Mesangial cell protein kinase C isozyme activation in the diabetic milieu

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Whiteside, Catharine I., and John A. Dlugosz. Mesangial cell protein kinase C isozyme activation in the diabetic milieu. Am J Physiol Renal Physiol 282: F975–F980, 2002; 10.1152/ajprenal.00014.2002.—High-glucose-induced activation of mesangial cell protein kinase C (PKC) contributes significantly to the pathogenesis of diabetic nephropathy. Excess glucose metabolism through the polyol pathway leads to de novo synthesis of both diacylglycerol (DAG) and phosphatidic acid, which may account for increased mesangial cell PKC-α, -β, -δ, -ε, and -ζ activation/translocation observed within 48-h exposure to high glucose. Raised intracellular glucose causes generation of reactive oxygen species that may directly activate PKC isozymes and enhance their reactivity to vasoactive peptide signaling. In both diabetic rodent models of diabetes and cultured mesangial cells, PKC-β appears to be the key isozyme required for the enhanced expression of transforming growth factor-β1, initiation of early accumulation of mesangial matrix protein, and increased microalbuminuria. Enhanced collagen IV expression by mesangial cells in response to vasoactive peptide hormone stimulation, e.g., endothelin-1, requires PKC-β, -δ, -ε and -ζ. Loss of mesangial cell contractility to potent vasoactive peptides and coincident F-actin disassembly are due to high-glucose-activation of PKC-ζ. Inhibition of mesangial cell PKC isozyme activation in high glucose may prove to be the next important treatment for diabetic nephropathy.

diacylglycerol; polyol pathway; collagen IV; reactive oxygen species; endothelin-1

KIDNEY DISEASE ASSOCIATED with diabetes is the leading cause of chronic renal insufficiency in North America (69). Clinical trials have demonstrated that high glucose is the principal cause of renal damage in both type 1 (12) and 2 diabetes (68). Although the underlying genetic predisposition to this microvascular complication remains elusive (53), investigation of cellular and molecular mechanisms has identified an integrated group of signaling and gene expression systems triggered directly or indirectly by high glucose (65). Altered mesangial cell function in high glucose is central to the pathogenesis of progressive diabetic glomerulosclerosis. Progressive accumulation of mesangial matrix protein and decreased degradation of mesangial extracellular matrix proteins, including collagen IV (1, 42, 59) and fibronectin (49), ultimately obliterates the glomerular capillary loops, leading to renal failure. In the diabetic milieu, mesangial cells are transformed into a sclerotic phenotype by the direct effects of high glucose (4), including enhanced expression of autocrine growth factors (54), and by intraglomerular hypertension caused by high-glucose-induced loss of afferent arteriolar contractility (73). In the search for the cellular mechanisms of diabetic complications, a pivotal role for protein kinase C (PKC) is recognized in every cell type targeted by the toxic effects of high glucose (45). The following describes high-glucose-induced activation of mesangial cell PKC as a major contributor to the initiation and propagation of diabetic glomerulosclerosis.

EXPRESSION OF MESANGIAL CELL ISOZYMES

The PKC family of at least 12 isozymes is classified into conventional PKC-α, -βI, -βII, and -γ, which re-

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quire Ca\textsuperscript{2+} and are activated by diacylglycerol (DAG)
or phorbol ester; novel PKC-\(\delta\), \(\epsilon\), \(\eta\), and \(\theta\), which are
Ca\textsuperscript{2+} independent and activated by DAG or phorbol
ester (60); and atypical PKC-\(\zeta\) and \(\lambda\), which are nei-
ther Ca\textsuperscript{2+} nor DAG sensitive (75). The expression pat-
ttern and response of specific PKC isozymes to growth
factors confer unique cellular phenotypic characteris-
tics, including differentiation during development, main-
tenance of a normal differentiated state (32, 56), or
generation of an abnormal phenotype in response to
pathogenic stimuli, e.g., high glucose. The function of
individual PKC isozymes is likely conferred, in part,
by their subcellular localization and binding to speci-
cific anchoring proteins after activation and translocation
(9, 46). Furthermore, differences in the individual
domain structure of the PKC isozymes allows for the
identification of pharmacological agents that have
isozyme-selective modulation (30, 70).

In cultured mesangial cells, expression of PKC-\(\alpha\), -\(\beta\)II,
-\(\beta\)I, -\(\delta\), -\(\epsilon\), and -\(\zeta\) has been reported by most investiga-
tors (2, 6, 26, 33, 36, 55). Koya et al. (39) confirmed the
detection of PKC-\(\beta\)I in both cultured mesangial cells
and isolated glomeruli, although they reported that
PKC-\(\beta\)II expression was less abundant. We have con-
sistently demonstrated the presence of PKC-\(\alpha\), -\(\beta\)II, -\(\delta\),
-\(\epsilon\), and -\(\zeta\) in glomeruli isolated from normal and strep-
tozotocin (STZ)-induced diabetic rats (5) and the iden-
tical expression of the same PKC isozymes, as well as
PKC-\(\beta\)I, in primary cultured rat mesangial cells (11,
18, 31, 36). Using immunogold labeling of normal and
STZ-diabetic rat glomeruli examined by transmission
electron microscopy, we have identified that mesan-
gial, visceral epithelial, and endothelial cells all ex-
press PKC-\(\alpha\), -\(\beta\)II, -\(\delta\), and -\(\epsilon\) (5).

**ALTERNED RESPONSE OF MESANGIAL CELL-SPECIFIC
PKC ISOZYMES IN HIGH GLUCOSE**

The effects of high glucose on glomerular cell PKC
isozyme expression and activity have been identified
using animal models of diabetes and in cultured cells.
Kikkawa et al. (37) were the first to report increased
mesangial cell membrane content of PKC-\(\alpha\) and -\(\zeta\) after
3 and 5 days of high-glucose exposure, taken as evi-
dence of translocation and probable activation of these
isozymes. In primary cultured rat and human mesan-
gial cells, Koya et al. (39) detected increased cell mem-
brane PKC-\(\alpha\) and -\(\beta\)I after 4 days of high glucose.
After exposure of primary cultured rat mesangial cells to
30 mM glucose, our laboratory has observed that total
cellular PKC-\(\delta\) is significantly increased at 24 h (13)
and total PKC-\(\alpha\), -\(\beta\)II, and -\(\epsilon\) are increased by 48 h (36).
These findings indicate enhanced expression or de-
creased degradation of these particular isozymes in
high glucose. Immunoblotting of mesangial cellular
fractions revealed increased membrane and nuclear
PKC-\(\alpha\), -\(\delta\), and -\(\epsilon\) after 48 h in 30 mM glucose (36) and
enhanced recovery of membrane-associated PKC-\(\zeta\) as
early as 24 h of high-glucose exposure (13). Under the
same conditions, we observed by confocal immunofluo-
rescence imaging a high-glucose-induced pattern of
mesangial cell PKC -\(\alpha\), -\(\beta\)II, -\(\delta\), and -\(\epsilon\) translocation
characterized by enhanced fluorescence labeling of the
plasma membrane, the nucleus (including nuclear
membrane), and, possibly, cytoskeletal elements (36,
76). By immunoblotting the cytosolic and membrane
cellular fractions of glomeruli isolated from the STZ-
diabetic rat, we and others have demonstrated in-
creased membrane recovery of PKC-\(\alpha\), -\(\delta\), and -\(\epsilon\), sug-
gesting an activation/translocation pattern of these
PKC isozymes in the diabetic milieu (5, 24, 37, 39).

Many laboratories have identified increased total
PKC activity in both glomeruli from STZ-diabetic rats
(10, 39, 61) and primary mesangial cells cultured in
high glucose (4, 8, 11, 25, 36) by analyzing PKC-specific
substrate phosphorylation in total cell lysates or cellular
fractions. Recently, our laboratory reported in-
creased PKC-\(\delta\) and -\(\zeta\) activity in primary rat mesangial
cells exposed to high glucose for 24 h, as analyzed by
immunoprecipitating these isozymes from total cell
lysate or membrane fractions and measuring phos-
phorylation of isozyme-specific substrates. This ap-
proach enables a more sensitive and specific detection
of the activity of individual PKC isozymes. The exact
mechanisms whereby mesangial cell PKC isozymes
appear to be sequentially activated in high glucose over
24–48 h remain to be elucidated.

**HOW DOES HIGH GLUCOSE CAUSE MESANGIAL CELL
PKC ACTIVATION?**

Entry of glucose into mesangial cells through
GLUT1 transport raises the intracellular concentra-
tion of glucose, reflecting the hyperglycemic state (28,
29). Entry of excess glucose into intermediate meta-
bolic pathways stimulates interactive signaling mech-
anzisms, which directly or indirectly activate PKC
isozymes. The following describes the roles of the
polyol pathway and the generation of reactive oxygen
species contributing to the enhanced responsiveness of
mesangial cell PKC isozymes in high glucose.

*The Polyol Pathway*

The polyol pathway converts glucose to sorbitol and,
subsequently, fructose, generating an increased ratio
of NADH/NAD\textsuperscript{+} (64). This change in redox poten-
tial drives conversion of glycolytic triose phosphate inter-
mediates, produced during glycolysis or metabolism
through the pentose phosphate pathway, into the sec-
ond messengers phosphatidic acid and DAG. De novo
synthesized DAG may directly activate conventional
and novel PKC isozymes, whereas phosphatidic acid
may activate PKC-\(\zeta\) (43). In cultured mesangial cells,
aldose reductase inhibition prevents the accumulation
of membrane-associated DAG observed within 24 h of
exposure to high glucose (11) and also prevents the
translocation pattern of PKC-\(\delta\) and -\(\epsilon\) caused by 48 h of
high glucose (36). By contrast, in the same mesangial
cell preparation, two different aldose reductase inhib-
itors failed to prevent high-glucose-enhanced translo-
cation of PKC-\(\alpha\) and -\(\beta\) (36). These data are in keeping
with high-glucose-induced activation of PKC-\(\alpha\) and -\(\beta\)
by a mechanism(s) that is independent of the polyol pathway.

In diabetic animal models and human studies where aldose reductase inhibitors were used to treat or prevent diabetic nephropathy, the outcomes remain controversial (44, 50, 51, 52). We have demonstrated that treatment of STZ-diabetic rats with tolrestat prevents glomerular hyperfiltration, glomerular hypertrophy, and increased microalbuminuria but does not prevent fractional mesangial expansion in the first 12 wk of diabetes (15). Our results indicate that the polyol pathway may be linked to some, but not all, mechanisms of diabetes-induced early progressive glomerulosclerosis in this model. The finding of increased aldose reductase mRNA in the peripheral blood mononuclear cells of subjects with type 1 diabetes and nephropathy, compared with levels of mRNA in age- and diabetes-duration-matched subjects without nephropathy or in nondiabetic controls, suggests that upregulation of aldose reductase expression is associated with an increased risk for nephropathy (58).

Another potential action of aldose reductase is to detoxify dicarbonyls, such as methylglyoxal and 3-deoxyglucosone, and lipid dialdehydes generated in excess by raised intracellular glucose (16). Hence, the role of the polyol pathway, and in particular the contribution of aldose reductase activity, to the pathogenesis of diabetic nephropathy is complex. Further studies are needed to identify the independent contributions to, and relative importance of, the polyol pathway to individual PKC isoform activation and to the detoxification of dicarbonyls.

**Reactive Oxygen Species**

Reactive oxygen species, such as hydrogen peroxide (H$_2$O$_2$), superoxide anion, and hydroxyl radical, are generated during oxidative stress and implicated in the mechanisms of diabetic nephropathy (47, 48). Excess glucose metabolism, including oxidation, is required for mesangial cell generation of reactive oxygen species in the diabetic milieu (22). During conversion of glucose to sorbitol, the NADP$^+$/NADPH ratio is increased and causes glutathione depletion and accumulation of reactive oxygen species (41). In many cell types, reactive oxygen species are generated as second messengers during signal transduction by agonists implicated in microvascular complications of diabetes, e.g., angiotensin II (20), platelet-derived growth factor (63), and advanced glycation end products (AGE) (72). The role of reactive oxygen species in mediating the effects of high glucose is inferred by the efficacy of antioxidants in preventing phenotypic changes in cultured mesangial cells, e.g., increased transforming growth factor (TGF)-β1 and collagen production (62, 66) and early progressive diabetic nephropathy in the STZ-diabetic rat (23, 40). Collagen production in cultured mesangial cells exposed to high glucose is prevented by taurine (67) or vitamin E (66).

Evidence is mounting for a mechanistic link between the generation of reactive oxygen species and consequent activation of mesangial cell PKC isoforms in high glucose (21). Ha and Lee (22) reported that in cultured rat mesangial cells, high glucose generates H$_2$O$_2$ within 1 h and inhibition of PKC blocks high-glucose- or H$_2$O$_2$-induced TGF-β1 and fibronectin mRNA expression and protein synthesis. These findings suggest that reactive oxygen species produced in high glucose directly or indirectly stimulate PKC. One mechanism for direct activation by reactive oxygen species is through redox changes in sulphydryl groups on PKC isoyme cysteine-rich regions. These redox changes may also render individual PKC isoymes more responsive to DAG activation during signal transduction (19). Taken together, these data support a role for high-glucose activation of PKC and subsequent matrix protein expression through reactive oxygen species-dependent pathways.

**OUTCOMES OF MESANGIAL CELL PKC ISOZYME ACTIVATION**

The activation of mesangial cell PKC, through either the direct effects of high glucose described above or the autocrine and paracrine action of vasoactive peptides such as angiotensin II and endothelin (ET)-1, results in the enhanced expression of TGF-β1 and extracellular matrix proteins (54, 71). The importance of individual PKC isoyme activation in the diabetic milieu initiating early diabetic nephropathy was first identified by Ishii et al. (34). This group discovered that the compound LY-333531, which specifically inhibits PKC-β1 and -β2, when administered orally to STZ-diabetic rats, not only prevents an elevated glomerular filtration rate, increased albumin excretion rate, and increased retinal circulation time but also overexpression of mRNA for glomerular TGF-β1 and extracellular matrix proteins (39). LY-333531 also prevents progressive mesangial expansion in the type 2 diabetic db/db mouse model (38). Scivittaro et al. (57) have identified that proteins rich in intracellular advanced glycation end products selectively activate mesangial cell PKC-βII through a mechanism involving oxidative stress, implicating no requirement for DAG. Cohen et al. (7) have identified that glycated albumin stimulates mesangial cell PKC-β activity, which is linked to the increased expression of collagen IV. The exact role of PKC-β in the early pathogenesis of diabetic retinopathy and nephropathy is presently being investigated in human clinical trials using LY-333531.

Vasoactive peptides such as angiotensin II are expressed normally in small quantities to maintain hemodynamic stability and may contribute to glomerular autoregulation. In the diabetic state, enhanced expression of peptide growth factors by mesangial cells, e.g., ET-1 (27), and other renal cells, e.g., angiotensin II (74), may contribute to the pathogenesis of diabetic nephropathy because mesangial cells constitutively express high-affinity receptors for these vasoactive peptides. Both ET-1 (14) and angiotensin II (Whiteside C, unpublished observations) stimulate mesangial cell PKC-α, -δ, and -ε. We have observed that the pattern of
mesangial cell PKC-δ and -ε translocation in response to ET-1 changes from cytosol-to-membrane distribution in normal glucose to cytosol-to-particulate (cytoskeleton/nucleus) in high glucose (18), in keeping with our confocal immunofluorescence imaging data (76). These findings suggest a change in compartmentalization and, possibly, the function of these PKC isoforms in the diabetic milieu.

Downstream of DAG-sensitive PKC isoforms is their activation of mitogen-activated protein kinases, particularly extracellular signal-regulated kinase (ERK)1/2, which are necessary for mesangial cell growth and enhanced gene expression, including growth factors and extracellular matrix proteins (17, 35). In both mesangial cells cultured in high glucose (18, 25) and glomeruli isolated from STZ-diabetic rats (3), ERK1/2 protein expression is unchanged but its activity (basal) is significantly increased compared with normal-glucose controls and is PKC dependent. Isono et al. (35) identified that high-glucose-enhanced mesangial cell TGF-β1 and extracellular matrix protein expression is mediated through ERK1/2. In cultured mesangial cells, high-glucose-enhanced ERK1/2 activity in response to ET-1 is entirely PKC dependent (18). Hence, high glucose causes amplification of signaling through PKC pathways. Recently, we have reported that high-glucose-enhanced expression of collagen IV by mesangial cells, when stimulated with ET-1, requires the action of specific PKC isoforms (31). The independent effect of each PKC isoform was revealed by transient transfection of dominant negative cDNAs, which inhibit the action of individual PKC isoforms. ET-1-stimulated collagen IV expression is also dependent on the activation of ERK1/2. Using this dominant negative-transfection strategy, we demonstrated that ET-1 activation of ERK1/2 is necessary and dependent on PKC-δ, -ε, and -ζ. We also showed that PKC-β is necessary for ET-1 stimulation of mesangial cell collagen IV expression but that this is ERK1/2 independent (31). These data suggest that the PKC isoforms stimulated by ET-1 have hierarchical or redundant functions in the signaling cascades leading to collagen IV expression and that basal activities of PKC-β and -ζ are required for the high-glucose-enhanced mesangial cell expression of this key extracellular matrix protein, contributing to diabetic glomerulosclerosis.

Mesangial cells exposed to high glucose for 24 h lose their normal contractile responsiveness to vasoactive peptides and demonstrate a cytoskeletal pattern of F-actin disassembly and depolymerization (13). The lack of a mesangial cell contractile response in high glucose mimics the hypocontractility observed in afferent and, to a lesser extent, efferent glomerular arteriolar smooth muscle cells in the diabetic milieu (73). The exact cellular mechanism linking high glucose to loss of normal contraction in response to vasoactive peptides or to arterial pressure in the afferent arteriole remains elusive. We recently reported that mesangial cell PKC-ζ demonstrates increased membrane association and enhanced activity in high glucose after 24 h (13). Inhibition of PKC-ζ with a cell-permeant, myristoylated PKC-ζ peptide inhibitor restored the normal contractile response of cultured mesangial cells to stimuli such as ET-1 (13) and prevented mesangial cell F-actin disassembly. These are the first data to link the effects of high glucose causing mesangial cell hypocontractility and cytoskeletal dysfunction to a specific PKC isoform. Further investigation is required to identify whether a similar mechanism causes glomerular arteriolar vascular smooth muscle cell dysfunction in high glucose.

SUMMARY AND FUTURE DIRECTIONS

In high glucose, mesangial cell PKC isoform activity is enhanced both in the basal state and in response to vasoactive growth peptides. Increasing evidence supports a linkage between abnormal glucose metabolism through the polyol pathway and other mechanisms that generate reactive oxygen species and the increased activity of PKC isoforms in the diabetic state. PKC-β is a major pathogenic PKC isoform contributing to early progressive diabetic nephropathy. Other PKC isoforms, including PKC-ζ, may also be important. Future investigation will focus on elucidating the relevance of PKC isoforms in maintaining normal mesangial cell function and further delineating their contributions to high-glucose-mediated transformation of mesangial cells into a sclerotic phenotype. The development of specific PKC isoform inhibitors, in addition to LY-333531, may prove useful for future targeted drug interventions in the treatment and prevention of diabetic nephropathy.

REFERENCES


