Perspectives in Diabetes

Protein Kinase C Activation and the Development of Diabetic Complications

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Recent studies have identified that the activation of protein kinase C (PKC) and increased diacylglycerol (DAG) levels initiated by hyperglycemia are associated with many vascular abnormalities in retinal, renal, and cardiovascular tissues. Among the various PKC isoforms, the β- and δ-isoforms appear to be activated preferentially in the vasculatures of diabetic animals, although other PKC isoforms are also increased in the renal glomeruli and retina. The glucose-induced activation of PKC has been shown to increase the production of extracellular matrix and cytokines; to enhance contractility, permeability, and vascular cell proliferation; to induce the activation of cytosolic phospholipase A_2; and to inhibit Na^+-K^+-ATPase. The synthesis and characterization of a specific inhibitor for PKC-β isoforms have confirmed the role of PKC activation in mediating hyperglycemic effects on vascular cells, as described above, and provide in vivo evidence that PKC activation could be responsible for abnormal retinal and renal hemodynamics in diabetic animals. Transgenic mice overexpressing PKC-β isoform in the myocardium developed cardiac hypertrophy and failure, further supporting the hypothesis that PKC-β isoform activation can cause vascular dysfunctions. Interestingly, hyperglycemia-induced oxidative stress may also mediate the adverse effects of PKC-β isoforms by the activation of the DAG-PKC pathway, since treatment with D-α-tocopherol was able to prevent many glucose-induced vascular dysfunctions and inhibit DAG-PKC activation. Clinical studies are now in progress to determine whether PKC-β inhibition can prevent diabetic complications. *Diabetes* 47:859–866, 1998

Hyperglycemia induces a bewildering list of changes in vascular or neuronal cells in animal models of diabetes or diabetic patients. The pleotypic nature of the changes is not surprising, since the flux of glucose and its metabolites are known to affect many cellular pathways. The main challenge in this area has been to identify those hyperglycemia-induced biochemical changes that are significant in causing vascular dysfunctions and pathologies. Multiple theories have been proposed to explain the pathogenesis of the various complications involving retina, glomeruli, peripheral nerves, cardiovascular tissues, wound healing, and pregnancy. Although a single theory has not been demonstrated to explain all these changes, a few have emerged that can encompass most of the data that have accumulated in this area (Table 1). Extracellularly, glucose has been demonstrated to react nonenzymatically with primary amines of proteins forming glycated compounds or oxidants (1). These products can secondarily act on inflammatory cells or vascular cells directly via receptor- or non–receptor-mediated processes to cause vascular dysfunctions (2,3).

Excessive glucose can also be transported intracellularly, mainly by the glucose transporter GLUT-1, and metabolized to change redox potential, increase sorbitol production via aldose reductase, or alter signal transduction pathways, such as the activation of diacylglycerol (DAG) and protein kinase C (PKC) levels (4–10). It is possible and likely that the common pathway by which all the intra- and extracellular changes induced by hyperglycemia are mediating their adverse effects is by altering various signal transduction pathways. Surprisingly, the effect of hyperglycemia on signal transduction pathways has not been studied intensely, except for the activation of DAG-PKC, which is known to be important in vascular cells to regulate permeability, contractility, extracellular matrix (ECM), cell growth, angiogenesis, cytokine actions, and leukocyte adhesions, all of which are abnormal in diabetes (11,12). In this article, the available data regarding the effects of hyperglycemia on DAG-PKC and its association with vascular functions will be reviewed. In addition, a perspective on the relative role of DAG-PKC activation in other proposed theories on the adverse effects of hyperglycemia will be evaluated.

PKC

The family of PKCs includes at least eleven isoforms (α, β1, β2, γ, δ, ε, ζ, η, θ, λ, μ), representing the major downstream targets for lipid second messengers or phorbol esters (11–13). PKC isoforms are classified into conventional PKCs (α, β1, β2, γ) (C1 region), which are Ca^{2+} dependent, contain two cysteine-rich, zinc finger–like motifs, and are binding sites of DAG or phorbol ester, and Ca^{2+}/phospholipids (C2 region). New PKCs (δ, ε, η, θ, μ) are DAG sensitive but Ca^{2+} independent because of the absence of C2 region, and atypical PKCs (ζ, λ) are rather insensitive to DAG and lack one cysteine-rich motif in their C1 region but can be activated by phosphatidylserine.

The source of DAG that activates PKC can be derived from the hydrolysis of phosphatidylinositides (PIs) or from the metabolism of phosphatidylcholine (PC) by phospholipase C

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Received for publication 28 October 1997 and accepted in revised form 31 March 1998.

G.L.K. serves as a consultant for Eli Lilly.

cGMP, cyclic guanosine monophosphate; $cPLA_2$, cytosolic phospholipase A_2; DAG, diacylglycerol; ECM, extracellular matrix; ET-1, endothelin-1; GFR, glomerular filtration rate; iNOS, inducible nitric oxide synthase; NO, nitric oxide; PC, phosphatidylcholine; PGE_2, prostaglandin E_2; PI, phosphatidylinositide; PKC, protein kinase C; PLC, phospholipase C; PLD, phospholipase D; TGF-β, transforming growth factor-β; VEGF, vascular endothelial growth factor; VPF, vascular permeability factor.

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TABLE 1
Possible mechanisms to explain the adverse effects of hyperglycemia

Nonenzymatic glycation process
Oxidative-reductive stress
Aldose-reductase activation
DAG-PKC activation

(PLC) or D (PLD), although recent evidence has demonstrated that each isoform could be regulated by more than one lipid second messenger (9), such as the activation of PKC-ζ by phosphoinositide 3,4,5 phosphate (PIP3) (12–14) (Fig. 1). Multiple isoforms are expressed in each cell, but in spite of extensive studies, attribution of a specific function to a specific isoform cannot be consistently established, suggesting that several isoforms can mediate a similar range of functions and that their actions may be cell specific (12,15).

MECHANISMS OF HYPERGLYCEMIA-INDUCED PKC ACTIVATION

Increases in total DAG contents have been demonstrated in a variety of tissues associated with diabetic vascular complications, including retina (16), aorta, heart (17), and renal glomeruli (18,19) from diabetic animal models and patients (Table 2) and in classically termed "insulin sensitive" tissues, such as the liver and skeletal muscle (20,21). In all vascular cells studied, increasing glucose levels from 5 to 22 mmol/l in the media elevated the cellular DAG contents (17), an effect that may not occur immediately but which reaches maximum in 3–5 days after elevating glucose levels (18,22). The elevation of DAG in the vasculature induced by hyperglycemia or diabetes is maintained chronically, as reported by Xia et al. (22), who have shown that DAG contents were still increased in the aorta of diabetic dogs even after 5 years of disease. In fact, Inoguchi et al. (17) have reported that euglycemic control by islet cell transplant after 3 weeks was not able to reverse the increases in DAG or PKC levels in the aorta of diabetic rats. These results clearly suggest that the activation of DAG-PKC can be sustained chronically.

Cellular DAG contents can be increased by agonist-stimulated hydrolysis of PI or PC by PLC or PLD (Fig. 1) (11–13). Because inositol phosphate products are not increased by hyperglycemia in aortic cells and glomerular mesangial cells, increases in PI hydrolysis are most likely not involved in diabetes (22,23). The increases in DAG content could also be from PC metabolism, since Yasunare et al. (24) reported that PLD activity was increased by elevation of glucose level in aortic smooth muscle cells, but the amount of total DAG was not quantitated. However, most studies have shown that the source of glucose-induced DAG was from the de novo pathway, as shown in Fig. 1. Labeling studies using [6-^{3}H]- or [U-^{14}C]glucose have demonstrated that elevation of glucose level increased the incorporation of labeled glucose into the glycerol backbone of DAG in aortic endothelial cells (25), aortic smooth muscle cells (22), and glomeruli (18), clearly establishing that the increased DAG contents were partially derived from glycolytic intermediates (26–28). Palmitic acid and oleic acid are the predominant fatty acids that are incorporated into DAG from the de novo pathway and the metabolism of PC, again consistent with the findings in vascular tissues from diabetic animals (25). In contrast, DAG derived from PLC activations consist mainly of 1-stearolyl, 2-arachidonyl fatty acid, which was not altered by glucose (25,29).

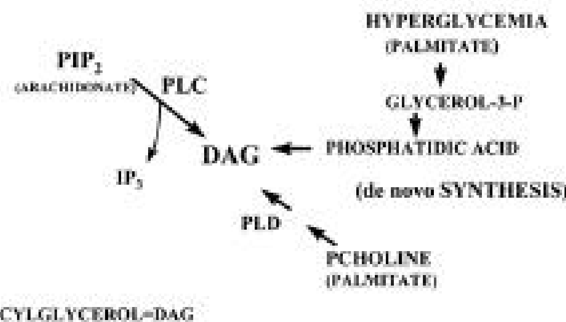

FIG. 1. Possible sources of DAG in the vascular cells. Hyperglycemia increased DAG synthesis mainly from glucose to glycerol 3-phosphate (glycerol-3-p), and possibly from PC (pcholine), by the actions of PLC, PLD, phosphoinositol diphosphate (PIP$_2$), and inositol triphosphate (IP$_3$).

The activation of PKC by hyperglycemia may be tissue specific, since it was noted in the retina (16), aorta, heart (17), and glomeruli (8,18), but may not occur in the brain (16) and peripheral nerves (30) (Table 2). Similar increases in DAG and PKC levels have also been shown in multiple types of cultured vascular cells when glucose levels were increased (Table 2) (8,16,22,31). Thus, it is likely that the DAG-PKC pathway is activated by hyperglycemic-diabetic states in all vascular cells. Of the various PKC isoforms in vascular cells, PKC-β and -δ isoforms appear to be preferentially activated by immunoblotting studies in aorta and heart of diabetic rats (17) and in cultured aortic smooth muscle cells (32) exposed to high levels of glucose. However, increases in other isoforms, such as PKC-α, -β2, and -ε isoforms in the retina (18) and PKC-α, -β1, and -δ in the glomerular cells (33,34) of diabetic rats have also been found. These results have clearly demonstrated that diabetes and hyperglycemia will activate DAG-PKC pathways in many types of tissues, including vascular tissues. As stated earlier, glucose and its metabolites can cause many cellular parameters to change. For a hyperglycemia-induced change to be credible as a causal factor of diabetic complications, it has to be shown to be chronically altered, to be difficult to reverse, to cause similar vascular changes when activated without diabetes, and to be able to prevent complications when it is inhibited. So far, we have presented evidence on the DAG-PKC activation that fulfills the first two criteria. In the following, supporting data will be presented to suggest that the last two criteria will also be fulfilled in the near future.

CELLULAR AND FUNCTIONAL ALTERATIONS IN VASCULAR CELLS INDUCED BY DAG-PKC ACTIVATION

Multiple cellular and functional abnormalities in the diabetic vascular tissues have been attributed to the activation of DAG-PKC pathways. Some of these adverse changes in the vascular cells or tissue have been diagrammed in Fig. 2.

VASCULAR BLOOD FLOW

Abnormalities in vascular blood flow and contractility have been found in many organs of diabetic animals or patients, including the kidney, retina, peripheral arteries, and

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TABLE 2
Summary of DAG level and PKC activity in cultured cells exposed to high glucose and tissues isolated from diabetic animals

	DAG	PKC
Cultured cells		
Retinal endothelial cells		
Aortic endothelial cells		
Aortic smooth muscle cells		
Renal mesangial cells		
Pericytes		
Tissues		
Retina (diabetic rats and dogs)		
Heart (diabetic rats)		
Aorta (diabetic rats and dogs)		
Renal glomeruli (diabetic rats)		
Brain (diabetic rats)	ND	No change
Peripheral nerve		

ND, not determined; , increase; , decrease; , no change.

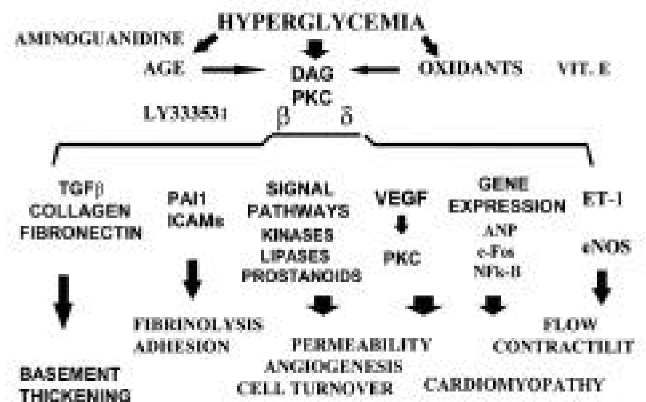

FIG. 2. Outline of some potential changes that can be induced by hyperglycemia-induced activation of the DAG-PKC pathway. Some possible inhibitors of this process are aminoguanidine, an inhibitor of glycation process; vitamin E (Vit. E), an antioxidant; and LY333531, a specific inhibitor of PKC-β isoform. ANP, atrial natriuretics peptide; ICAM, intracellular adhesion molecule; PAI-1, plasminogen activator inhibitor 1.

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microvessels of peripheral nerves. In the retina of diabetic patients (35,36) and animals (37–39) with short durations of disease and without clinical retinopathy, retinal blood flows have been shown to be decreased. However, retinal blood flow may be normal or increased with longer duration of retinopathy (40). Multiple lines of evidence have supported that the decreases in retinal blood flow are due to PKC activation. For example, introduction of a PKC agonist, such as phorbol esters, into the retina will decrease retinal blood flow (16). Decreases in retinal blood flow in diabetic rats have been reported to be normalized by PKC inhibitors (16,19). In addition to the retina, decreases in blood flow have also been reported in the peripheral nerves of diabetic animals that were normalized by PKC inhibition (41,42), although some reports have noted increases in neuronal blood flow in diabetic rats as well (6).

One of the potential mechanisms by which PKC activation could be causing vasoconstriction in the retina is by increasing the expression of endothelin-1 (ET-1) (43). We have reported that the expression of ET-1, a potent vasoconstrictor, is increased in the retina of diabetic rats and that intravitreous injection of endothelin-A (ET-A) receptor antagonist BQ123 prevented the decrease in retinal blood flow in diabetic rats (43). The decrease in blood flow to the retina could lead to local hypoxia, which is a potent inducer of vascular endothelial growth factor (VEGF), causing increases in permeability and microaneurysms (44,45).

Abnormalities in hemodynamics have been clearly documented to precede diabetic nephropathy (46,47). Elevated renal glomerular filtration rate (GFR) and modest increases in renal blood flow are characteristic findings in IDDM patients (46,47) and experimental diabetic animals (48). Diabetic glomerular hyperfiltration is likely to be the result of hyperglycemia-induced decreases in arteriolar resistance, especially at the level of afferent arterioles (49,50), resulting in an elevation of increases of glomerular filtration pressure. Multiple mechanisms have been proposed to explain the increases in GFR and glomerular filtration pressure, including an enhanced activity of angiotensin (51) and culturation in prostanoid productions (52–54). It is possible that the activation of DAG-PKC may also play a role in the enhancement of angiotensin actions, since angiotensin mediates some of its activity by the activation of the DAG-PKC pathway (52). In addition, increases in vasodilatory prostanoids, such as prostaglandin E_2 (PGE_2) and prostaglandin I_2 (PGI_2), could also be involved in causing glomerular hyperfiltration in diabetes (53,54). The enhanced production of PGE_2 induced by diabetes and hyperglycemia could be the result of sequential activation of PKC and cytosolic phospholipase A_2 ($cPLA_2$), a key regulator of arachidonic acid synthesis (55–58).

In the microvessels, increases in the activities of nitric oxide (NO), a potent vasodilator, may also enhance glomerular filtration (59). Urinary excretions of NO_2 and NO_3, stable metabolites of NO, have been reported to be increased in diabetes of short duration (59–61), possibly due to enhanced expression of inducible NO synthase (iNOS) gene and increased production of NO in mesangial cells (62). In addition, both increases in iNOS gene expression and NO production can be mimicked by PKC agonists and inhibited by PKC inhibitors when induced by hyperglycemia (62), suggesting that NO production might be increased in diabetes through PKC-induced iNOS overexpression. In addition, Graier et al. (63) have suggested that NO production was enhanced by the elevation of glucose levels, possibly by the increased flux of Ca^{2+} and its activation of iNOS . However, Craven et al. (64) reported that the production of glomerular NO and its second messenger, cyclic guanosine monophosphate (cGMP), were decreased in diabetic rats in response to cholinergic agents and that PKC inhibitors restored the glomerular cGMP production. Several authors have also reported that elevated levels of glucose decreased iNOS expression in vascular smooth muscle cells and that these effects of glucose were reversed by general PKC-β inhibitors (65,66). Thus, PKC can regulate renal hemodynamics by increasing or decreasing NO production dependent on the cell type and tissue location.

In the macrovessels, increases in contractility found in diabetes are due to a delay in the relaxation response after

contraction induced by cholinergic agents (67–70). These abnormal responses can also be prevented by PKC inhibitors (71), suggesting that PKC activation plays a general role in causing abnormal peripheral hemodynamics in diabetes.

VASCULAR PERMEABILITY AND NEOVASCULARIZATION

Increased vascular permeability is another characteristic systemic vascular abnormality in diabetic animals, in which increased permeability to albumin can occur as early as 4–6 weeks of diabetes (72), suggesting endothelial cell dysfunctions. PKC activation can directly increase the permeability of albumin and other macromolecules through barriers formed by endothelial cells (73,74) and skin chamber granulation tissues (75), probably by phosphorylating cytoskeletal proteins forming the intracellular junctions (76,77). Interestingly, phorbol ester–induced increases in endothelial permeability may be regulated by PKC-β1 activation (78), which is consistent with the preferential activation of PKC-β isoforms in diabetes.

PKC activation could also regulate vascular permeability and neovascularization via the expression of growth factors, such as the VEGF/vascular permeability factor (VPF), which is increased in ocular fluids from diabetic patients and has been implicated in the neovascularization process of proliferative retinopathy (44). We have reported that both the mitogenic and permeability-inducing actions of VEGF/VPF are partly due to the activation of PKC-β isoform via the tyrosine phosphorylation of PLC. Inhibition by PKC-β–selective inhibitor LY333531 can decrease endothelial cell proliferation, angiogenesis, and permeability induced by VEGF (79). In addition, Williams et al. (45), have shown that the expression of VEGF was increased in aortic smooth muscle cells by elevating glucose concentration and was inhibited by PKC inhibitors.

In the kidney, the expression of transforming growth factor-β (TGF-β) has been shown to be increased in the glomeruli of diabetic patients and experimental animals. Similar increases of TGF-β have also been reported in cultured mesangial cells exposed to high glucose levels (9). Because TGF-β can directly cause the overexpression of ECM, PKC inhibitors have been shown to inhibit TGF-β expression by hyperglycemia and may prevent the mesangial expansion observed in diabetic nephropathy (7,9,11).

NA^+-K^+-ATPASE

Na^+-K^+-ATPase, an integral component of the sodium pump, is involved in the maintenance of cellular integrity and functions such as contractility, growth, and differentiation (5). It is well established that Na^+-K^+-ATPase activity is generally decreased in the vascular and neuronal tissues of diabetic patients and experimental animals (5,80–82). However, studies on the mechanisms by which hyperglycemia inhibited Na^+-K^+-ATPase activity have provided some conflicting results regarding the role of PKC.

Phorbol esters, activators of PKC, have been shown to prevent the inhibitory effect of hyperglycemia on Na^+-K^+-ATPase (5), which suggests that PKC activity might be decreased in diabetic conditions. Yet we have recently provided evidence that elevated glucose levels (~20 mmol/l) will increase PKC and $cPLA_2$ activities, leading to increases in arachidonic acid release and PGE_2 production and decreases in Na^+-K^+-ATPase activity. Inhibitors of PKC or PLA_2 prevented glucose induced reduction in Na^+-K^+-ATPase activities in aortic smooth muscle cells or mesangial cells (56). The apparent paradoxical effects of phorbol ester and hyperglycemia are probably due to the quantitative and qualitative differences of PKC stimulation induced by these stimuli. Phorbol ester, which is not a physiological activator, can increase many PKC isoforms and PKC activity by 5–10 times, whereas hyperglycemia can only increase PKC by twofold, a physiologically relevant change (56) that appears to affect only a few isoforms. Thus, the results derived from the studies using phorbol esters are difficult to interpret with respect to their physiological significance.

ECM COMPONENTS

Thickening of capillary basement membrane is one of the early structural abnormalities observed in almost all the tissues, including the vascular system, in diabetes (83). Because basement membrane can affect numerous functions, such as in structure support, vascular permeability, cell adhesion, proliferation, differentiation, and gene expression, alterations in its components may cause vascular dysfunctions (84).

Histologically, increases in type IV and VI collagen, fibronectin, and laminin and decreases in proteoglycans are observed in the mesangium of diabetic patients (85,86). These effects can be replicated in mesangial cells incubated in increasing glucose levels (5–20 mmol/l) that were prevented by general PKC inhibitors (87–92). As described above, increased expression of TGF-β has been implicated in the development of mesangial expansion and basement membrane thickening in diabetes (93–98). Ziyadeh et al. (99) and Sharma et al. (100) reported that neutralizing TGF-β antibodies significantly reduced collagen synthesis and gene expression of type IV collagen and fibronectin in the renal cortex of diabetic rats and in cultured mesangial cells exposed to high glucose level. Because PKC activation can increase the production of ECM and TGF-β expression, it is not surprising that several reports have shown that PKC inhibitors can also prevent hyperglycemia- or diabetes-induced increases in ECM and TGF-β in mesangial cells or renal glomeruli (32).

USE OF PKC-β ISOFORM SELECTIVE INHIBITOR

Numerous studies have used PKC inhibitors, such as staurosporine, H-7, and GF109203X, to examine the role of PKC activation in diabetic vascular complications, but long-term studies involving PKC inhibitors have not been possible because of their toxicities, which are the result of their nonspecificity for other kinases (19,101). Because analysis of retina, kidney, and cardiovascular tissues of diabetic rats showed that PKC-β isoforms were preferentially activated (17,19,32), a specific inhibitor for PKC-β isoforms should be more effective and less toxic than a non–isoform-specific PKC inhibitor.

Recently, we have reported that abnormal retinal and renal hemodynamics and the increases in albuminuria in diabetic rats can be ameliorated by an orally available PKC-β isoform selective inhibitor, LY333531, in parallel with the inhibition of diabetes-induced PKC activation in retina and renal glomeruli (19). LY333531 prevented the overexpression of TGF-β, α_1 (IV) collagen, and fibronectin in renal glomeruli of diabetic rats (33). These results again suggested that activation of PKC-β isoforms are involved in the development of some of the early abnormalities of diabetic vascular complications. PKC

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inhibitors could also mediate their effect by the inhibition of angiotensin actions, which may be increased because ACE inhibitors have been proven to delay the progression of nephropathy (101). However, long-term studies are needed to clarify the usefulness of LY333531 in preventing the chronic pathological changes of diabetic vascular complications.

VITAMIN E

There is a great deal of evidence that suggests that hyperglycemia may be mediating some of its adverse effects by producing excessive amounts of oxidants (102). Recent studies using antioxidants have provided some support that oxidative stress is increased in the diabetic state. The present discussion will only focus on the role of vitamin E, since it is a well-studied antioxidant that has the additional interesting property of inhibiting the activation of DAG-PKC in vascular tissues and cultured vascular cells exposed to high glucose levels (32,103,104). We have reported that vitamin E can inhibit PKC activation probably by decreasing DAG levels (32,103), since the direct addition of vitamin E to purified PKC-α or -β isoforms in vitro did not have any inhibitory effect (103). Recently, the activation of DAG kinase has been suggested to be one potential site of action for vitamin E to inhibit PKC, since DAG kinase metabolizes DAG to phosphatidic acid, thus attenuating PKC activity (105).

Biochemically, intraperitoneal injection of vitamin E prevented the increases in both DAG and active PKC levels in the retina, aorta, heart, and renal glomeruli of diabetic rats (103,105). Functionally, vitamin E treatment prevented the abnormal hemodynamics in retina and kidney of diabetic rats in parallel with the inhibition of DAG-PKC activation (103,105). In addition, increased albuminuria was prevented by vitamin E treatment in diabetic rats (105). Thus, it is possible that some of the PKC activation induced by diabetes could also be the result of excessive oxidants, which are known to activate PKC and can be produced by hyperglycemia, leading to the development of vascular dysfunctions in early stages of diabetes (106). Multicenter trials are needed to determine whether high doses of vitamin E can prevent the microvascular complications of diabetes.

CARDIOMYOPATHY

The development of cardiac dysfunctions in the absence of significant coronary artery disease and the high mortality rate after myocardial infarction in diabetic patients have led to the postulate of a specific diabetic cardiomyopathy (107). Multiple studies have reported that a host of cardiac dysfunctions can occur in diabetic rats, suggesting that hyperglycemia can cause myocardium dysfunction directly. We have reported that the diabetic state will induce the activation of PKC-β isoform in the hearts of rats (17). To determine whether activation of PKC-β isoform can cause cardiac abnormalities, we have made transgenic mice overexpressing PKC-β2 isoform specifically in the myocardium by the use of tissue-specific promoter myosin heavy chain α(MHC-α) (108). These mice developed cardiac hypertrophy, cardiomyocyte injuries, and fibrosis at 8–12 weeks of life. At the 20th week, cardiac atrophy and severe fibrosis were observed (108). Treatment with PKC-β isoform inhibitor (LY333531) prevented most of the functional and pathological changes in the hearts of the transgenic mice (108), clearly demonstrating that excessive PKC-β activation can cause cardiomyopathy.

CONCLUSION AND SPECULATION

These results have firmly established that diabetic conditions will activate the DAG-PKC signal transduction pathway. The initiating factors are chiefly metabolic, with hyperglycemia as the main element, although other metabolic changes, such as free fatty acids, may also be involved. The finding that the secondary metabolic products of glucose, such as glycation products and oxidants, can also increase DAG-PKC suggest that the activation of DAG-PKC could be a common downstream mechanism by which multiple byproducts of glucose are exerting their adverse effects. It is not surprising that changes in DAG-PKC may serve this role, since this signal transduction pathway is known to regulate many vascular actions and functions, as described above (11,12). It is also likely that hyperglycemia and diabetes may also affect other signal transduction pathways besides DAG-PKC, since many of these pathways can regulate vascular functions and can be affected by changes in PKC activities.

More than one PKC isoform was activated by diabetes or hyperglycemia, which again is not surprising, since many isoforms are DAG sensitive, and each cell usually contains several PKC isoforms (11,12). However, it is surprising that the results of immunoblotting and the use of PKC-β isoform inhibitor appear to suggest that PKC-β isoforms are predominantly activated in all vascular tissues and may be responsible for many of the vascular dysfunctions.

Although the correlation between the activation of DAG-PKC and diabetic vascular and neurological complications is fairly substantial in rodent models of diabetes (7,8,17–19,31), there is not a great deal of reported evidence that DAG-PKC levels are increased in the vasculature of diabetic patients because of the difficulty in obtaining fresh vascular or neurological tissues for the measurement of DAG-PKC levels. Several lines of evidence are still needed to determine whether DAG-PKC activations are important for the development of diabetic complications. First, the activation of DAG or PKC needs to be chronically inhibited in an animal model of diabetes to determine which of the various retinal and renal pathologies can be prevented. These experiments to inhibit PKC chronically can be accomplished by using specific inhibitors to PKC isoform or by making transgenic mice strains lacking a specific PKC isoform. These experimental approaches are now possible, since specific and relatively nontoxic oral inhibitors of PKC-β isoforms are now available to test which of the vascular dysfunctions are due to PKC-β isoform activation (19).

Second, most of the findings in the literature so far are not in human vascular tissues, which may be different from animal vessels with regard to glucose metabolism and PKC isoform expression. Thus, a PKC-β isoform inhibitor is only useful in diabetic patients if the same profile of PKC isoforms are activated or expressed in these patients and in the diabetic rodent models. In addition, the secondary markers of PKC activation need to be identified, since they can be used to monitor the effectiveness of PKC inhibition when treated with intensive glycemic control or PKC inhibitors. Progress has been made to identify some of these potential secondary parameters of vascular pathologies, such as the levels of VEGF, changes in retinal hemodynamics, and endothelial cell functions (38,44,45,56).

Third and finally, the most important evidence in determining the role of the activation of DAG-PKC in the vascular

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complication of diabetic patients would be clinical trials using PKC isoform–specific inhibitors that are now in progress. This important point needs to be stressed, since multiple agents have been shown to be capable of reversing vascular abnormalities induced by hyperglycemia in rodent models of diabetes, but none have succeeded in clinical trials (109,110), clearly pointing out the inadequacy of most of the rodent models for diabetic complications. An additional potential problem with any drugs that will be used by patients chronically is toxicity. For the inhibition of PKC, this could potentially be a major hurdle, since PKC activation is involved in so many vital functions of the cell.

Thus, a tremendous amount of information suggests that the activation of the DAG-PKC pathway by hyperglycemia and diabetes can cause some of the vascular dysfunctions and possibly neurovascular changes and insulin resistance. These results are only suggestive. Definitive studies, as described above, are ongoing and should determine clearly the role of DAG-PKC in the development of the various complications of diabetic patients.

ACKNOWLEDGMENTS

This work was supported by National Eye Institute of Health Grant EY-05110-11, Diabetes and Endocrinology Research Center Grant DK-36836, and a grant from Eli Lilly (to G.L.K.), as well as by support from the Adler Foundation.

The authors wish to thank Joan C. Taylor for assistance in preparation of this manuscript.

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