all viable. In addition, isoform-specific activation or inhibition of PKC translocation can be achieved by peptides representing domains in PKC adaptor proteins.19

**Endothelium-Dependent Vasomotor Function**

In diabetes, endothelial vasodilator function is compromised because of changed production of vasodilator and vasoconstrictor substances, particularly nitric oxide (NO), prostacyclin,20 cyclooxygenase-dependent vasoconstrictors,20–22 and ET-1.23 Dysfunction of endothelium-dependent vasodilation in diabetes characterizes a proatherosclerotic state. It likely also plays a causal role in atherogenesis because the underlying changes in vascular intracellular signal transduction and in secretion of endothelium-derived vasoactive factors predispose to atherosclerosis. Through such mechanisms, PKC may mediate proatherosclerotic processes by causing increased production of reactive oxygen species (ROS),24,25 decreased NO production, or increased ET-1 production.26 The role of PKC activation in endothelial dysfunction in diabetes has been demonstrated both in animal models and in humans in vivo. In one study of aorta isolated from rabbit, high-glucose concentrations decreased endothelium-dependent vasodilation.27 PMA produced a similar effect, whereas inhibitors of PKC prevented this phenomenon.27 In aorta of rats with streptozotocin-induced diabetes, PKC activity in the membrane fraction was increased because of upregulation of the PKCβ2 isoform.12 Endothelial dysfunction by hyperglycemia in healthy humans has been prevented by pretreatment with the PKCβ inhibitor ruboxistaurin.28

**Vascular NAD(P)H Oxidase Activation and Induction**

Vascular NAD(P)H oxidase has been shown to be the source of a major part of ROS production in VSMC and endothelial cells during physiological and pathological conditions.29 Vascular NAD(P)H oxidase is a homologue of neutrophil NADPH oxidase. PKC regulation of this enzyme was suggested 2 decades ago when it was shown that neutrophil NADPH oxidase is stimulated by phorbol ester.30 It is possible that neutrophils or monocytes contribute to vascular oxidative stress. Thus, increased monocyte p22phox mRNA was demonstrated in diabetic patients and was partly normalized after lowering of plasma glucose.31 However, this section focuses on regulation of vascular NAD(P)H oxidase.

In VSMC, angiotensin II stimulates vascular NAD(P)H oxidase.32 Angiotensin II is involved in the development of cardiovascular disease in people with and without diabetes, perhaps through mechanisms independent of hypertension.33 A very rapid angiotensin II-stimulated activation of NAD(P)H oxidase involving PKC may cause redox activation of c-Src, leading to a more sustained activation of NAD(P)H oxidase by mobilizing a PKC-independent pathway.34 Angiotensin II also increases NAD(P)H oxidase gene expression. Vascular cells express several catalytic subunit isoforms, including nox2 (synonymous with gp91phox), the
catalytic subunit of neutrophil NADPH oxidase; the suffix stands for phagocyte oxidase). In rats, subcutaneous infusion of angiotensin II for 7 days increased PKC activity in aorta and increased ROS production from the aortic lamina media; gp91phox and p22phox were increased by 7-fold and 3-fold, respectively. Continuous infusion of the isoform-nonspecific PKC inhibitor chelerythrine decreased superoxide production and prevented approximately half the increase in NAD(P)H oxidase subunit expression. Angiotensin II infusion caused hypertension in these animals. Even though increased intra-arterial pressure was shown to increase p47phox Ser phosphorylation (which precedes p47phox membrane translocation and NAD(P)H oxidase activation) and superoxide production in a PKC-dependent manner, the VSMC culture studies described suggest that angiotensin II may stimulate activation of NAD(P)H oxidase independent of increased blood pressure.

High concentrations of glucose and nonesterified fatty acids can also increase superoxide production from NAD(P)H oxidase in cultures of aortic endothelial cells and vascular smooth muscle cells. Rats with streptozotocin-induced diabetes were shown to have a 7-fold increase of gp91phox mRNA expression and increased activity of NAD(P)H oxidase in the aorta. These abnormalities could be partly normalized, without changes in plasma glucose, after treatment of rats with the PKC inhibitor N-benzoylestaurorosporine (synonymous with CGP41251, a PKC inhibitor with selectivity toward conventional PKC isoforms). Induction of several NAD(P)H oxidase subunits have also been shown in endothelial cell culture exposed to constant or intermittent high-glucose concentrations, and such upregulation was inhibited by LY379196, a specific PKCβ inhibitor. Evidence for increased activity of vascular NAD(P)H oxidase in Otsuka Long-Evans Tokushima Fatty rats, a model of insulin resistance, has also been published. In mammmary arteries and saphenous veins from diabetic patients undergoing coronary artery bypass surgery, superoxide production was doubled compared with vessels from nondiabetic controls, likely because of increased endothelial expression of NAD(P)H oxidase subunits.

Only limited information is available about which PKC isoform is involved in neutrophil NADH oxidase activation. In isolated neutrophils, PKCζ inhibition prevented oxidant production, and in monocytes superoxide production caused by incubation in high-glucose concentrations was dependent on PKCα. In endothelial and VSMC culture, the temporal association of increasing DAG concentrations and PKC activation—and the fact that increased superoxide production can be recapitulated by stimulation with PMA—suggest activation of conventional or novel PKC. This is consistent with data from renal glomeruli from rats with streptozotocin-induced diabetes, in which ruboxistaurin treatment prevented increased membrane-associated p47phox and p67phox without changing total p47phox or p67phox mRNA or protein expression. In contrast, tumor necrosis factor-α-stimulated p47phox membrane translocation in cultured endothelial cells was inhibited by antisense oligonucleotides or kinase-dead mutants targeting PKCζ, but not by PKCα, θ, or ε oligonucleotides or prolonged PMA stimulation.

**Endothelial Nitric Oxide Synthase Expression and Dysfunction**

The abnormal metabolic milieu of diabetes and insulin resistance results in the PKC-dependent dysregulation of many endothelium-derived substances that are primarily known vasodilators or vasoconstrictors. For example, in aortic endothelial cells, PKC activation may lead to increased expression of cyclooxygenase, decreased production of the vasodilator prostacyclin, and increased production of the vasoconstrictor thromboxane A2. However, in the next 2 sections, the focus is on regulation of NO and ET-1, for which extensive evidence exists for an implication of PKC. A schematic representation of endothelial NO synthase (eNOS) regulation by PKC is shown in Figure 2.

**Figure 2.** Overview of PKC-dependent activation and induction of eNOS and vascular NAD(P)H oxidase. Please see text for details. IRS-1 indicates insulin receptor substrate-1; eNOS, endothelial nitric oxide synthase.
may uncouple eNOS so that it reduces molecular oxygen rather than L-arginine, producing superoxide. Second, superoxide, from vascular NAD(P)H oxidase or other sources, may react with NO and form peroxynitrite, which potently oxidizes tetrahydrobiopterin, causing uncoupling of eNOS and further eNOS superoxide production.\(^{49,50}\) Third, peroxynitrite may release Zn\(^{2+}\) from the Zn\(^{2+}\)-thiolate complex, breaking the eNOS homodimer and uncoupling eNOS.\(^{51}\) Each mechanism is thus a positive feedback cycle, in which superoxide production causes even more superoxide production. Although PKC activation decreases NO production in cell culture,\(^{47,52}\) it remains to be directly demonstrated that this occurs by uncoupling eNOS.

The cause of induction of eNOS expression in streptozotocin-induced diabetes\(^{35}\) is unclear. In endothelial cells cultured in 5.5 mmol/L glucose, the PKC inhibitors staurosporine,\(^{53}\) chelerythrine,\(^{53}\) and GF109203X\(^{54}\) increased eNOS expression. PMA treatment for 24 hours, expected to downregulate PKC, decreased eNOS expression in endothelial cell culture.\(^{53}\) In another study, the same effect was present after PMA treatment with durations between 6 and 20 hours,\(^{55}\) consistent with PKC downregulation (although PKC expression was not measured at these time points, except for PKC\(\alpha\) and \(\epsilon\)). Ruboxistaurine (LY333531)—used at a dose in which it specifically inhibits PKC\(\beta\)—also increased eNOS mRNA, although overexpression of PKC\(\beta\) did not decrease eNOS mRNA.\(^{54}\) Thus, it seems that PKC activation in itself likely tends to downregulate eNOS expression. However, PKC inhibition was found to prevent eNOS upregulation in aortic endothelial cells cultured in high-glucose concentrations.\(^{22}\) One possible explanation is that PKC increases eNOS transcription and eNOS mRNA half-life through promotion of oxidative stress.\(^{56}\)

To further complicate this issue, it is controversial how eNOS expression is changed in nondiabetic insulin resistance. In the Zucker fatty rat, a model of nondiabetic obesity-associated insulin resistance, eNOS mRNA was found to be decreased to 29% of control levels in microvessels isolated from fat.\(^{54}\) In contrast, eNOS protein was increased 1.7-fold in brain arteries in another study of nondiabetic Zucker fatty rats.\(^{57}\) Thus, it is possible that eNOS expression is regulated differently in large and small vessels or in different tissues. The eNOS expression is unchanged in skin biopsy samples from patients with insulin resistance or diabetes,\(^{58}\) but we are not aware of any published data on endothelial eNOS expression in human conduit vessels.

**ET-1 Expression**

ET-1 is primarily produced by endothelial cells and is one of the most potent vasoconstrictors known.\(^{34}\) Plasma concentrations of ET-1 are increased in patients with type 2 diabetes complicated with atherosclerosis, compared with nondiabetic patients with atherosclerosis and compared with healthy control subjects.\(^{59}\) Infusion of an ET\(_1\) receptor blocker increased forearm blood flow in patients with type 2 diabetes, but not in healthy control subjects, indicating that ET-1 has a basal vasoconstrictor effect only in diabetes.\(^{60}\)

A likely causal role for ET-1 in the development of atherosclerosis was established with a study of apolipoprotein E knockout mice treated with an ET\(_\alpha\) receptor antagonist for 30 weeks. This intervention did not change blood pressure or plasma lipids but reduced ET-1 protein expression, increased NO-dependent vasorelaxation, and decreased atherosclerotic lesion area in the aorta.\(^{61}\)

In microvascular cells, induction of ET-1 caused by high-glucose concentration is associated by increased membrane-associated PKC\(\beta\) and \(\delta\) and can be blocked by GF109203X,\(^{62}\) as well as an inhibitor of the upstream activator of Erk. The nuclear factors downstream of Erk activation, fos and jun, are responsible for induction of ET-1.\(^{63}\) PKC also mediates other stimuli for ET-1 induction. Thus, in heart endothelial cells, PKC mediated angiotensin II-stimulated induction of ET-1 expression, because this could be blocked by calphostin C.\(^{64}\) Furthermore, oleic acid increased ET-1 expression in human aortic endothelial cells, and this could be blocked by inhibition of PKC or nuclear factor \(\kappa B\) (NF\(\kappa B\)). Oleic acid also increased membrane-associated PKC\(\alpha\) and \(\beta\) and increased NF\(\kappa B\) binding activity, and the latter was prevented by treatment with a PKC inhibitor.\(^{65}\) Finally, ET-1 increases its own expression through a PKC-dependent mechanism.\(^{66}\)

**Vascular Insulin Resistance**

Insulin stimulates skeletal muscle blood flow in healthy, lean individuals,\(^{67}\) but this effect is blunted in people with obesity\(^{67}\) or type 2 diabetes.\(^{68}\) Insulin-stimulated vasodilation is dependent on NO.\(^{69–71}\) Systemic or extravascular effects may contribute to insulin-stimulated vasodilation,\(^{72}\) for example, through platelet release of vasodilatory factors mediated by platelet-derived NO.\(^{73}\) However, at least part of the vasodilatory effect of insulin is likely caused by stimulation of endothelial-derived NO as it occurs in isolated vascular rings, and only when the endothelium is intact.\(^{69,74}\) and because insulin-stimulated NO production is dependent on insulin signaling in endothelial cell culture, including activation of the insulin receptor, insulin receptor substrate (IRS), 1-phosphatidylinositol 3-kinase (PI3K), 3-phosphoinositide-dependent kinase-1, Akt, and ultimately eNOS Ser\(^{1177}\) phosphorylation.\(^{75–77}\) Insulin also induces eNOS gene expression through the PI3K signaling pathway.\(^{58}\) In mice with conditional deletion of the insulin receptor gene only in endothelial cells, eNOS expression in aorta is decreased by 62%.\(^{78}\) Thus, insulin-stimulated regulation of endothelium-derived NO may be a factor necessary for vascular homeostasis that is absent in insulin resistance.

Although Ser/Thr phosphorylation of the insulin receptor with ensuing inhibition of insulin receptor autophosphorylation can inhibit insulin signaling, most attention has been given to modification of IRS in attempts to explain diabetes-related inhibition of PI3K-Akt signaling. In endothelial cell culture, PMA inhibits IRS-2–associated PI3K activity,\(^{54}\) and in VSMC, PMA or PKC\(\alpha\) overexpression inhibits IRS-1 tyrosine phosphorylation,\(^{79}\) necessary for binding PI3K. Mutations of IRS-1 are sufficient to alter insulin-stimulated eNOS function, demonstrated by elegant experiments in human umbilical vein endothelial cells with a common single nucleotide polymorphism in IRS-1. Cultures with this polymorphism had decreased insulin-stimulated activation and gene expression of eNOS through IRS-1, PI3K, and Akt.\(^{77}\)
However, studies of insulin signaling compromised by PKC-mediated IRS modification in vascular cells are sparse. Currently, the most definite evidence for PKC-mediated inhibition of IRS comes from studies of insulin action in skeletal muscle of PKCθ knockout mice and shows that PKCθ activation during hyperlipidemia is necessary for inhibition of skeletal muscle IRS-1 tyrosine phosphorylation and insulin-stimulated glucose uptake. Although PKCθ can directly phosphorylate IRS-1, it may also activate other kinases, and the mechanism may include downregulation of IRS-1 expression. However, the PKC isoform involved may well be dependent on species, because lipid infusion in humans activates skeletal muscle PKCβ1, β2, and δ, but not PKCε, θ, or ζ.

In insulin-resistant states, impairment of vascular insulin signaling is selective for insulin-stimulated PI3K pathways, leaving insulin-stimulated mitogen-activated protein kinase (MAPK) signaling unaffected. It is possible that insulin-stimulated ET-1 expression is preserved in insulin resistance, because ET-1 expression is dependent on nuclear factors fos/jun downstream of MAPK signaling.

**Proliferation and Apoptosis of VSMCs**

The MAPK Erk1/2 is a major regulator of growth and proliferation. In VSMCs, Erk1/2 activation and proliferation stimulated by oxidized low-density lipoprotein (oxLDL), glycated serum albumin, oleic acid, and angiotensin II is dependent on PKC. Angiotensin II and high-glucose concentrations may stimulate VSMC growth by PKC-dependent induction of transforming growth factor-β. Other MAPK pathways are activated in diabetes. In rats with streptozotocin-induced diabetes, p38 MAPK activation in aorta was increased compared with control rats. p38 activation was also found in VSMC cultured in 16.5 mmol/L glucose, in which overexpression of PKCθ, but not PKCβ1, increased p38 activation.

During cyclic stretch of VSMC cultures, apoptosis is mediated by PKC-dependent p38 activation. In fact, PKCθ has been shown to promote VSMC apoptosis and limit intimal neoplasia in a model of vascular remodeling in response to increased vascular pressure. Thus, allografting of vena cava to the carotid artery in mice resulted in marked neointimal hyperplasia in the vein graft after 8 weeks. However, vein grafts from PKCθ knockout mice showed exaggerated neointimal hyperplasia and decreased apoptosis compared with vein grafts from wild-type mice. Whether these findings are relevant for diabetes is unclear because VSMCs grown in high-glucose concentrations show decreased apoptosis. This antiapoptotic effect was prevented by prolonged incubation with PMA or treatment with calphostin C, suggesting that conventional or novel PKC were involved. When cultured in 5.5 mmol/L glucose, apoptosis of VSMCs as a result of serum starvation was prevented by overexpression of PKCα or β2, but not PKCδ. Whether VSMC apoptosis is increased or decreased in diabetes remains to be resolved.

The contribution of VSMC apoptosis to clinical atherosclerosis is complex, because intimal neoplasia may promote early atherosclerosis but result in instability of advanced plaques. It is more readily accepted that endothelial apoptosis promotes atherosclerosis. In endothelial cell culture, high-glucose concentrations have been shown to increase apoptosis through activation of caspase-3 and p38.

**Induction of Adhesion Molecules**

A critical early step in atherogenesis is adhesion of monocytes to endothelium, followed by migration of monocytes to the intima where they transdifferentiate into macrophages that take-up lipids and become foam cells. This process is initiated by binding of monocyte integrins to endothelial adhesion molecules. Vascular cell adhesion molecule-1 (VCAM-1) was first associated with early atherogenesis as an inducible adhesion molecule in endothelium covering fatty streaks in hyperlipidemic rabbits. In rabbits, VCAM-1 and E-selectin expression in aortic endothelium is increased by diabetes and further upregulated by diet-induced hyperlipidemia. The clinical relevance of these observations is supported by many human studies. For example, in healthy men, the future risk of myocardial infarction is associated with plasma concentrations of soluble intercellular adhesion molecule-1 (ICAM-1) and in patients with type 2 diabetes, soluble VCAM-1 concentrations are independently associated with cardiovascular mortality.

Cell culture studies have firmly established a role for PKC in mediating leukocyte–endothelial interaction. Adhesion of monocytes to the endothelium is dependent on PKC activation in monocytes and PKC is necessary for monocyte differentiation into macrophages. In endothelial cells cultured in high-glucose concentrations, neutrophil adherence and expression of ICAM-1, E-selectin, and P-selectin are increased, but this can be prevented by incubation with PKC inhibitors.

High-glucose concentrations was shown to increase NFκB activation and VCAM-1 expression in endothelial cell culture, and these events were inhibited by a selective PKCθ inhibitor. However, VCAM-1 induction stimulated by thrombin has been shown to be mediated by PKCδ and PKCζ through binding of NFκB and GATA-2, respectively, to the VCAM-1 promotor. Both conventional and atypical PKC may be involved in regulation of ICAM-1 expression as well. In endothelial cells, ICAM-1 expression is induced by NFκB binding to the ICAM-1 promotor, which may be mediated by PKCζ after tumor necrosis factor-α stimulation or PKCθ after thrombin stimulation. The pathways regulating ICAM-1 transcription during high-glucose concentrations have not yet been described.

**Monocyte–Macrophage LDL Uptake and Foam Cell Formation**

The earliest lesion in atherosclerosis consists of lipid accumulation in the lamina intima and formation of foam cells through uptake of modified LDL, including oxLDL, by monocyte-derived macrophages, dependent on macrophage scavenger receptors. The oxLDL increase induction of ICAM-1 in isolated porcine coronary arteries and leukocyte adhesiveness to endothelium, as well as growth of cultured macrophages. ICAM-1 induction and macrophage...
growth\(^{112}\) can be partly prevented with calphostin C and other PKC inhibitors.

In cultured macrophages, oxLDL was shown to also increase expression of scavenger receptor CD36, and this could be prevented by calphostin C.\(^{113}\) High-glucose concentrations caused induction of another scavenger receptor, LOX-1, in cultures of monocyte-derived macrophages.\(^{114}\) This increase in LOX-1 expression was associated with increased membrane location of PKC\(\beta_2\) and could be prevented by calphostin C or the PKC\(\beta\)-specific inhibitor LY379196.\(^{114}\) The study pointed to ROS production as an upstream event of PKC\(\beta_2\) activation and LOX-1 induction because both could be prevented by the antioxidant N-acetylcysteine.\(^{114}\) Thus, PKC signaling may be involved in foam cell formation by mediating several different effects of oxLDL.

Pharmacotherapy

The PKC\(\beta\) inhibitor ruboxistaurine is currently being tested in ongoing clinical trials with microvascular end points. In the future, clinical trials may determine whether PKC inhibition can prevent atherosclerosis. However, PKC activation may be modified by strategies other than with PKC inhibitors. For example, troglitazone\(^{115}\) and other PPAR\(\gamma\) agonists\(^{116}\) inhibit high-glucose–induced PKC activation in VSMCs\(^{115}\) and endothelial cells,\(^{116}\) and this may be caused by activation of DAG kinase, resulting in decreased DAG concentrations.\(^{116}\)

Conclusions

A large body of literature supports that vascular DAG accumulation and ensuing PKC activation in diabetes lead to endothelial dysfunction by causing activation of vascular NAD(P)H oxidase, dysfunction of eNOS, induction of ET-1, or by other means (Figure 1). The information about eNOS expression and dysfunction in diabetes and high-glucose conditions is contradictory and more studies are clearly needed. Vascular remodeling by VSMC proliferation and apoptosis is regulated by PKC, either through activation of the DAG–PKC pathway or as intermediary signaling, eg, after angiotensin II stimulation. Lastly, leukocyte adhesion, monocyte transdifferentiation, and macrophage growth leading to intimal foam cell formation are critical events in early atherogenesis, and all may be partly regulated through PKC activation. With regard to most of these mechanisms, the characterization of which PKC isoforms are involved is only partial at best.

Earlier studies of the role of vascular PKC activation in diabetes were primarily focused on microvascular dysfunction.\(^{1}\) Preliminary data show that PKC\(\beta\)/apolipoprotein E double knockout mice have decreased atherosclerosis in the aorta compared with apolipoprotein E knockout mice,\(^{117}\) confirming a role for PKC\(\beta\) in atherogenesis, even in the nondiabetic condition of hypercholesterolemia. Before deciding whether the role of PKC deserves to be tested in clinical studies of atherosclerosis, animal models will help evolve this field from the current suggestive evidence concerning proatherosclerotic signaling to studies of actual atherothrombosis.

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