Deletion of Protein Kinase C-ε Signaling Pathway Induces Glomerulosclerosis and Tubulointerstitial Fibrosis In Vivo

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Protein kinase C (PKC), a family of 12 distinct serine-threonine kinases, is an important intracellular signaling pathway involved in various cellular functions, such as proliferation, hypertrophy, apoptosis, and adhesion. PKC-ε, a novel PKC isoform that is activated in the diabetic kidney, has been demonstrated to have a central role in the underlying signaling infrastructure of myocardial ischemia and hypertrophy. The renal phenotype of PKC-ε−/− mice was studied with regard to renal hypertrophy and fibrosis. PKC-ε−/− deficient knockout mice were generated and then killed after 6, 16, and 26 wk of life. Kidney/body weight ratio did not show any significant group difference compared with appropriate wild-type controls. Urinary albumin/creatinine ratio remained normal in wild-type mice, whereas PKC-ε−/− mice showed elevated albuminuria. Masson-Goldner staining revealed that tubulointerstitial fibrosis and mesangial expansion were significantly increased in PKC-ε−/− mice. However, this profibrotic phenotype was not observed in other organs, such as liver and lung. Immunohistochemistry of the kidneys from PKC-ε−/− mice showed increased renal fibronectin and collagen IV expression that was further aggravated in the streptozotocin-induced diabetic stress model. Furthermore, TGF-β, phospho-Smad2, and phospho-p38 mitogen-activate protein kinase expression was increased in PKC-ε−/− mice, suggesting a regulatory role of PKC-ε in TGF-β, and its signaling pathway in the kidney. These results indicate that deletion of PKC-ε mediates renal fibrosis and that TGF-β1 and its signaling pathway might be involved. Furthermore, these data suggest that activation of PKC-ε in the diabetic state may rather represent a protective response to injury than be a mediator of renal injury.


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pathway was recently proposed in the development of interstitial lung fibrosis in patients with systemic sclerosis (10). These results Implicate a role for PKC-ε in the development of other fibrotic diseases such as chronic renal disease (e.g., diabetic nephropathy). In this study, we therefore investigated the functional role of PKC-ε in renal physiology by means of PKC-ε−/− KO mice and tested the hypothesis that PKC-ε is involved in chronic renal fibrosis in the STZ-induced diabetic mouse model.

Methods and Materials

Animal Studies

Experiments were performed with male hybrid C57BL/6 × 129/Ola wild-type (WT) littermates and PKC-ε−/− KO mice (9,11). PKC-ε−/− mice were developed as described elsewhere and published previously (9,11). In brief, embryonic day 14 stem (ES) cells (129/Ola) were used for the targeting experiment. Homologue-recombined ES cell clones were then introduced by injection into C57BL/6 blastocytess. The possible germline transmission of the injected ES cells was identified by crossing the observed chimeric males to C57BL/6 females and subsequently the presence of agouti coat color in the F1 progeny. F1 heterozygote breeding gave rise to homozygote mice, which were finally used in this study and correspond to the hybrid (C57BL/6 × 129/Ola) background. KO of the PKC-ε gene in the individual KO mice was confirmed using real-time PCR. The mice received a standard diet with free access to tap water. All procedures were carried out according to guidelines from the American Physiologic Society and were approved by local authorities.

To induce a diabetic state in the rodents, 7-wk-old weight-matched mice received either 50 mg/kg body wt STZ (Sigma-Aldrich, Munich, Germany) in 50 mM sodium citrate (pH 4.5) or sodium citrate buffer intraperitoneally on five consecutive days. Blood glucose measurement was performed from tail blood using the glucometer Elite (Bayer, Leverkusen, Germany) every other day. Mice with glucose levels >15 mmol/L on two consecutive measurements were regarded as hyperglycemic, and glucose measurements were extended to once weekly. Mice that had no hyperglycemia 20 d after the last STZ injection were not used for our experiments. The mice received no insulin within the complete study period. Ketonuria did not occur (data not shown). After 8 wk of hyperglycemia, the mice were killed according to a standardized protocol as previously published (8). Briefly, after anesthesia with Avertin (2.5%), a laparotomy was performed and urine was collected by puncturing the bladder with a 23-G needle. Then, the abdominal aorta was cannulated again with a 23-G needle and the organs were perfused with lactated Ringer solution. After ligation of the left renal artery, the left kidney was removed, weighed, and snap-frozen in isopentane (−40°C). Afterward, the right kidney was perfused with 3% paraformaldehyde in 0.1 M Sörensen’s phosphate buffer. The right kidney was fixed for an additional 20 h in 3% paraformaldehyde in Sörensen’s phosphate buffer and then paraffin embedded.

Cell Culture of Isolated Vascular Smooth Muscle Cells

Aortic vascular smooth muscle cells (VSMC) were prepared from WT and PKC-ε−/− mice and cultured as previously published (12). The differentiated phenotype of the cultured VSMC was determined by staining cells for α-actin and desmin. Cells were washed with ice-cold PBS and scraped off on ice. Cell lysis was done by boiling the cell pellet in lysis buffer (50 mM Tris HCl [pH 7.4], 2% SDS, 1 mM EDTA, and 1 mM EGTA). After short high-speed centrifugation (5 min, 15,000 × g), protein concentration of the supernatant was estimated by the Bradford method and 30 μg of total protein was loaded in each lane. Nonreducing conditions resulted in dimer formation of TGF-β1 as proved in control experiments. Quantification was done with Scion Image (Frederick, MD) by correction for loading differences using a housekeeper protein (α-actin).

Urine Analysis

Albumin concentration in spot urine samples was measured with a commercially available competitive ELISA following the instructions of the manufacturer (Exocell, Philadelphia, PA) and was normalized to urine creatinine defined as albumin/creatinine ratio. Urine creatinine was measured with the Beckman Creatinine Analyzer (Beckman Instruments, Fullerton, CA) using a modified Jaffé reagent. The rate of increase in absorbance as a result of the formation of alkaline creatinine picrate complex was measured. The rate of complex color formation was directly proportional to the concentration of the creatinine in the sample.

Morphometric Studies

For morphologic evaluation, 3-μm paraffin sections were stained with trichrome Masson-Goldner standard procedure (8). Immunohistochemistry was performed using the following primary antibodies: TGF-β1 (sc-146; Santa Cruz Biotechnology, Santa Cruz, CA), fibronectin (14-109-0568; Paesel + Lorei, Frankfurt, Germany), type IV collagen (1340-01; Southern Biotechnology, Birmingham, AL), phospho-p38 mitogen-activated protein kinase (phospho-p38 MAPK; 9211; Cell Signaling, Danvers, MA), and phospho-Smad2 (3101; Cell Signaling). For indirect immunofluorescence, nonspecific binding sites were blocked with 10% normal donkey serum (Jackson Immunoresearch, West Grove, PA) for 30 min. Then cryosections were incubated with the primary antibody for 1 h. All incubations were performed in a humid chamber at room temperature. For fluorescence visualization of bound primary antibodies, sections were further incubated with Cy3-conjugated secondary antibodies (Jackson Immunoresearch) for 1 h. Specimens were analyzed using a Zeiss Axioplan-2 imaging microscope with the computer program AxioVision 3.0 (Zeiss, Jena, Germany).

Western Blotting

Frozen kidneys were pulverized in liquid nitrogen and resuspended in 2 ml of lysis buffer (20 mM Tris buffer [pH 7.5] that contained 10 mM glycercol-phosphate, 2 mM pyrophosphate, 1 mM sodium fluoride, 1 mM PMSF, 1 g/ml leupeptin, 1 mM dithiothreitol, and 1 mM EDTA) as described previously (8). Homogenates were sonicated for three 20-s bursts on ice and centrifuged at 500 × g for 1 min to remove cell debris. Aliquots of the supernatants were stored at −80°C. The protein amount was measured using the Lowry assay. A total of 70 μg of protein of each sample was suspended in loading buffer and run on a 10% polyacrylamide gel and electrophoretically transferred to nitrocellulose membrane. Membranes were blocked in 5% skim milk and 1% BSA for 1 h at room temperature. Primary antibody against factor TGF-β1 (sc-146; Santa Cruz Biotechnology) was applied with gentle rocking overnight at 4°C. After three, 10-min washing steps with TBST buffer (50 mM Tris Hepes [pH 7.5], 150 mM NaCl, and 0.01% Tween 20), incubation with horseradish peroxidase–conjugated goat anti-rabbit secondary antibody (Dianova, Hamburg, Germany) was performed for 1 h at room temperature. After three additional TBST washes, the membrane was incubated with Renaissance reagent (NEN Life Science, Zaventem, Belgium) according to the manufacturer’s instructions and exposed to x-ray film (Kodak, Stuttgart, Germany). Quantitative analysis was done by measuring relative density (Scion Image).
**Confocal Microscopy**

Confocal microscopy was performed as described previously (9). The preparation was mounted with medium (Aqua Polymount; Polysciences, Warrington, PA) under a glass coverslip and analyzed with a Nikon-Diaphot microscope. A confocal imaging system (Bio-Rad MRC 1024, Bio-Rad Laboratories, Munich, Germany) with a ultraviolet source and an argon/krypton laser was used. At least 30 cells from each of at least three independent experiments were examined under each experimental condition. For each set of experiments, identical settings for power of the light source, confocal aperture, gain, and black level were used. Quantification of the signal intensity of single cells was done with histogram/area functions in the available software (Laser-sharp or NIH Image). The cells were outlined manually, and the mean fluorescence intensity was obtained for the delineated regions. Data are presented as relative fluorescence intensity.

**Statistical Analyses**

The data were compared by ANOVA and the unpaired t test as appropriate, and values are displayed as mean ± SEM. Significant differences were accepted at P < 0.05. Data analysis was performed using SPSS 11.0 software (SPSS, Chicago, IL).

**Results**

We started our investigation of PKC-ε⁻/⁻ (KO) mice at the ages of 6, 16, and 26 wk in comparison with appropriate WT littermates to analyze changes of the renal phenotype over time. All mice were screened for clinical parameters such as body and kidney weight with subsequent calculation of the kidney/body weight ratio. Furthermore, the albumin/creatinine ratio was measured in spot urine that was sampled before the mice were killed and kidneys were removed. Subsequent morphometric studies of the kidney tissue were performed using Masson-Goldner staining to evaluate structural renal morphometric studies of the kidney tissue were performed the mice were killed and kidneys were removed. Subsequent nine ratio was measured in spot urine that was sampled before time. All mice were screened for clinical parameters such as littermates to analyze changes of the renal phenotype over ages of 6, 16, and 26 wk in comparison with appropriate WT controls. It is interesting that only KO mice after 16 wk showed a significantly increased tubulointerstitial fibrosis and glomerulosclerosis in the KO mice (Figure 1, top) compared with the appropriate WT control mice (Figure 1, bottom) at the ages of 6, 16, and 26 wk. Figure 1, top, displays already increased tubulointerstitial fibrosis as displayed as more pronounced blue staining in 6-wk-old KO mice (Figure 1A) which is further aggravated in the KO groups at the age of 16 (Figure 1B) and 26 wk (Figure 1C) compared with appropriate WT controls (Figure 1, D through F). In conclusion, the observed profibrotic renal phenotype of PKC-ε⁻/⁻ explains the increased albuminuria that was observed in the clinical examination reported previously. However, renal fibrosis is more pronounced than albuminuria in PKC-ε⁻/⁻ mice.

We then extended our morphometric studies to other organs that are susceptible to fibrosis in humans, mainly liver and lung. In contrast to the observed changes in the kidneys from PKC-ε⁻/⁻, light microscopy of liver tissue did not show any significant fibrosis in KO mice compared with WT controls, whereas lung tissue exhibits slightly increased pulmonary fi-

**Table 1. Body weight and albumin/creatinine ratio of baseline (day 42, week 6; n = 5), intermediate (day 112, week 16; n = 13), and final (day 168, week 26; n = 10) groups**

<table>
<thead>
<tr>
<th>Study Group</th>
<th>Body Weight (g)</th>
<th>Albumin/Creatinine Ratio (g/mol)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Day 42 (n = 5)</td>
<td>Day 112 (n = 13)</td>
</tr>
<tr>
<td>WT control</td>
<td>21.5 ± 2.2</td>
<td>32.1 ± 0.7</td>
</tr>
<tr>
<td>PKC-ε⁻/⁻</td>
<td>21.2 ± 2.7</td>
<td>35.7 ± 0.6b</td>
</tr>
<tr>
<td></td>
<td>4.0 ± 2.1</td>
<td>3.8 ± 1.3</td>
</tr>
<tr>
<td></td>
<td>25.9 ± 21.9</td>
<td>30.0 ± 10.2c</td>
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*Body weight increases significantly with age because of general growth but to a similar extent in wild-type (WT) and knockout (KO) mice compared with the baseline group. It is interesting that only KO mice after 16 wk showed a significantly increased albumin/creatinine ratio (P < 0.01) whereas albumin/creatinine ratio was only slightly increased after 6 wk and normal after 26 wk compared with appropriate WT controls (P > 0.05). Data are means ± SD.

bP > 0.05; cP < 0.01.
brosis in the KO mice. Figure 2 displays representative Masson-Goldner stains of liver and elastic van Gieson stains of lung tissue from 26-wk-old PKC-ε−/− mice. The latter demonstrates only a minor increase of pulmonary fibrosis in the PKC-ε−/− mice. The predominant profibrotic phenotype that was observed in the kidneys from PKC-ε−/− mice therefore represents organ specificity to the kidney rather than a systemic effect. This conclusion goes ahead with the observation that the general life span of this KO breed is not abbreviated. Furthermore, these data suggest that stimulated PKC-ε signaling might be able to reduce or prevent chronic (murine) kidney disease but does not seem to be critical in other organ systems that are prone to fibrosis.

Because PKC isoform specificity is of considerable importance in vascular complications in diabetes mellitus (4) and because we and others previously showed that the expression and activation in PKC-α and -ε are increased in the diabetic rat kidney (13,14), we next studied the role of PKC-ε signaling in chronic renal disease such as diabetic nephropathy. We tested the hypothesis that deletion of PKC-ε exacerbates experimental diabetic nephropathy, mainly renal fibrosis, in the STZ-induced diabetic mouse model. Hyperglycemia was induced in 7-wk-old mice by intraperitoneal injection of STZ (50 mg/kg body wt) on days 1 through 5. Blood glucose levels >15 mmol/L were achieved within 20 d after the last STZ injection and

### Table 2. Kidney weight and kidney/body weight ratio of baseline (day 42, week 6; n = 5), intermediate (day 112, week 16; n = 13), and final (day 168, week 26; n = 10) groups

<table>
<thead>
<tr>
<th>Study Group</th>
<th>Kidney Weight (g)</th>
<th>Kidney/Body Weight Ratio (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Day 42 (n = 5)</td>
<td>Day 112 (n = 13)</td>
</tr>
<tr>
<td>WT control</td>
<td>163 ± 33</td>
<td>216 ± 11</td>
</tr>
<tr>
<td>PKC-ε−/−</td>
<td>159 ± 12</td>
<td>235 ± 5</td>
</tr>
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</table>

*aKidney weight and kidney/body weight increase over time in both WT and KO mice to a comparable degree (P > 0.05). Data are means ± SD.*
persisted in both WT littermates and PKC-ε−/− (KO) mice, diabetic groups as compared with the appropriate, sham-injected control mice. After 8 wk of diabetes, urinary albumin/creatinine ratio was significantly increased in diabetic KO mice compared with healthy and diabetic WT mice (44.9 ± 11.4 versus 3.8 ± 1.3 [P < 0.01], respectively; 12.0 ± 1.6 mmol/L [P < 0.05]) and further increased compared with nondiabetic PKC-ε−/− mice (30.0 ± 10.2 mmol/L). These data indicate that this chronic diabetic stress model is able to exacerbate and outline further the previously demonstrated renal changes in the PKC-ε−/− mice. Second, no group difference regarding kidney/body weight ratio was observed between the nondiabetic and diabetic KO mice (8.94 ± 0.7 versus 8.60 ± 1.1; P > 0.05), indicating also no increased renal hypertrophy in the diabetic state in PKC-ε−/− mice. Masson-Goldner staining revealed a more pronounced tubulointerstitial fibrosis and glomerulosclerosis in diabetic KO mice compared with appropriate WT control mice (Figure 3). These results suggest that the increased expression and activation of the PKC-ε isoform that previously was shown in experimental diabetic nephropathy (13,14) does not induce profibrotic changes in the (diabetic) kidney but rather maintains antifibrotic functions even in the nondiabetic kidney.

We then investigated matrix expansion by means of immunohistochemistry of ECM proteins to evaluate further the molecular mechanisms that lead to the profibrotic phenotype of PKC-ε−/− compared with WT control mice at the age of 16 wk.

Figure 3. Streptozotocin (STZ)-induced diabetes (8 wk diabetes duration) leads to further exacerbated tubulointerstitial fibrosis (top) and glomerulosclerosis (bottom) in PKC-ε−/− mice in light microscopy after Masson-Goldner staining. KO mice (B) displayed increased tubulointerstitial fibrosis compared with WT controls under diabetic condition (A). Comparable results are also observed on the glomerular level in the diabetic state (WT control [C] and KO mice [D]).

Figure 4, top, displays increased tubulointerstitial expression of fibronectin in KO mice compared with WT mice. Similar expression patterns are also shown in Figure 4, bottom, where mesangial expression of collagen IV is shown. These results clearly indicate that PKC-ε signaling is directly involved in profibrotic mechanisms, such as ECM expansion, that are observed in chronic renal diseases.

TGF-β1 represents a key cytokine that mediates renal hypertrophy and accumulation of ECM in chronic renal disease such as diabetic nephropathy (4,12). For further extrapolation of how deletion of PKC-ε leads to increased glomerulosclerosis and tubulointerstitial fibrosis, immunohistochemistry of TGF-β1 was subsequently performed. We revealed that TGF-β1 expression is increased in PKC-ε−/− mice compared with WT controls (Figure 5). These changes mainly occur on the glomerular level (Figure 5, bottom) where, again, the KO mice displayed a significantly higher expression of TGF-β1 than in controls. Figure 5, top, displays increased tubulointerstitial TGF-β1 staining of WT and PKC-ε−/− mice at the age of 16 wk as well.

To study quantitatively the increase of TGF-β1 protein expression, we next performed Western blot analysis (Figure 6A) and confocal microscopy (Figure 6B) in VSMC that were isolated from WT and PKC-ε−/− mice. Furthermore, cells were starved under normal as well as high-glucose conditions for 16 h to outline the phenotype change. Again, quantitative analysis revealed that VSMC from KO mice showed significantly increased TGF-β1 expression compared with VSMC from WT mice, which is further stimulated by the high-glucose condition. These results indicate that the TGF-β1 signaling pathway is modified by the deletion of PKC-ε.
We also studied whether deletion of PKC-ε signaling not only leads to increased expression of TGF-β1 but also induces activation of the TGF-β1 signaling pathway (Figure 7). We therefore performed immunohistochemistry of kidneys from 16-wk-old WT and KO mice to study phospho-Smad-2 expression. The results clearly demonstrated increased expression of phospho-Smad2 in PKC-ε−/− mice, proving activation of the TGF-β1 receptor signaling pathway when PKC-ε is deleted (Figure 7, top). Furthermore, immunohistochemistry using a specific phospho-antibody against p38 MAPK (Figure 7, bottom), another signaling cascade downstream of the TGF-β1 receptor that is postulated to be involved in cellular processes such as growth and differentiation (15), was subsequently performed. Again, increased phospho-Smad-2 and phospho-p38 MAPK expression is further increased when diabetes mellitus is induced in the PKC-ε−/− mice (data not shown). Deletion of PKC-ε was shown to activate the p38 MAPK signaling pathway mainly in the glomeruli. These results indicate that the enhanced signaling of this inflammatory pathway might be an important mechanism involved in the increased glomerulosclerosis and tubulointerstitial fibrosis in the PKC-ε KO mice.

Discussion

In this study, we investigated the functional role of PKC-ε in renal physiology by means of PKC-ε−/− KO mice and tested the hypothesis that PKC-ε is involved in chronic renal fibrosis in the STZ-induced diabetic mouse model. It is interesting that the results demonstrate an organ-specific renal phenotype in PKC-ε−/− mice with increased tubulointerstitial fibrosis and glomerulosclerosis. Deletion of PKC-ε signaling thereby might interfere with activation of the TGF-β1 pathway. The diabetic stress model was able further to enhance renal fibrosis and

Figure 5. Immunohistochemistry of expression. KO mice (B and D) displayed a significantly increased TGF-β1 expression, mainly on the glomerular level, compared with WT controls (A and C).

Figure 6. Quantitative protein analysis of TGF-β1 confirmed increased expression levels in PKC-ε−/− mice using Western blot (A) and confocal microscopy (B). (A) Increased protein expression of TGF-β1 under high-glucose condition in cultured vascular smooth muscle cells (VSMC) from KO mice compared with VSMC from WT controls. (B) Confocal microscopy of TGF-β1 expression in cultured VSMC from WT (top) and KO mice (bottom). Comparable to the observed changes in vivo, the most abundant expression level of TGF-β1 was found in the VSMC from PKCe−/− KO mice that were starved under high-glucose condition.
pathway was recently proposed in the development of interstitial lung fibrosis in patients with systemic sclerosis (10,20,21). It has been demonstrated that the regulation of an ECM molecule (tenascin-C) is controlled by the PKC-ε isoform in lung fibroblasts from patients with systemic sclerosis (21). In addition, the signaling pathway involving PKC-ε and phase 2 detoxification enzymes heme oxygenase 1 and glutathione S-transferase P1, is defective, leading to decreased PKC-ε levels in scleroderma lung fibroblasts in vitro and in fibrotic lung tissue in vivo (10). Furthermore, PKC-ε plays critical roles in protecting against ischemic damage, whereas overexpression and activation of PKC-ε results in myocardial hypertrophy (22,23). In conclusion, our in vivo data add to the somewhat complex situation that deletion of PKC-ε leads to organ-specific renal fibrosis.

So far, it is largely accepted that high-glucose-induced expression and translocation to the membranous compartment (activation) of PKC isoforms is a key mediator of microvascular complications in the diabetic state, leading to subsequent induced expression and stimulated action of various cytokines such as TGF-β (4,5,14). Several studies previously showed increased renal expression and activation of PKC-ε in STZ-induced experimental diabetic nephropathy in various rodent models, indicating an important functional role of this novel PKC isoform in the development of chronic kidney disease (13,18,24,25). We therefore induced diabetes with STZ in our mouse model, which further enhanced the profibrotic phenotype in the PKC-ε−/− mice. The diabetic state was able to pronounce further tubulointerstitial fibrosis and glomerulosclerosis when PKC-ε was deleted. This result suggests that increased expression and activation of PKC-ε, which was previously demonstrated in experimental STZ-induced diabetes, represents a protective response to injury rather than a mediator of diabetic nephropathy (4,5,8,12–14,16,17).

TGF-β causes the transformation of interstitial fibroblasts into myofibroblasts and leads to the overproduction of ECM followed by tubulointerstitial fibrosis (15). Binding of TGF-β to its TGF-βRII and subsequent recruitment of the TGF-β-RI results in phosphorylation and activation of receptor-regulated Smads, a series of transducer proteins that associate to heteromultimers when activated (26). The Smad complex then translocates to the nucleus, where it regulates the expression of TGF-β target genes through direct binding to DNA via binding to Smad-binding element and/or interaction with other transcription factors (26). Yakymovych et al. (27) demonstrated that Smad2 and Smad3, the targets of TGF-β receptors, can be phosphorylated in their MHI domain by PKC, indicating potential cross-talk between classical TGF-β and PKC signaling cascades. Furthermore, it has been shown that TGF-β1 activates PKC-δ in human mesangial cells and that this activation plays a role in TGF-β1-stimulated transcriptional activity and collagen I gene transcription (28). PKC, however, is also known to be involved in hyperglycemia-stimulated TGF-β1 promoter activity in mesangial cells (29). This could be the mechanism that leads to increased TGF-β1 mRNA expression and protein synthesis that have been observed in murine mesangial cells that were cultured in high glucose (30).

PKC-ε−/− mice showed only inconsistent albuminuria com-

Figure 7. TGF-β1 signaling pathway is activated in PKC-ε−/− mice. Immunohistochemistry of phospho-Smad2 (top) and phospho-p38 mitogen-activated protein kinase (phospho-p38 MAPK, bottom) revealed increased activation of the TGF-β1 signaling pathway compared with nondiabetic (A and E) and diabetic (C and G) controls when PKC-ε is deleted (B and F). Again, in the diabetic state, the activation level is further increased (D and H).
pared with WT controls. However, induction of STZ diabetes mellitus as a chronic stress model led to a significantly increased urinary albumin/creatinine ratio in PKC-ε−/− mice. We previously showed that deletion of PKC-α in vivo leads to protection against the development of microalbuminuria in STZ-induced diabetic mice, indicating a specific role of this classical isoform in the breakdown of the glomerular filtration barrier in the diabetic state (8). Furthermore, we have shown that high glucose leads to a PKC-α-dependent expression of TGF-β1 in VSMC under in vitro conditions (12) but failed to demonstrate a role for PKC-α in the regulation of TGF-β1 expression in vivo in murine diabetic nephropathy (8). Such controversy results might be explained by the fact that glucose stimulates different PKC isoenzymes in various cell types or that short-term activation of PKC isoform by high glucose concentrations in vitro has a different cellular effect than the long-term effects of hyperglycemia in vivo. Complex diversity in tissue expression and cellular compartmentalization that is critical for the activation of specific PKC isoforms therefore results in controversial in vitro studies (4,5). Thus, murine KO models of various PKC isoforms now represent an exciting option for studying in vivo effects of such single-gene deletions and how they contribute to the high-glucose-mediated transformation of the kidney into a sclerotic phenotype (6,8).

Conclusion

Our results suggest a regulatory role of the PKC-ε isoform in the development of renal fibrosis and that TGF-β1 and its signaling pathway might be involved. Therefore, PKC-ε activation may also have a potential key regulatory function in chronic renal disease such as diabetic nephropathy. However, the detailed molecular mechanism for how deletion of PKC-ε results in this valuable profibrotic KO model awaits further studies.

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Disclosures

None.

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