Tandem Inhibition of PKC in Diαβetic Nephropathy

It Takes Two to Tango?

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It is now more than 15 years since the initial reports of upregulation of protein kinase C (PKC) isoforms in the diabetic kidney and positive preclinical data with the PKC isoform inhibitor, ruboxistaurin. However, subsequent clinical studies with ruboxistaurin that failed to reach key primary end points and the failure of the PKC-α or PKC-β isoform knockout mice in the presence of diabetes to totally prevent the diabetes-associated renal lesions have reduced the enthusiasm for targeting PKC isoforms in diabetic nephropathy. However, as reported in this issue of *Diabetes*, Menne et al. (1) have provided positive provocative data suggesting that dual inhibition of PKC-α and -β isoforms is a novel approach that warrants consideration in diabetic nephropathy. The work is strengthened by using not only a genetic approach, i.e., a double PKC isoform knockout mouse, but by also using a PKC-α/β inhibitor, albeit its specificity remains questionable.

PKC has been implicated in the pathogenesis of diabetic nephropathy. PKC is a family of closely related enzymes that phosphorylate serine or threonine residues of various intracellular proteins and thus is involved in a wide range of cellular functions that may be relevant to the pathophysiology of diabetes complications including basement membrane production and signal transduction for hormones and growth factors (2). At least 11 isoforms have been identified and classified into three groups. The first group is the conventional PKCs, including the isoforms studied by Menne et al. (1) such as α, βI, βII, as well as γ. The second group is composed of δ, ε, η, θ, and μ, and these are the novel PKCs. There is a third group or atypical PKCs, which include ζ, ι, and λ (3). Although hyperglycemia is one of the major players in PKC activation, a number of other stimuli have been demonstrated to play a role, including advanced glycation end products (AGEs) (4), AGE receptors (5), angiotensin II (6), and reactive oxygen species (7,8) (Fig. 1).

Debate has been raging over many years to determine which PKC isoform(s) are primarily upregulated and directly implicated in diabetes complications. One of the earliest in vivo studies showed an increase in PKC activity in renal glomeruli from streptozotocin (STZ) diabetic rats within 1 week after the induction of diabetes (9). The first inhibitor developed for potential clinical use was against the PKC-β isoform, LY333531, now known as ruboxistaurin. In 1996 it was demonstrated that this competitive reversible inhibitor to PKC-βI and -βII was able to improve albuminuria, glomerular filtration rate, and retinal circulation in diabetic rats when administered orally for 2 weeks (10). In a longer study in the *db/db* mouse model, treatment with LY333531 ameliorated albuminuria and mesangial expansion possibly by attenuating the expression of transforming growth factor (TGF)-β leading to reduced fibronectin and collagen IV (11). Subsequently, a study in diabetic transgenic Ren-2 rats' inhibition of PKC-β with ruboxistaurin resulted in amelioration of albuminuria, structural injury, and TGF-β expression despite continued hyperglycemia and hypertension (12). Clinical studies with ruboxistaurin in patients with diabetic nephropathy have been disappointing with at best a modest effect on albuminuria (13,14).

Though there have been a number of supporters of the prime importance of the PKC-β isoform, other researchers have implicated PKC-α as a major culprit for diabetes complications. PKC-α was shown to be increased in glomerular mesangial cells cultured under high glucose conditions (15). This was followed by a study by Kang et al. (2) who demonstrated an increase in PKC-α and -ε in the kidney after 4 weeks of STZ diabetes but no increase in the PKC-β isoforms. Furthermore, a link between PKC-α and activation of reactive oxygen species was identified (16).

Generation of PKC isoform–specific knockout mice (17) initiated the next phase of the investigation into PKC isoform specificity in diabetes. It was demonstrated in PKC-α knockout mice that glucose-induced albuminuria is mediated at least in part by PKC-α signaling via downregulation of proteoglycans and vascular endothelial growth factor (VEGF) (18). Our own group demonstrated renal activation of PKC-α in a STZ diabetic rat model, which was attenuated by an AGE inhibitor and correlated with renal VEGF expression (4). Further studies by Menne et al. (19) demonstrated no loss of nephrin with the deletion of PKC-α in diabetic mice, further linking PKC-α to previously identified mechanisms of diabetes-associated albuminuria. To further elucidate the differences in deleting various PKC isoforms, a study by Meier et al. (20) in 8-week-old diabetic and control PKC-β knockout mice showed that this isoform is linked to the regulation of renal hypertrophy with no effect on albuminuria or nephrin loss. These findings are consistent with an article by King and colleagues (21) in which a longer duration study in diabetic PKC-β knockout mice for up to 24 weeks revealed reduced glomerular and renal hypertrophy, although there was a modest effect on albuminuria.

These studies on PKC-α and PKC-β knockout mouse models led the investigators to hypothesize that PKC-α–dependent signaling is involved in perlecan and nephrin expression leading to albuminuria in diabetes, whereas the

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DOI: 10.2337/db12-1666

See accompanying original article, p. 1167.

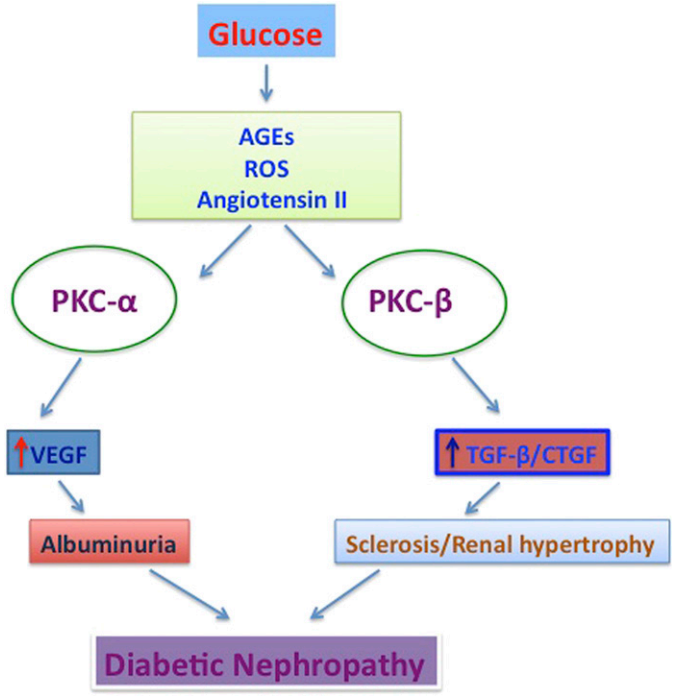

FIG. 1. In diabetic nephropathy, hyperglycemia initiates various intracellular signaling pathways resulting in further downstream activation of different PKC isoforms. ROS, reactive oxygen species; CTGF, connective tissue growth factor.

PKC-β isoform is involved in TGF-β–mediated renal hypertrophy (22). This postulate led to experiments involving the generation of double knockout mice (PKC-$\alpha/\beta^{-/-}$) in which glomerular hypertrophy, TGF-β, and extracellular matrix were diminished when compared with wild-type mice after 8 weeks of diabetes (1). Interestingly—and as yet not fully explained—albuminuria was not completely prevented with the double α/β PKC isoform knockout mice when compared with the single diabetic PKC-α knockout mice. To strengthen these findings in the knockout mice, treatment with the PKC-α/β inhibitor CGP41252 in the STZ 129/SV and the *db/db* mice was associated with improvement in albuminuria but only a mild reduction in renal hypertrophy. These findings were interpreted as occurring as a result of CGP41252 being a pan-PKC inhibitor rather than specific for PKC-α/β (1). Such an agent may inhibit novel PKC isoforms such as PKC-ε, whose upregulation has been shown to be protective in diabetic nephropathy (23).

As diabetic nephropathy continues to be a major complication of type 1 and type 2 diabetes and represents the major cause of end-stage renal disease in the Western world, new therapies are urgently needed. Improved control of hyperglycemia and blood pressure are critical for preserving renal function in diabetes, and interruption of the renin-angiotensin system remains the gold standard for the treatment of diabetic nephropathy. However, this new study highlights the need for further development of isoform-specific PKC inhibitors with dual PKC-α/β action, which could potentially be used in diabetic nephropathy.

ACKNOWLEDGMENTS

V.T.-B. is a recipient of a JDRF Advanced Postdoctoral Fellowship. M.E.C. is a National Health and Medical Research Council Australian Fellow and the recipient of a JDRF Scholar Award.

No potential conflicts of interest relevant to this article were reported.

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