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Differential expression of protein kinase C isoforms in streptozotocin-induced diabetic rats

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Background. The cellular effects of hyperglycemia are mediated by protein kinase C (PKC). However, PKC consists of several distinct isoforms, and their contribution to the pathogenesis of diabetic complications in different organs is not clear. We investigated the expression and translocation of PKC isoforms α, βI, βII, δ, ϵ, and ζ in kidney, heart, and aorta from diabetic rats.

Methods. Hyperglycemia was induced with streptozotocin (70 mg/kg) in the rat. After four weeks, PKC isoform expression was assessed by Western blot after tissue fractionation and by immunohistochemistry.

Results. Streptozotocin increased blood glucose from 117.0 ± 3.6 to 510.0 ± 19.4 mg/dl ($N = 8$, $P < 0.01$) and induced albuminuria. PKC isoforms α, β, δ, ϵ, and ζ were all detected in control animals. Western blot showed increased PKC α expression in kidney and heart (160% and 170%, respectively). PKC βI, βII, and δ expression was not influenced by hyperglycemia. PKC ζ was decreased in diabetic animals in both tissues by 60%. The membrane association of PKC α and PKC ϵ was increased; however, the relative amount of PKC in the particulate fraction was not influenced by hyperglycemia. Immunohistochemistry revealed a marked increase in PKC α immunoreactivity in renal glomeruli and interstitial capillaries, cardiac capillaries, and skeletal muscle, as well as in the endothelial cells of larger arteries. PKC β showed a small decrease in the glomeruli. PKC ϵ was increased in renal tubules in diabetic rats but was decreased in the myocardium. PKC ζ was expressed in both myocardial and glomerular cells but was decreased during hyperglycemia. Our results demonstrate that PKC isoforms are differentially regulated in kidney and heart in diabetes. High glucose increases PKC α expression, whereas PKC ζ is down-regulated. The finding that PKC α is mostly increased in endothelial cells supports a role for PKC α in functional endothelial disturbances observed in diabetes.

Key words: diabetes, PKC isoform α, gene transfer, antisense oligonucleotides, hyperglycemia, endothelium, kidney.

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Hyperglycemia is the major causal factor in the pathogenesis of diabetic vascular complications. An elevated blood glucose may mediate its adverse effects through multiple pathways [1–5]. Studies in isolated glomeruli and in cultured endothelial, smooth muscle, and mesangial cells have demonstrated that high ambient concentrations of glucose activate protein kinase C (PKC) and thereby implicate hyperglycemia *per se* as a mediator of PKC activation [2, 6]. Several authors have demonstrated that both *in vitro* and *in vivo* increased glucose concentrations lead to the *de novo* synthesis of diacyl glycerol (DAG) [7–13]. High glucose concentrations activate PKC by increasing cellular DAG levels, the major mediator of this signaling system [14–27]. The activation of PKC regulates various vascular functions by modulating enzymatic activities such as cytosolic phospholipase A_2 and Na^+-K^+-ATPase, as well as gene expression of extracellular matrix components and contractile proteins [6, 28–31]. PKC may thereby be involved in the pathogenesis of both the functional and structural alterations of the kidney in diabetes.

In vitro, increased extracellular glucose leads to a rapid PKC activation within minutes, and its activity remains increased for several days [16, 24, 32]. *In vivo*, increased PKC activity has been found in glomeruli, heart, and retinal vessels within days of streptozotocin-induced hyperglycemia [14, 33, 34]. The sustained activation of the enzyme has been linked to increased DAG generation in diabetic tissue [6]. However, activated PKC is inactivated by enzymatic cleavage; sustained activation of the enzyme has been generally associated with down-regulation of the protein and loss of PKC activity. A possible explanation of the sustained increase in PKC activity is an enhanced expression of the enzyme during hyperglycemia. In fact, increased PKC expression has been observed in the diabetic kidney [35] and in the retinal circulation [17]. We have previously shown that increased PKC expression is associated with an increase in transforming growth factor-β (TGF-β) [36].

The PKC issue is further complicated by the fact that PKC is not a single entity but consists of several isoforms with distinct cellular functions and different expression patterns [37]. Considerable debate exists about which isoform is involved in the glucose-induced activation. Inoguchi et al observed an increased expression of the PKC isoform βII in tissue from diabetic animals and hypothesized that this PKC isoform is primarily responsible for the glucose-induced effects in diabetes [34]. Further evidence for this hypothesis stems from recent reports that a specific PKC β inhibitor ameliorates hyperglycemia-induced changes in the vasculature [17, 38, 39]. However, PKC β may not be expressed in all cell types of the vascular wall. We were not able to detect PKC β expression in endothelial cells, even though these cells responded to changes in extracellular glucose concentration [40]. In addition, we recently reported that high glucose increases endothelial cell permeability via PKC α [41] and that PKC α plays an important role in vascular smooth muscle cell (VSMC) differentiation [36]. Whiteside et al have recently described an increased membrane association of PKC α, but also of PKC δ and ϵ, in a diabetic rat model [42]. We were therefore especially interested in investigating the expression and translocation of PKC isoforms in a rat model of diabetes and to analyze their cellular distribution in kidney and heart.

METHODS

Animal protocol

Experiments were performed in accordance with the Helsinki Declaration and The Guiding Principles in the Care and Use of Animals. Male Sprague-Dawley rats (Ivanovas, Kisslegg, Germany) aged eight weeks were maintained in cages at 24 ± 2°C. Animals were fed a standard pellet diet and were allowed free access to water. Diabetes was induced by a single intraperitoneal injection of 70 mg/kg body wt streptozotocin (Sigma Chemical Co., Deisenhofen, Germany). Blood glucose concentrations were determined 48 hours after streptozotocin using an automated glucose analyzer (Eppendorf, Hamburg, Germany). Rats with a blood glucose concentration above 300 mg/dl during the study period were declared "diabetic," whereas age-matched normoglycemic animals were used as controls. Body weight, 24-hour urine protein excretion, and blood glucose concentrations were determined weekly. Twenty-four–hour albumin excretion was measured using an enzyme-linked immunosorbent assay technique.

Western blotting

The excised organs were divided with forceps and scissors. The renal tissue was treated with cold buffer [50 mM Tris-HCl, pH 7.4, 2% sodium dodecyl sulfate (SDS), 1 mM ethylenediaminetetraacetic acid (EDTA), 10 mM egtazic acid (EGTA), 25 mM leupeptin, and 5 mM phenylmethylsulfonyl acid (PMSF)] and immediately homogenized. The homogenate was then incubated for one hour with benzonase (Merck, Darmstadt, Germany) and boiled for 10 minutes at 95°C. Afterward, it was spun at 1600 *g* for 15 minutes. The cardiac tissue was homogenized in phosphate-buffered saline (Seromed, Berlin, Germany). Subsequently, boiling lysis buffer was added (1% sodium dodecyl sulfate, 1 mM sodium vanadate, 10 mM Tris-HCl, pH 7.4), and the samples were microwaved for 30 seconds. The homogenate was then spun at 12,000 *g* for five minutes. After determination of protein content, the immunoblotting was carried out as previously published [42]. Briefly, 60 μg of each sample were run on a 10% polyacrylamide gel and electroblotted onto a Poly Screen polyvinylidine difluoride (PVDF) Transfer Membrane (NEN, Boston, MA, USA). The membrane was then treated according to a commercially available protocol (Serva, Heidelberg, Germany). The membrane was incubated with antibodies directed against defined epitopes of six different PKC isoforms (α, β, δ, ϵ, and ζ; Transduction Laboratories, Lexington, KY, USA). The antibody against PKC β is a polyconal antibody. For the detection of the two variants PKC βI and PKC βII, we used monoclonal antibodies from GIBCO (Eggenstein, Germany). A final incubation was carried out with peroxidase conjugated antimouse IgG (Pierce Chemicals, Oud-Beijerland, Netherlands). Visualization was achieved by chemiluminescence (Renaissance; DuPont, Boston, MA, USA).

Immunocytochemistry

Experiments were carried out as described previously [43]. Frozen renal and cardiac tissues specimens were cryosectioned at 6 μm thickness and air dried. The sections were fixed in cold acetone, air dried, and washed in Tris-buffered saline (TBS; 0.05 M Tris-buffer, 0.15 M NaCl, pH 7.6). The sections were incubated for 60 minutes in a humid chamber at room temperature or overnight at 4°C with primary monoclonal antibodies against PKC isoforms (discussed earlier in this article). After washing with TBS, the sections were incubated with a bridging antibody (rabbit-antimouse IgG; Dako, Hamburg, Germany) for 30 minutes at room temperature and washed again with TBS. The APAAP-complex (alkaline-phosphatase-antialkaline-phosphatase; Dako) was applied, and the sections were incubated for 30 minutes at room temperature. The immunoreactivity was visualized by development in a mixture of napthtol-AS-BI-phosphate (Sigma) with neufuchsin (Merck). Endogenous alkaline phosphatase was blocked by addition of 10 mM levamisole (Sigma) to the substrate solution. The sections were slightly counterstained in Mayer's hemalaun (Merck), blued in tap water, and mounted with GelTol (Coulter-Immuotech, Hamburg, Germany). For immunofluorescence, detection of bound primary antibodies

was performed with preabsorbtion against rat serum proteins affinipure goat antirabbit IgG (H + L) conjugated with dichlortriazinyl aminofluorescein (DTAF) at a dilution of 1:100. For fluorescent microscopy, DTAF-labeled cryosections were mounted with Citifluor (Plano GmbH, Wetzlar, Germany) with or without nuclear counterstaining with propidium iodide. Preparations were examined under a Zeiss Axioplan-2 microscope (Zeiss, Jena, Germany) and photographed using a color reversal film Agfa CTX 100. The data shown are representative of at least five independent experiments that gave similar results. The controls were as follows: (*a*) omission of incubation with primary AB and (*b*) substitution of primary AB by the corresponding mouse IgG subclass (Dianova, Hamburg, Germany) at the same final concentration.

Statistical analysis

Statistical analysis was carried out with a commercially available program (SPSS Inc., Chicago, IL, USA). To standardize densitometric values for each Western blot, the results from diabetic animals were calculated as the percentage as compared with control animals. These values were then tested by univariate *t*-tests against 100% under the null hypothesis of no difference. These comparisons were carried out for the different PKC isoforms and the different tissues. The results (mean ± SEM) represent duplicate measurements made on 7 to 10 separate rats from each group. Nonparametric (Wilcoxon test) and parametric (two-way analysis of variance) were used as appropriate. $P \leq 0.05$ was considered significant.

RESULTS

Induction of hyperglycemia in the eight-week-old rats by streptozotocin resulted in an increased blood glucose concentration after one week (356.9 ± 20.9 vs. 116.7 ± 8.8 mg/dl, $N = 16$, $P < 0.01$). Hyperglycemia persisted in the treated animals and was 510.0 ± 19.4 mg/dl at four weeks as compared with 117.0 ± 3.6 mg/dl in the control animals. The hyperglycemic animals developed albuminuria. Twenty-four hour urinary albumin excretion in the hyperglycemic animals was 2329 ± 1015 mg/24 hr after four weeks, whereas in the control animals, 343 ± 73 mg/24 hr were measured ($N = 16$, $P < 0.01$). After four weeks, the animals were killed, and the organs were assessed for PKC isoform α, βI, βII, δ, ϵ, and ζ expression. We first investigated the expression of PKC isoform α in kidney and heart of diabetic animals. These results are shown in Figure 1. The left panels show immunostains of renal (Fig. 1 A, C) and cardiac (Fig. 1E) tissue from normoglycemic control rats. The right panels show the respective immunostains from diabetic rats. PKC α was weakly expressed in the tubules with a preponderance toward the apical surface (Fig. 1 A, C). A faint expression of PKC α was also visible in focal areas of the glomerular loops and in the adventitia. In hyperglycemic animals, the expression of PKC α was greatly enhanced (Fig. 1 B, D, and F). The most prominent increase was present in the glomeruli and the endothelium of larger vessels (Fig. 1 B, D). In addition, tubular PKC immunoreactivity was enhanced, and more immunoreactive material was present in the vascular lumen and the adventitia. An increased expression in endothelial cells was also seen in the heart (Fig. 1F).

In Figure 2, the expression of PKC isoforms β (Fig. 2 A–D) and δ (Fig. 2 E–H) are shown. In renal tissue of normoglycemic animals (Fig. 2A), PKC β was present both in the tubules and glomeruli. A more prominent expression of PKC β was observed in cardiac myocytes (Fig. 2C). Hyperglycemia induced a modest decrease of PKC β immunoreactivity in the glomeruli (Fig. 2B). A decreased PKC β expression was also observed in the cardiac myocytes from diabetic animals. In vessels in both the kidney and the heart, a weak, nonsignificant staining for PKC β was observed. The influence of hyperglycemia on PKC β in the vessels was not significant (data not shown). PKC δ showed only a faint expression in normal kidney and heart (Fig. 2 E, G), which was not influenced by hyperglycemia.

We then used two monoclonal antibodies to investigate the expression of the two PKC β variants. The results for PKC βI are shown in Figure 3 A–D. PKC βI was more prominently expressed in tubular cells compared with glomeruli. In the heart, a more homogenous distribution was observed. PKC βII showed an increased immunoreactivity in the glomeruli, whereas the staining pattern in the heart was not different from PKC βI in the heart. Hyperglycemia had no significant effect on the immunoreactivity of both β isoforms in the kidney (Fig. 3 A, B and E, F), whereas a small increase of immunoreactivity for βI was observed in the heart (Fig. 3 C, D and F, G).

In Figure 4, the expression of PKC isoforms ϵ (Fig. 4 A–D) and ζ (Fig. 4 E–H) are shown. PKC ϵ was prominently expressed in the renal tubules and was almost undetectable in the glomeruli. Hyperglycemia induced a significant increase in tubular PKC ϵ expression, but had little effect in the glomeruli. In contrast to the results with the other PKC isoforms, PKC ϵ was differentially influenced by hyperglycemia in the kidney and in the heart. The strong expression of PKC ϵ in cardiac myocytes was reduced during hyperglycemia and showed a patchier pattern. Finally, we examined the expression of PKC ζ (Fig. 4 E–H). This isoform was evenly distributed in renal tubules and glomeruli (Fig. 4E). In the heart, a strong expression in the cardiomyocytes was observed (Fig. 4G). Hyperglycemia decreased the expression of PKC ζ in the glomeruli and caused a slightly increased immunoreactivity in the tubules (Fig. 4F). In the heart,

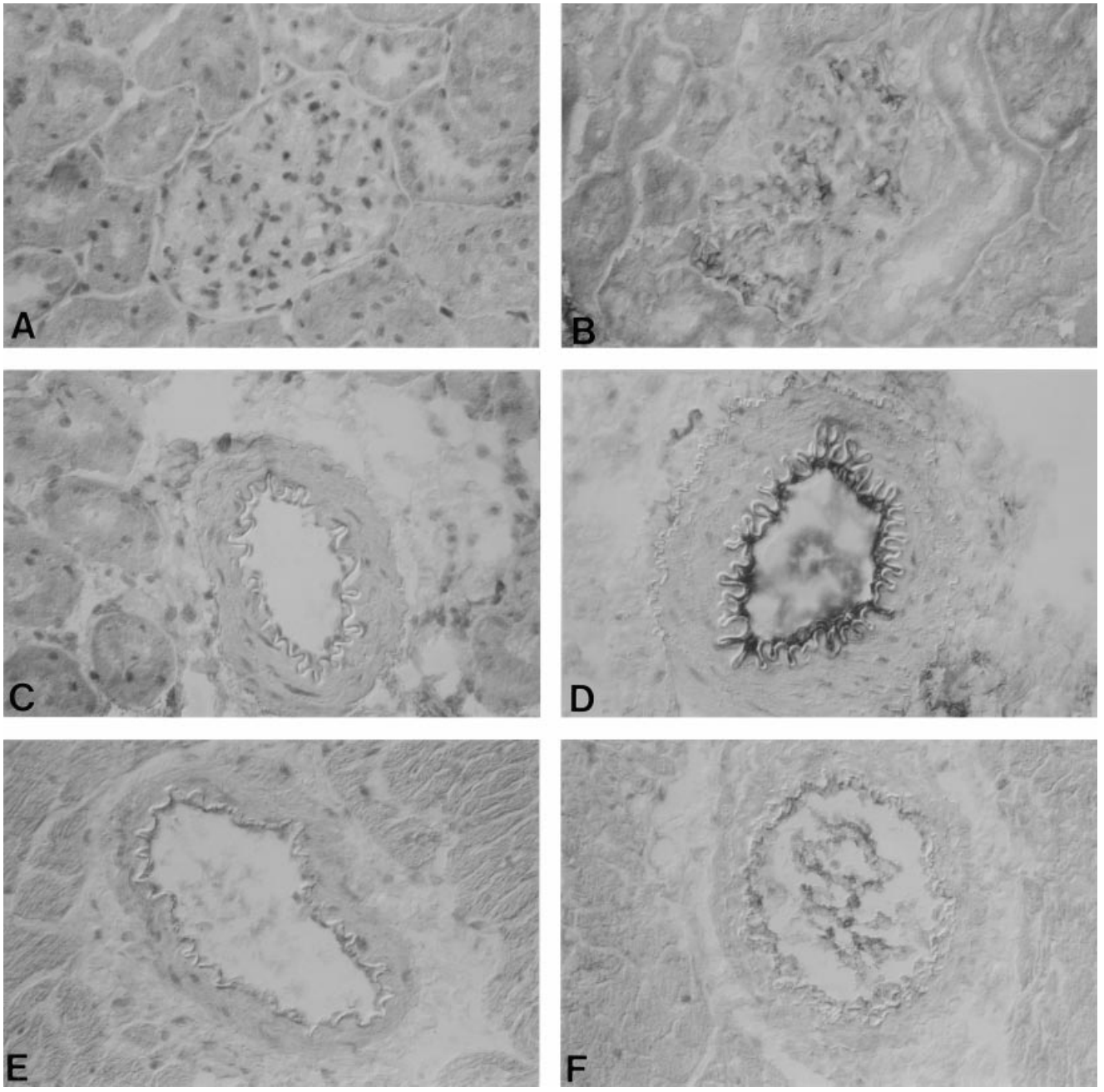

Fig. 1. Immunohistochemistry of PKC isoform α in kidneys (*A–D*) and heart (*E* and *F*) from normoglycemic (left panels) and hyperglycemic animals (right panels). In renal tissue from normoglycemic rats, PKC α was faintly expressed in the glomeruli and apical tubules (A). Hyperglycemia increased PKC α expression in the glomeruli and the interstitial space (B). Hyperglycemia also induced and increased expression in the endothelium and in the perivascular space (C and D). Similar changes were present in the vessels of the heart (E and F).

a relative decrease of PKC ζ expression in the myocytes was observed (Fig. 4H).

After the immunohistochemical analysis of the tissue was performed, we used Western blot analysis to substantiate further the influence of hyperglycemia on PKC isoforms in the kidney and the heart. These results are shown in Figure 5 for the kidney and in Figure 6 for the heart. Results are expressed in percentage enzyme expression in the normoglycemic (control) animals. In both the kidney and the heart, PKC α was detected as an isolated band with a molecular weight of 82 kDa. In diabetic animals, PKC α expression was significantly increased in the kidney and the heart ($N = 8$, $P < 0.05$). PKC β and its two splicing variants were detected as a single band with a molecular weight of 80 kDa. Expression of PKC β in diabetic animals was not significantly

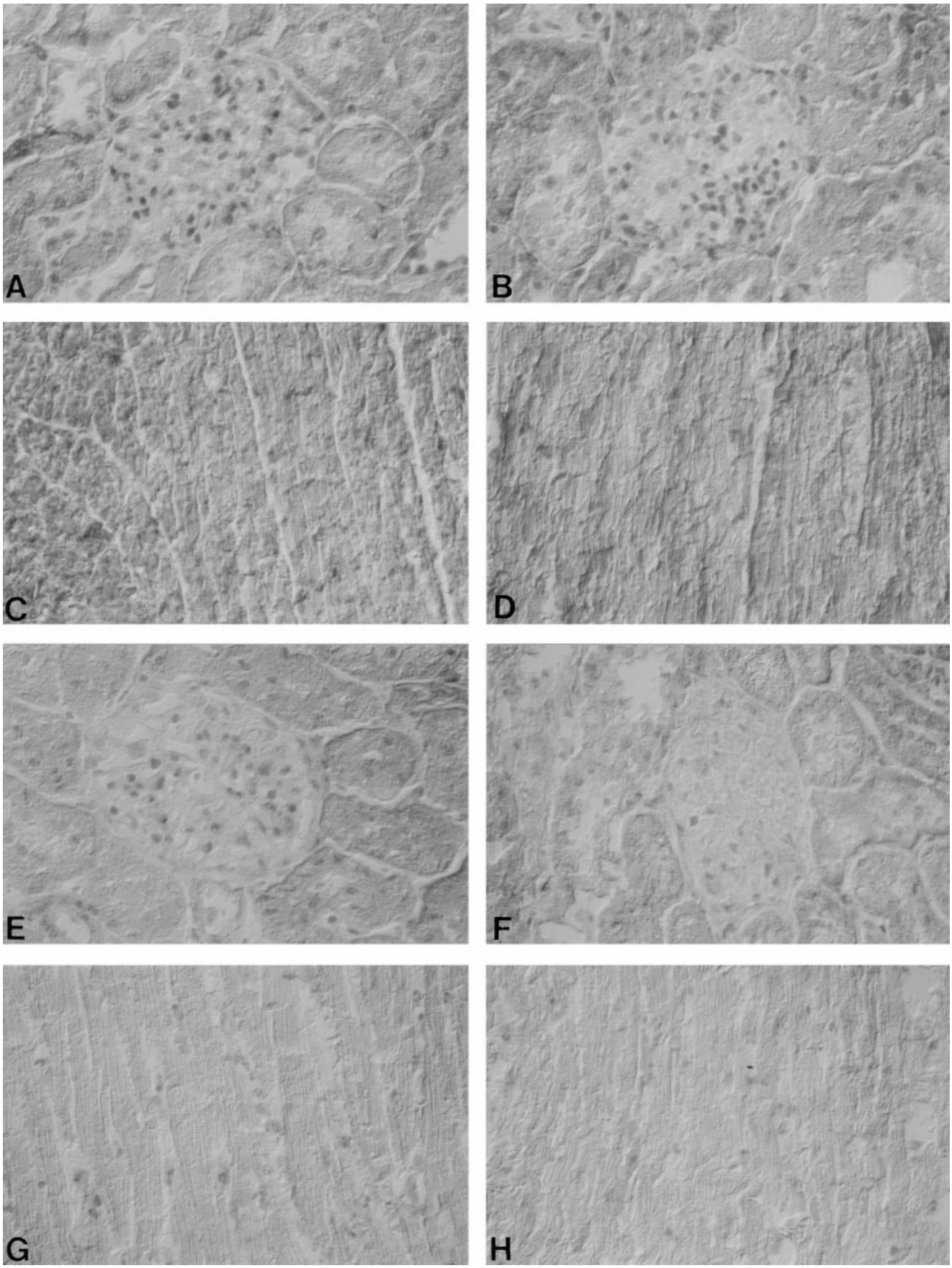

Fig. 2. Immunohistochemistry of PKC β (common antibody; *A–D*) and PKC δ (*E–H*) in kidney (A and B, E and F), and myocardium (C and D, G and H) of normoglycemic (left panel) and hyperglycemic rats (right panel).

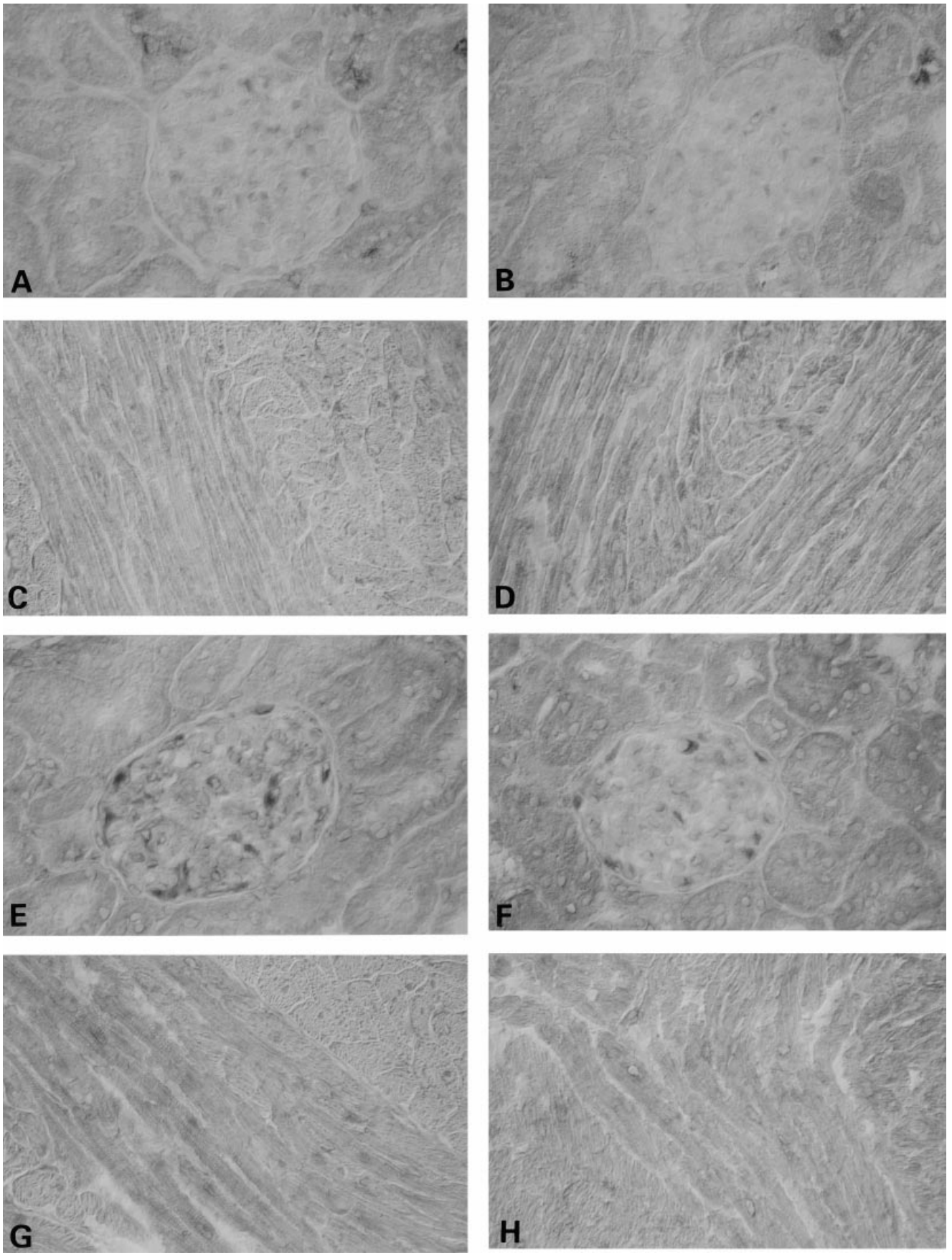

Fig. 3. Immunohistochemistry of PKC βI (*A–D*) and PKC βII (*E–H*) in kidney (A and B, E and F), and myocardium (C and D, G and H) of normoglycemic (left panel) and hyperglycemic rats (right panel).

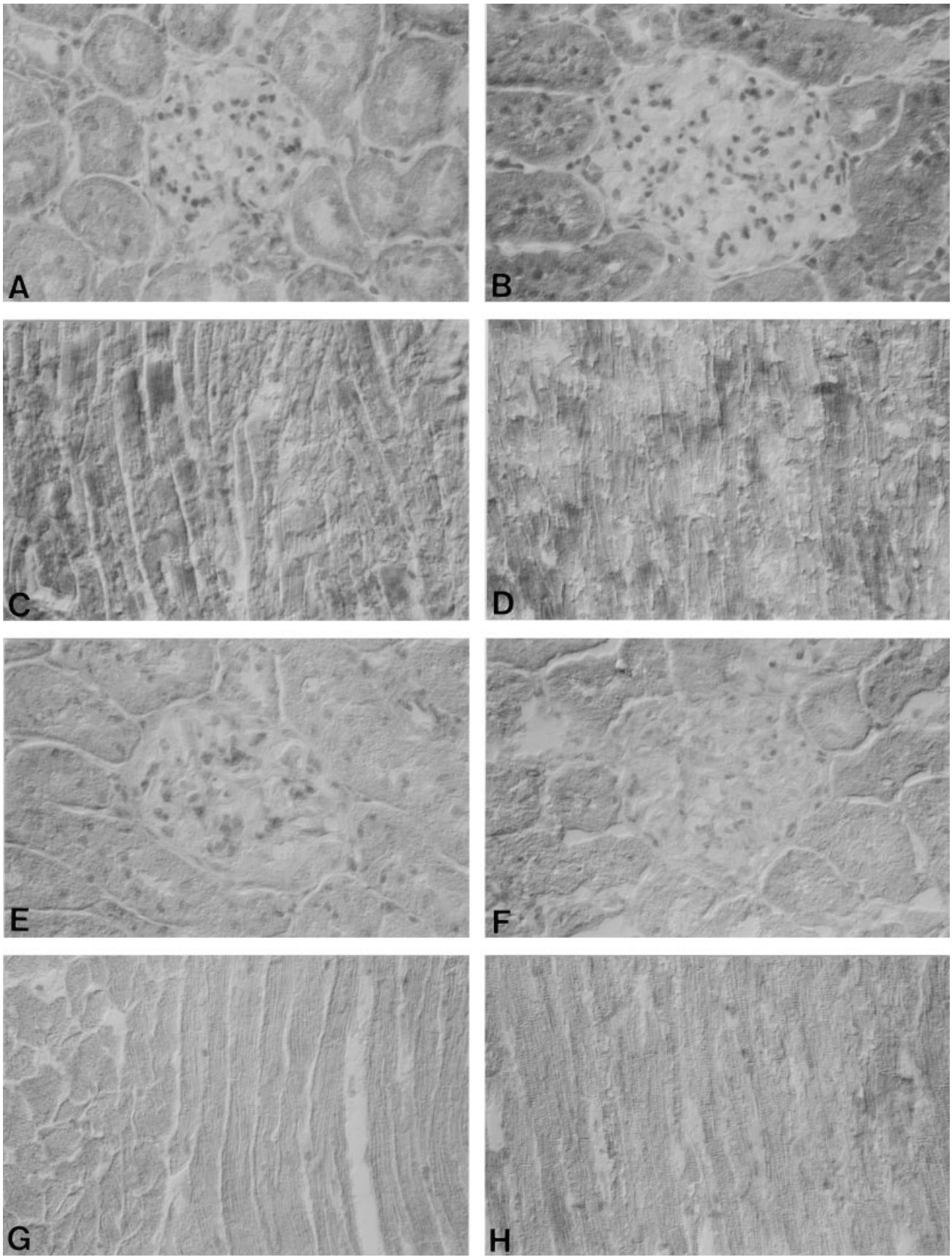

Fig. 4. Immunohistochemistry of PKC ϵ (*A–D*) and PKC ζ (*E–H*) in kidney (A and B, E and F), and myocardium (C and D, G and H) of normoglycemic (left panel) and hyperglycemic rats (right panel).

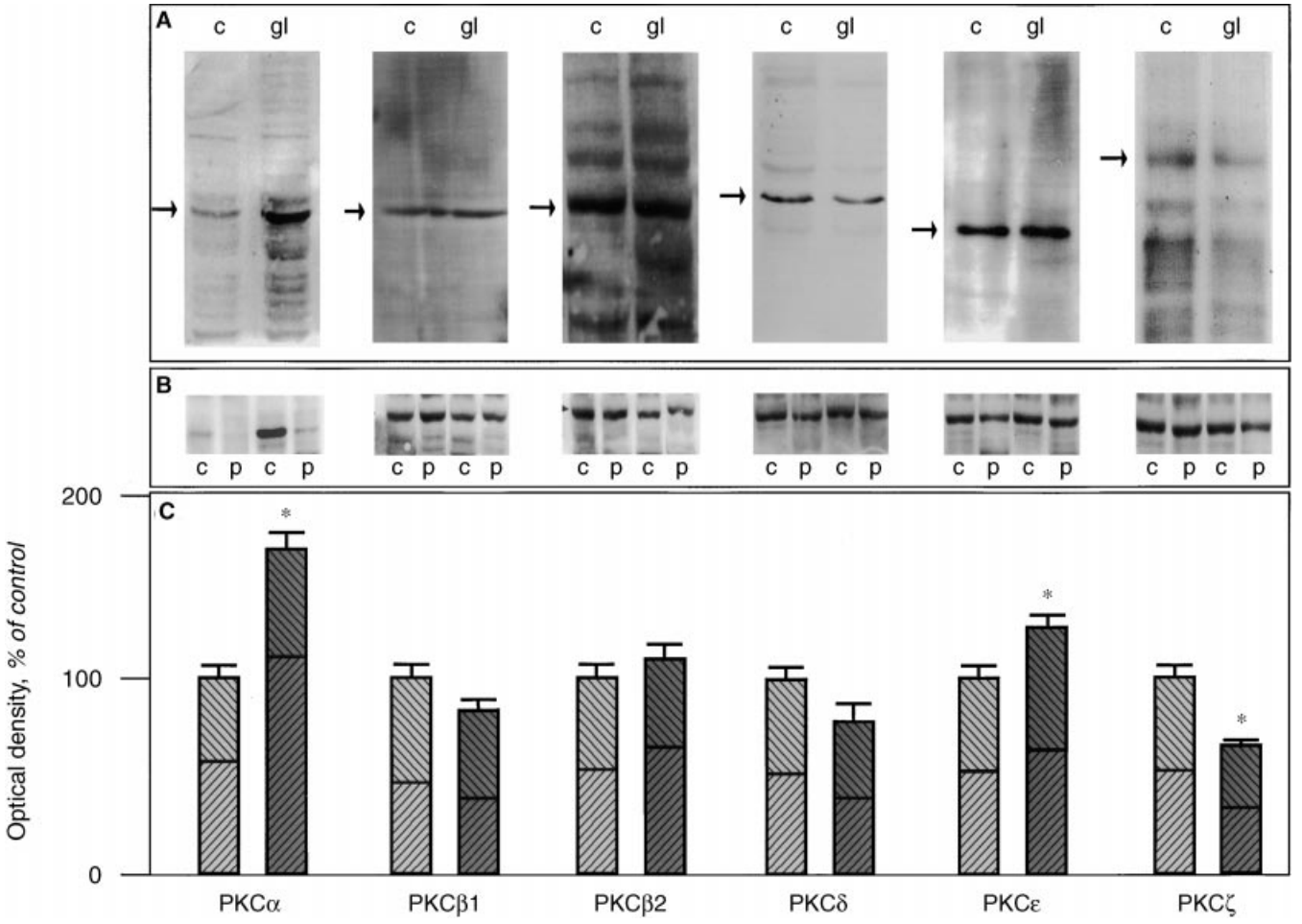

Fig. 5. Protein expression (*A*) and translocation (*B* and *C*) of PKC isoforms α, βI, βII, δ, ϵ, and ζ in renal tissue of normoglycemic control (c) and hyperglycemic (gl) rats. For the translocation experiments, renal cortical tissue was fractionated by ultracentrifugation, and the cytosolic and particulate fractions were analyzed. Bars represent the densitometric analysis of more than experiments. Symbols in C are: (▧) particulate fraction; (▨) cytosolic fraction; (□) control; (■) hyperglycemia.

changed ($N = 8$). PKC δ in kidney and heart had a molecular weight of 78 kDa and was also not influenced by hyperglycemia ($N = 8$). PKC ϵ was detected at 90 kDa. As described in the immunohistochemical analysis, immunoreactivity of this isoform was increased in the kidney but showed a decrease in the heart ($N = 8$, $P < 0.05$). Finally, PKC ζ was detected at a molecular weight of 72 kDa and was less expressed in diabetic animals both in the kidney and the heart ($N = 8$, $P < 0.05$).

We then analyzed the influence of hyperglycemia on the translocation of the PKC isoforms in renal tissue. These results are shown in Figure 5B. We were able to confirm that the expression of PKC α was increased. The translocation experiments showed an increased membrane-association of PKC α and PKC ϵ in hyperglycemic animals; however, the percentage of cytosol versus particulate remained unaltered by hyperglycemia.

The Western blot analysis demonstrated an increased expression of PKC α in diabetic animals. Immunohistochemistry had indicated that the increased PKC α expression was mostly localized in the endothelium. Therefore, we next used immunofluorescence to test the hypothesis that PKC α expression is increased in the endothelium of diabetic animals. The results of these experiments are shown in Figure 7. Tissue from normoglycemic control animals is shown in the left panel, and in tissue from hyperglycemic animals is shown in the right panel. Hyperglycemia induced an increase in PKC α immunoreactivity in the kidney, which was mostly localized in the glomerular and peritubular capillaries (Fig. 7 A–D). Especially in Figure 7D, the distinct increase in immunoreactivity of peritubular capillaries is evident. We then examined skeletal muscle. Figure 7 E and F demonstrate that PKC α immunoreactivity was increased in capillaries of skeletal muscle. Finally, we analyzed the endothelium of larger blood vessels and demonstrated an increased PKC α immunoreactivity in the endothelium of the aorta (Fig. 7 G, H).

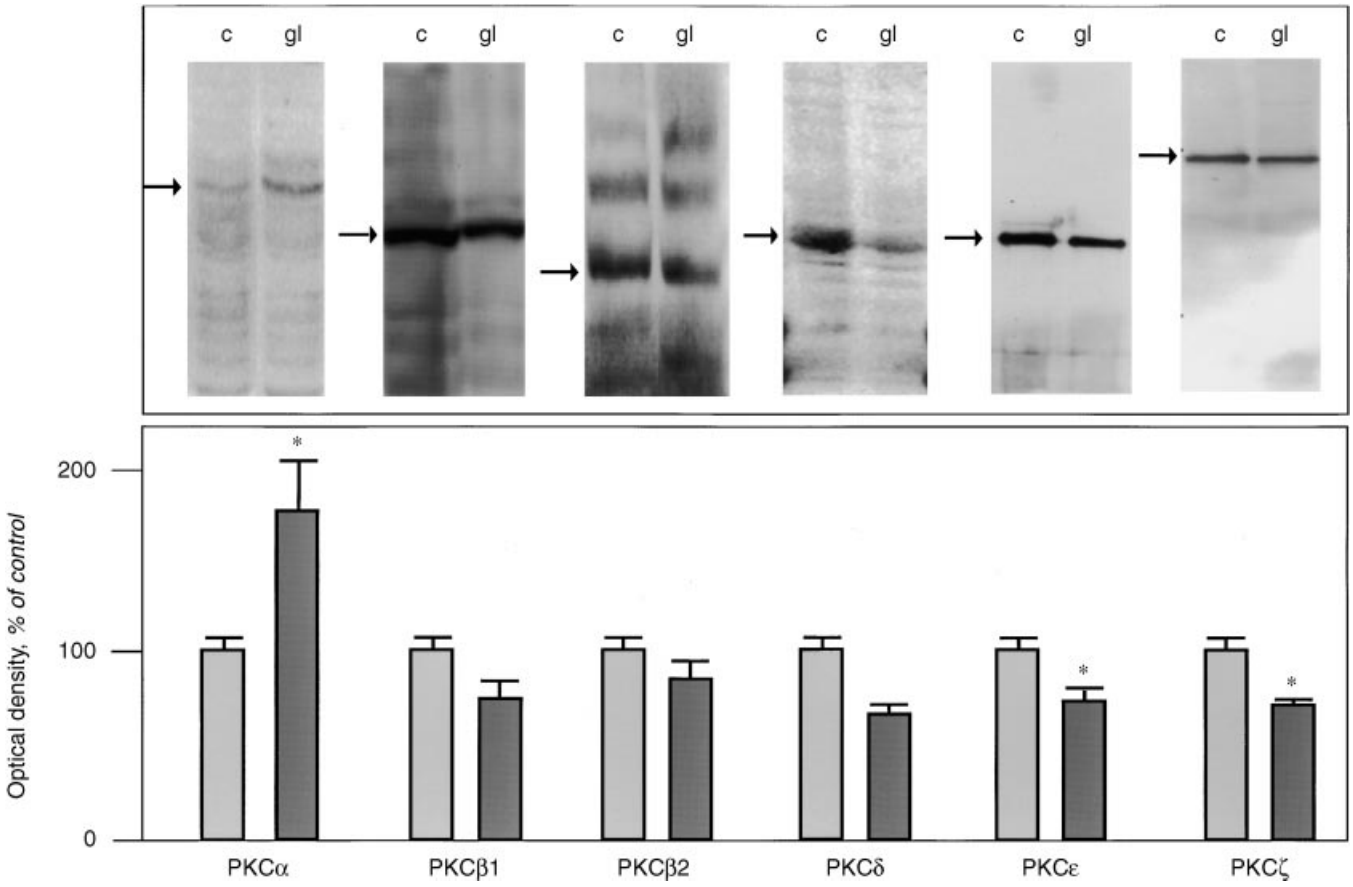

Fig. 6. Western blotting of PKC isoforms α, βI, βII, δ, ϵ, and ζ in myocardial tissue of normal and hyperglycemic rats. Bars are densitometric analysis of more than experiments. Symbols are: (□) control; (■) hyperglycemia.

DISCUSSION

We investigated PKC isoform expression in the kidney, heart, blood vessels, and skeletal muscle from hyperglycemic animals. Our most important finding was an increased PKC α expression during hyperglycemia, whereas PKC β was not significantly influenced. We demonstrated that the increased PKC α expression was localized to endothelial cells and especially enhanced in capillaries. In addition, we found that PKC ϵ was increased in tubules of diabetic animals but was decreased in the myocardium and that PKC ζ was down-regulated under hyperglycemic conditions. These results underscore the importance of PKC isoforms in diabetes-induced organ damage and suggest an important role for the PKC isoform α in endothelial function in diabetes.

Several *in vitro* studies have previously demonstrated that the intracellular effects of high glucose concentration are mediated by PKC [23, 32]. Because PKC is an important regulatory molecule in several cellular reactions important to diabetic complications, such as expression of matrix proteins and metalloproteases, PKC activation by hyperglycemia may play a role in the pathogenesis of diabetes-associated organ damage [6]. Our results, together with earlier observations, suggest that high glucose concentrations mainly affect PKC isoform α [41, 45]. This finding is supported by our recent observation that the glucose-induced increase in endothelial cell permeability is also mediated by PKC α [41]. Glucose-induced activation of this PKC isoform has also been described by Ganesan et al [46]. However, another PKC isoform, namely PKC βII, has been implicated in the intracellular effects of glucose-induced activation of this enzyme family. In two rat diabetic models, the PKC βII isoform was found to be preferentially increased in aorta and heart, whereas PKC α did not change significantly [17, 38]. An increased expression of PKC βII was also recently reported in cultured VSMCs [18]. Further support for a role of PKC βII in hyperglycemia comes from a study by Ishii et al, who recently demonstrated that an oral inhibitor of PKC β ameliorates vascular dysfunction in diabetic rats [39]. However, most of the evidence for a role of PKC β in glucose-induced cell activation stems from experiments using a selective PKC β inhibitor [18]. Conceivably, the compound used in these studies could

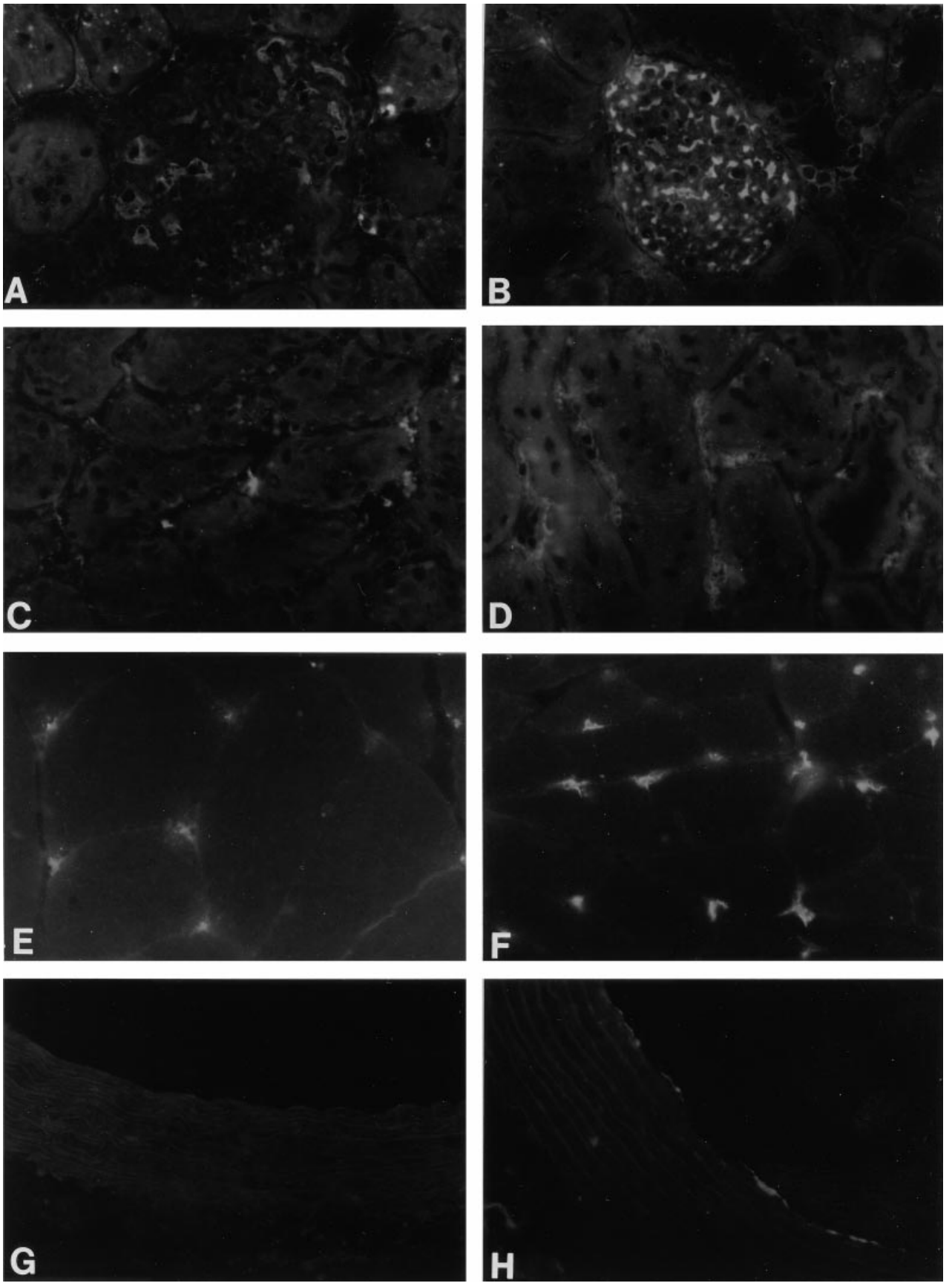

Fig. 7. Immunofluorescence of PKC α in renal tissue (*A–D*), skeletal muscle (*E* and *F*), and aorta (*G* and *H*) of normoglycemic (left panel) and hyperglycemic rats (right panel).

also have inhibited PKC α. Another explanation for the discrepant results in these studies and our observations could be a differential expression of PKC isoforms in different vascular cell types. Our previous finding, that high glucose increases permeability via PKC α, was obtained in endothelial cells [41]. We could not detect expression of PKC β by Western blot and reverse transcription-polymerase chain reaction (RT-PCR) in endothelial cells in an earlier study [40]. It is therefore conceivable that glucose influences PKC α in cell types in which PKC β is not present. However, we have previously shown that high glucose concentration induces translocation of both PKC α and β in VSMCs [16]. In the present study, we could not detect an increase in PKC β expression, but rather a modest decrease in the glomeruli. Although this finding does not rule out an increase in PKC β activity in the diabetic animals, these data support our hypothesis that PKC α is an important intracellular mediator of high glucose concentrations. Our observations concerning the role of PKC α are further supported by the recent observations from Whiteside et al [42]. They demonstrated a significant increase in membrane-associated PKC α, δ, and ϵ after two and four weeks of hyperglycemia in the rat, whereas PKC β was not influenced. In contrast to our observations, they found most of the increased PKC α immunoreactivity in the membrane fraction. This discrepancy, however, could be explained by methodological differences because we have previously shown that PKC translocation studies in whole tissue are difficult, and small changes in the homogenization procedure may influence the amount of membrane-bound enzyme [43]. We have previously shown that in vascular smooth muscle cells, inhibition of PKC α by antisense oligonucleotides resulted in a decreased expression of TGF-β, while down-regulation of PKC β had no influence [44]. Whiteside et al did not observe a decrease in PKC ζ expression [42], whereas Kikkawa et al have previously shown that PKC ζ is translocated by high glucose in mesangial cells [45].

The effects of hyperglycemia on PKC in different cell types may vary. In skeletal muscle from hyperglycemic animals, a down-regulation of PKC activity was observed, whereas in other tissues, PKC activity was found to be increased [46–50]. Furthermore, Donnelly et al recently observed a distinct increase of PKC isoform τ in skeletal muscle from diabetic animals [50]. Therefore, we cannot rule out the possibility that other PKC isoforms besides PKC α mediate the effects of high glucose and hyperglycemia in other cell types and tissues. However, in our animal experiments, we observed an increase in the expression of PKC α. This observation is supported by a recent report of increased PKC α expression in myocardial tissue from diabetic animals, suggesting a role of PKC α in the cellular response to hyperglycemia in the heart [51].

Most of the previous reports on a role of PKC as the cellular effector of hyperglycemia and diabetes have focused on the activation of PKC via glucose-generated diacylglycerol [reviewed in 6]. In contrast, we observed an increase in PKC α expression during hyperglycemia in the rat. This finding would indicate that a high glucose concentration not only induces activation of PKC but also enhances expression of PKC α. Indirect evidence for such a mechanisms comes from several studies in which long-term effects of glucose on PKC activity were described. Williams and Schrier demonstrated that continued exposure to D-glucose induced a sustained PKC activation for up to 48 hours [32]. In a previous report, we observed an increased PKC activity up to 72 hours of high glucose concentration [16]. Because PKC activation leads to a rapid down-regulation of the enzyme and its activity, the sustained activation during hyperglycemia argues for an increased PKC expression and/or protein stabilization. In support for such a hypothesis, several authors have reported a sustained increase in PKC activity in diabetes and hyperglycemia. Giles et al have recently shown increased total PKC activity in the hearts of diabetic animals [51]. Similar findings in the heart have been obtained by others [22]. However, Craven and Derubertis did not find an increase in total PKC activity, but rather an enhanced enzyme activity only in the membrane fraction of isolated glomeruli [14]. In order to solve this issue, we are currently cloning the PKC α promoter to investigate the regulation of PKC α gene expression.

We observed an increased PKC ϵ expression in the kidney of diabetic animals, whereas a decrease was observed in the heart. The role of PKC ϵ in the kidney is not well understood. PKC ϵ has been implicated in the signal transduction of proliferation, and both stimulatory and inhibitory functions have been suggested [52–54]. It seems that PKC ϵ is linked to raf-1 kinase activation and the mitogen-activated protein kinase pathway, and a role for this PKC isoform in mechano-sensitive signal transduction and expression of matrix proteins has been suggested [55–58]. We recently demonstrated that PKC ϵ, together with PKC α, may play a role in endothelial cell permeability, and the increased expression of both isoforms could indicate a cooperative effect in diabetes-induced changes of permeability [41]. However, PKC ϵ was mostly increased in the renal tubules. Several authors have recently implicated PKC ϵ in salt and water transport in the nephron and have shown that this isoform mediated the effects of vasopressin and angiotensin II [59, 60]. The decreased expression of PKC ϵ in myocardial tissue is interesting because recently a distinct role of this PKC isoform in preconditioning was suggested [61]. A decreased expression during hyperglycemia could indicate that the protection against ischemia is less pronounced in diabetes. In addition, PKC ϵ has been impli-

cated in stretch-mediated responses in the heart [62]. Malhotra et al have suggested that angiotensin II receptor-mediated activation of PKC ϵ may play a role in the contractile dysfunction seen in chronic diabetes [63].

In contrast to PKC isoform ϵ, PKC ζ was decreased in both myocardium and kidney. Although PKC ζ has been characterized in kidney [64] and has been implicated in mitogenic signaling [65], its function is not clear. PKC ζ also seems to stimulate the MAP/ERK pathway, however, via a different mechanism [66, 67]. Recently, a role for PKC ζ was demonstrated in the expression of COX-2, prostaglandin E_2 (PGE_2), and inducible nitric oxide synthase in mesangial cells [68]. Possibly, the altered expression of PKC ζ in diabetes influences the regulation of these enzymes; however, currently, the potential role of PKC ζ in diabetic nephropathy is unclear.

In summary, we found that hyperglycemia has a differential and specific effect on PKC isoform expression. We demonstrated increased PKC α expression both in kidney and heart during diabetes. No changes in PKC β and δ were observed. PKC ϵ showed a differential response to hyperglycemia, with an increase in the kidney and a decrease in the diabetic heart. PKC ζ was decreased in both tissues. Immunohistochemistry revealed that PKC ζ was decreased in the glomeruli and in cardiomyocytes. PKC α was most prominently expressed in endothelial cells in kidney and heart, but also in skeletal muscle and larger vessels. In view of our recent observations on the role of PKC α in endothelial and VSMCs [36, 41], we suggest a role for PKC α in the functional disturbance of the endothelium, capillaries, and arterioles in diabetes.

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