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Immunolocalization of protein kinase C isoenzymes α, βI, βII, δ, and ε in mouse kidney

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Redling, Stephanie, Imke L. Pfaff, Michael Leitges, and Volker Vallon. Immunolocalization of protein kinase C isoenzymes α, βI, βII, δ, and ε in mouse kidney. *Am J Physiol Renal Physiol* 287: F289–F298, 2004. First published March 23, 2004; 10.1152/ajprenal.00273.2003.—Localization of protein kinase C (PKC) isoenzymes α, βI, βII, δ, and ε was studied employing Western blot analysis and immunohistochemical methods including confocal laser-scanning microscopy in the kidney of two mice strains, namely, C57BL/6 and 129/Sv, which have recently been used as genetic backgrounds for respective knockout mice. Immunoblot analysis identified immunoreactive bands for each isoenzyme in total kidney cell extracts. Isoenzyme expression sites were identical for both strains. Glomeruli expressed PKC-α, -βI, and -ε. The latter isoenzme was also detected in apical aspects of proximal convoluted but not in proximal straight tubules. In contrast to rats, neither PKC-α nor PKC-βI was detectable in the proximal tubule. Immunofluorescence was observed in luminal membranes of medullary (MTAL) and cortical thick ascending limbs for PKC-βI and in MTAL for PKC-ε. The cortical collecting duct expressed PKC-α, -βI, and -δ in intercalated cells only. In the outer medullary collecting duct, PKC-α and -βI were detectable in principal cells, whereas PKC-δ was found in intercalated cells. In the inner medullary collecting duct, PKC-α, -βI, and -βII were detected. As described for the rat, the expression of PKC-βII was otherwise restricted to cortical and medullary interstitial cells. The specificity of all labeling was confirmed in respective PKC isoenzyme knockout mice. In summary, distinct expression patterns were shown for PKC isoenzymes α, βI, βII, δ, and ε in the mouse kidney.

immunohistochemistry; knockout; renal interstitial cells

THE MAMMALIAN protein kinase C (PKC) family comprises 10 isoenzymes and plays a central role in cell signaling. Assuming that every isoenzyme exerts special functions under physiological but possibly also under pathophysiological conditions, PKC isoenzyme-selective inhibitors may provide new avenues for therapy (24). Clearly, before one considers this approach, a very detailed understanding of PKC isoenzyme expression and function under physiological and pathophysiological conditions has to be established.

In the kidney, PKC is involved in the control of glomerular hemodynamics (32) as well as transport mechanisms in the tubular (4, 5, 26, 33) and collecting duct system (1, 6, 8, 11). Expression of various PKC isoforms has been reported in the kidney of rats and humans. By employing Western blotting, Northern blotting, or in situ hybridization in rat kidney, PKC isoenzymes α (10, 16, 20, 28, 30), βI (16, 20, 30), δ (10, 20, 28), ε (10, 20), ζ (10, 20, 28), λ (10), and ι (10) were detected. Erdbrügger et al. (10) provided evidence by PKC activity assay and Western blot analysis that PKC-α, -δ, -ε, -ζ, -λ. and -ι are expressed in the human kidney (10). All these findings point to a substantial role of PKC in renal function. Little is known, however, about which PKC isoenzymes are involved in the renal actions.

A first approach to gain insights into their renal function is to localize PKC isoenzyme protein expression along the nephron, i.e., to establish their renal site of action, as performed before in the rat kidney for PKC-α (9, 16, 17, 30), -βI (30), and -βII (30). Another promising approach to delineate the function of a gene or its protein is to study the phenotype of the respective gene knockout mouse. With regard to PKC isoenzymes expressed in the kidney, knockout mice have been generated for the isoenzymes α (22), β (including both splice variants, βI and βII) (23), δ (21), and ε (12, 27, Leitges M, unpublished observations). Therefore, the aim of the present study was to localize these PKC isoenzymes in the kidney of their genetic background mice, namely, C57BL/6 and 129/Sv, to *1*) establish their renal site of action in mouse kidney and thus provide a basis for the phenotypical analysis of the knockout mice (38) and *2*) look for potential species differences in the expression pattern. The respective PKC isoenzyme knockout mice have been used in the present study to confirm the specificity of the employed PKC isoenzyme antibodies.

MATERIALS AND METHODS

All animal experiments were conducted in accordance with the National Institutes of Health *Guide for the Care and Use of Laboratory Animals*. Western blot analysis and immunohistochemistry for PKC isoenzymes were adapted from the rat (30) to the mouse and were performed in male C57BL/6 and male 129/Sv mice (25- to 30-g body wt). Animals had free access to standard chow and tap water and were anesthetized on the day of the experiment by intraperitoneal injection of ketamine (60 mg/kg body wt; Cura Med, Karlsruhe, Germany) and xylazine (9 mg/kg body wt; Bayer Vital, Leverkusen, Germany).

Western Blot Analysis

Studies were performed in three C57BL/6 mice and three 129/Sv mice. After anesthesia and opening of the abdominal cavity, kidneys were rapidly excised and immediately immersed in liquid nitrogen. Total cellular proteins were obtained by pulverizing the tissue and dissolving the powder in lysis buffer [(in mM) 20 HEPES, 150 NaCl, 4 EGTA, 4 EDTA, 1 PMSF, and 2 DTT as well as 10% glycerol, 1%

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Triton X-100, 2 μg/ml aprotinin, and 0.5 μg/ml leupeptin]. Homogenization was followed by centrifugation (17.000 *g*, 15 min, 4°C), and the pellet was used for further analysis. Protein content was determined as described by Bradford using a commercial protein assay (Bio-Rad, Munich, Germany) and bovine serum albumin (Serva, Heidelberg, Germany) as a standard. Samples were diluted 1:3 with Roti-Load sample buffer (Roth, Karlsruhe, Germany) and boiled for 2.5 min at 95°C. The following steps were performed at room temperature. Samples of 10 μg of protein were subjected to SDS-PAGE using 7.5% acrylamide gels in a Mini-PROTEAN II Electrophoresis Cell (Bio-Rad). For determination of molecular mass, a 10-kDa protein ladder (Life Technologies, Eggenstein, Germany) was used.

After gel electrophoresis (60 mA/gel, 70 min), proteins were transferred to nitrocellulose paper of 0.45-μm pore size (Schleicher & Schuell, Dassel, Germany). Membranes were blocked for 30 min with PBS containing 5% low-fat milk powder and 0.02% Tween 20 and rinsed twice with PBS containing 0.02% Tween 20 (PBST). Thereafter, the PKC antibodies (anti-PKC-α at 0.25 μg/ml; anti-PKC-βI, -βII, -δ, and -ε at 0.5 μg/ml; Santa Cruz Biotechnology, Heidelberg, Germany) were incubated for 30 min in PBST. The secondary horseradish peroxidase-conjugated antibody was incubated at a concentration of 0.1 μg/ml for 30 min in PBST. Blots were rinsed twice with PBST and washed three times for 15 min with PBST. Immunoreactive bands were visualized by the enhanced chemiluminescence system onto a Hyperfilm BECL (Amersham, Buckinghamshire, UK). The specificity of the PKC binding was confirmed by neutralization of the antibodies with their corresponding peptide. For neutralization, antibodies were incubated with a 10-fold (by weight) excess of peptide antigen for 2 h at room temperature before being incubated with the blotting membrane.

Immunohistochemistry

Studies were performed in five C57BL/6 mice and five 129/Sv mice. After anesthesia, the left cardiac ventricle was exposed through a subcostal incision of the abdominal cavity and subsequent opening of the pericardium. The tip of the perfusion system was placed into the left ventricle, and the arterial system was perfused for 1 min with 3–5 ml PBS (120 mM NaCl, 16 mM Na_2HPO_4, 2.9 mM KH_2PO_4) to clear the kidneys of blood and subsequently for 10 min with 10–15 ml of a fixation solution (4% paraformaldehyde and 3% sucrose in phosphate buffer). Both solutions were at room temperature. Kidneys were removed, cut into slices (3 mm in thickness) displaying cortex and outer and inner medulla, and incubated for 15 min in the fixation solution at 4°C. After being rinsed in PBS for 15 min, kidney slices were dehydrated in 30% sucrose in PBS for 6 h at 4°C. Thereafter, kidney slices were frozen in isopentane precooled by liquid nitrogen and stored at −80°C until further use. Cryosections of ~16 μm were made at −20°C and transferred onto gelatin-coated glass slides. The following steps were performed at room temperature. After preincubation for 20 min in PBS containing 4% normal goat serum or normal donkey serum plus 1% bovine serum albumin and 0.25% Triton X-100, sections were incubated for 1.5 h with the respective anti-PKC antibody (anti-PKC-α at a final concentration of 1 μg/ml; anti-PKC-βI, -βII, -δ, and -ε at 2 μg/ml). For negative control, the primary PKC antibodies were preabsorbed with their corresponding peptides by incubating them with a 10-fold excess of peptide antigen in PBS for 2 h at room temperature before incubation of the sections with the antibody-peptide solution. After the sections were washed three times for 5 min in PBS, they were incubated for 1.5 h with donkey anti-rabbit IgG conjugated with indodicarbocyanine (final concentration 5 μg/ml; Dianova, Hamburg, Germany). Sections were then washed twice for 10 min in PBS and mounted in FluorSave (Calbiochem, San Diego, CA) as fading retardant. For further negative control, immunohistochemical analysis as described above was performed on kidneys from knockout mice for PKC-α (genetic background: 129/Sv) (22); -β (C57BL/6) (23); -δ (C57BL/6) (21); and -ε (C57BL/6–129/Sv hybrids) (Leitges M, unpublished observations).

Double-staining experiments were performed with each of the PKC antibodies and a primary antibody against the respective tissue epitope. For labeling of filamentous actin in the foot process of podocytes and proximal tubular brush border, Alexa Fluor 633-conjugated phalloidin (200 U/ml) was used. The thick ascending limb of Henle was labeled by a polyclonal antibody (goat antiserum) against Tamm-Horsfall protein (final concentration 250 μg total protein/ml; ICN, Eschwege, Germany). To study the expression of PKC in the cortical and medullary collecting duct, a polyclonal antibody against aquaporin-2 (AQP2; final concentration 2.0 μg/ml; Santa Cruz Biotechnology) was applied. The following secondary antibodies were used: donkey anti-rabbit IgG conjugated with indodicarbocyanine or goat anti-rabbit IgG conjugated with Alexa Fluor 488 for PKC antibodies; and donkey anti-goat IgG conjugated with Alexa Fluor 488 for anti-AQP2 antibody and for antibody against Tamm-Horsfall protein. The final concentrations were 5 μg/ml for indiocarbocyanine-conjugated secondary antibodies and 6.6 μg/ml for Alexa Fluor 488-conjugated secondary antibodies. Alexa Fluor 488- and Alexa Fluor 633-conjugated secondary antibodies were obtained from Molecular Probes (MoBiTec, Göttingen, Germany). Sections were studied by confocal laser-scanning microscopy (Zeiss LSM 410 Invert; Jena, Germany).

RESULTS

Western Blot Analysis

Immunoblot analysis identified immunoreactive bands corresponding to PKC-α, -βI, -βII, -δ, and -ε in total kidney cell extracts of both mouse strains (see Fig. 1). The observed molecular masses of ~80 kDa were in agreement with the literature (22, 25, 28, 30). The smaller proteins detected by antibodies against PKC-α and -βI were inhibited by antibody preabsorption with the respective peptide and most likely represent proteolytic fragments due to enzymatic degradation.

Immunohistochemistry

The distribution of PKC isoenzymes α, βI, βII, δ, and ε in the kidney was identical for C57BL/6 and 129/Sv mice and is depicted in Table 1 and illustrated, in part, for C57BL/6 mice in Figs. 2–6. The specificity of all five PKC isoenzyme

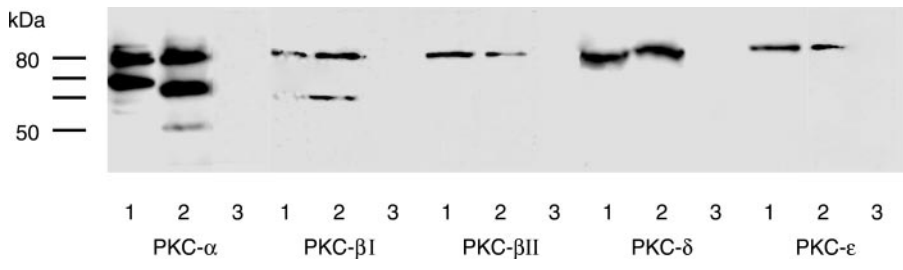

Fig. 1. Western blot analysis showing the expression of PKC-α, -βI, -βII, -δ, and -ε in total kidney cell extracts from C57BL/6 mice (*lane 1*) and 129/Sv mice (*lane 2*) at ~80 kDa. The specificity was confirmed by peptide neutralization of the antibodies with their corresponding peptide (*lane 3*).

Table 1. *Expression of PKC isoenzymes α, βI, βII, δ, and ε in mouse kidney*

	PKC Isoenzyme				
	α	βI	βII	δ	ε
Glomerulus	+	+	−	−	+
Proximal tubule					
Cortex	−	−	−	−	+
OSOM (S3)	−	−	−	−	−
TAL					
Cortex	−	+	−	−	−
Medulla	−	+	−	−	+
Collecting duct					
Cortex					
Principal cells	−	−	−	−	−
Intercalated cells	+	+	−	+	−
Outer medulla					
Principal cells	+	+	−	−	−
Intercalated cells	−	−	−	+	−
Inner medulla	+	+	+	−	−
Interstitial cells					
Cortex	−	−	+	−	−
Medulla	−	−	+	−	−

−, +, No or significant immunostaining, respectively; OSOM, outer stripe of outer medulla; TAL, thick ascending limb. Intercalated cells refer to cells of the cortical and outer medullary collecting duct, which are negative for aquaporin-2.

polyclonal antibodies was tested by preimmunoadsorption with the corresponding peptides. This treatment consistently abolished specific binding of the fluorescent secondary antibody. In accordance, incubation with the antibodies of kidney slices of respective PKC isoenzyme knockout mice (n = 2/knockout mouse) revealed no significant staining (not shown).

PKC-α. Immunostaining for PKC-α was detected in glomeruli (see Fig. 2, *left*). There was no significant signal detectable in proximal tubules, in either the cortex (see Fig. 2, *left*) or the outer stripe of the outer medulla. No visible immunofluorescence was detected in medullary or cortical thick ascending limb (MTAL and CTAL, respectively). PKC-α was found in intercalated but not principal cells of cortical collecting duct (CCD), identified by the expression of AQP2 (see Fig. 2, *middle*). AQP2-positive medullary collecting duct (MCD) cells also expressed PKC-α (see Fig. 2, *right*). PKC-α in the inner medullary collecting duct (IMCD) was restricted to basolateral aspects (see Fig. 5, *right*).

PKC-βI. Anti-PKC-βI stained glomeruli, including mesangial cell-like structures (see Fig. 3, *middle*). As observed for PKC-α, no significant PKC-βI immunofluorescence was observed in the proximal tubule (see Fig. 3, *left* and *middle*). Unlike PKC-α, PKC-βI was detected in MTAL and CTAL (see Fig. 3, *left* and *right*). Like PKC-α, PKC-βI was found in intercalated but not in principal cells of CCD (see Fig. 3, *middle*). In addition, PKC-βI was expressed in AQP2-positive MCD cells (see Fig. 3, *right*).

PKC-βII. In contrast to PKC-α and PKC-βI, PKC-βII could not be detected in glomerular structures. Furthermore, there was no detectable staining with anti-PKC-βII in proximal tubules, TAL, or CCD. In contrast to the other isoenzymes studied, anti-PKC-βII stained interstitial cells in both the cortex and medulla (see Fig. 4, all columns). The only other structure in which PKC-βII was detected was the IMCD (see Fig. 4, *right*).

PKC-δ. No significant staining could be detected for PKC-δ in glomeruli, proximal tubule, or TAL. PKC-δ was found, however, in AQP2-negative CCD cells, i.e., intercalated cells (see Fig. 5, *left*). PKC-δ was also detected in the outer medullary collecting duct (OMCD). In contrast to PKC-α and -βI, however, the expression was localized to AQP2-negative, i.e., intercalated, cells (see Fig. 5, *middle*).

PKC-ε. PKC-ε was detected in glomeruli and apical aspects of the proximal convoluted tubule (see Fig. 6, *left*), whereas straight portions in the outer stripe of outer medulla appeared immunonegative. PKC-ε was also detected in MTAL but, in contrast to the other isoenzymes studied, was not detected in CCD or MCD (see Fig. 6, *right*).

DISCUSSION

The present study revealed the expression of PKC-α, PKC-βI, and PKC-ε in glomeruli of C57BL/6 and 129/Sv mice in accordance with previous studies in the rat (3, 14, 16, 30). In further accordance with the findings in the rat (30), PKC-βI was detected in mesangial cell-like structures. The glomerular cell type expressing PKC-α or PKC-ε was not readily identifiable. Also in accordance with results in the rat (30), there was no labeling of glomerular cells for PKC-βII. Providing first evidence for species differences, PKC-δ was not detectable in glomeruli of the two mouse strains, whereas previous studies were able to detect PKC-δ in rat glomeruli (3, 14). Recent studies proposed a role for glomerular PKC-α and PKC-β in the pathogenesis of albuminuria and diabetic nephropathy (19). At least under normal, nondiabetic conditions, PKC-α knockout mice do not exhibit enhanced urinary albumin excretion (38). Studies in diabetic PKC knockout mice will allow further insights.

Several studies demonstrated that PKC plays an important role in proximal tubular reabsorption (4, 5, 26). Whereas PKC-α (9, 16, 17, 30) and PKC-βI (16, 30) were found to be highly expressed in the brush border of the proximal tubule in the rat, neither of these isoforms was detectable in the proximal tubule of either mouse strain in the present study, indicating significant differences between species with regard to the expression of PKC isoenzymes in the proximal tubule. Whereas PKC-βII (consistent with the finding in the rat) (30) and PKC-δ were also not found in proximal tubule of these two mouse strains, PKC-ε was detected in apical aspects of proximal convoluted but not in proximal straight tubule. In comparison, a previous study had detected PKC-α and -ε but also PKC-δ in rat proximal tubule (17). Furthermore, angiotensin II (17) and dopamine (26) were found to acutely induce translocation of PKC-α and PKC-ε to the membrane in rat proximal tubule and the proximal tubular-like cell line, LLC-PK_1, respectively. The authors proposed a role for PKC-α and PKC-ε in angiotensin II-mediated regulation of Na^+/H^+ and Na^+-HCO_3^- transporters (17) and dopamine-mediated regulation of Na^+-K^+-ATPase (26). Studies in PKC-ε-deficient mice can provide further insights into the functional importance, whereas studies on the respective transporters in proximal tubule of PKC-α knockout and wild-type mice would be helpful to confirm the presented immunohistochemistry by showing no functional evidence for PKC-α in mouse proximal tubule.

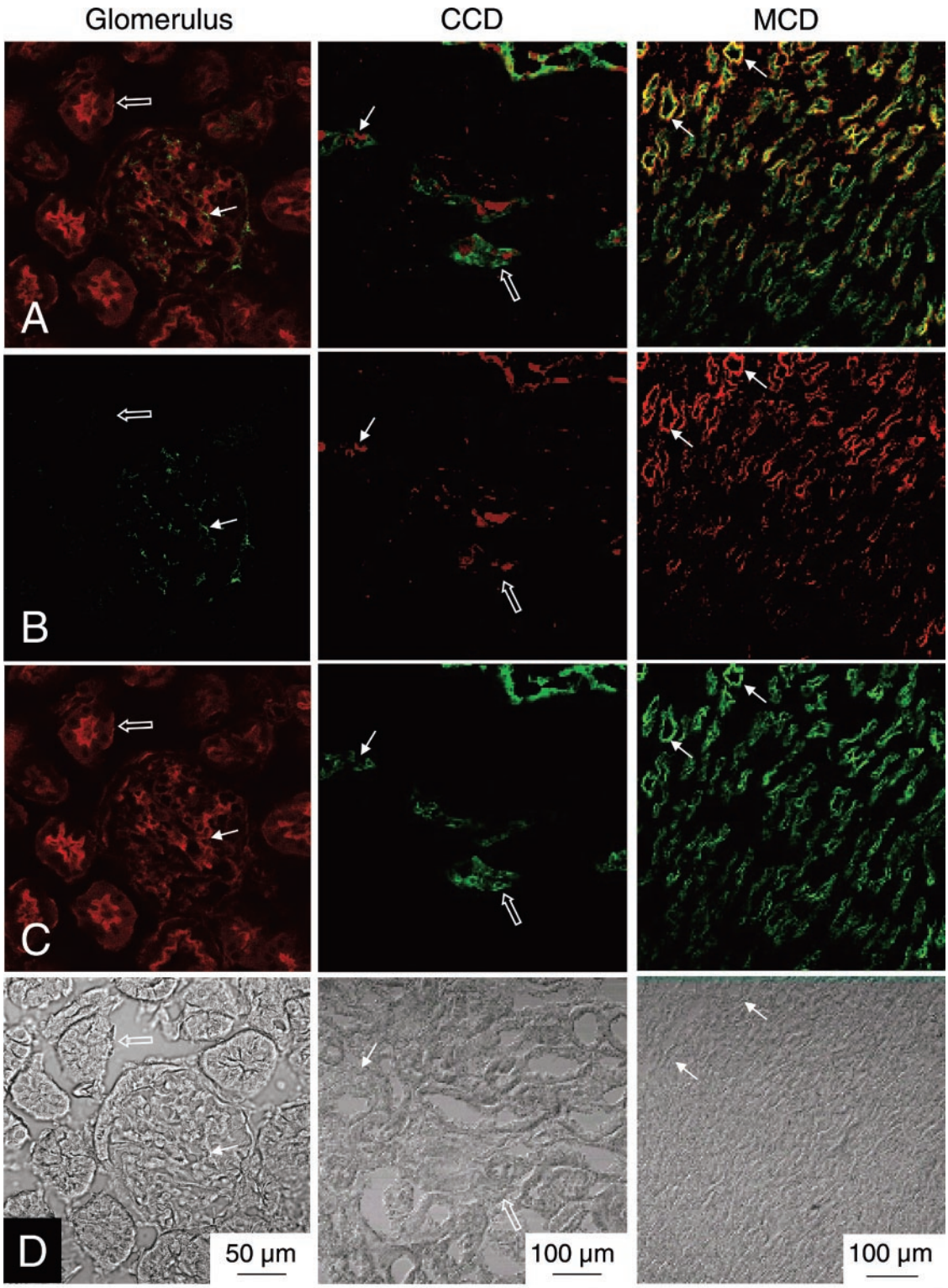

Fig. 2. Immunolocalization of PKC-α. *Row A*: superimposition. CCD and MCD, cortical and medullary collecting duct, respectively. *Row B*: PKC-α (green or red fluorescence). *Row C*: filamentous actin in foot processes of podocytes or proximal tubular brush border labeled by phalloidin (*left*, red fluorescence); principal cells of collecting duct expressing aquaporin-2 (AQP2; *middle* and *right*, green fluorescence). *Row D*: Numarski. *Left*: PKC-α was detectable in glomeruli (filled arrows) but not in proximal tubule (open arrows). *Middle*: in the CCD, PKC-α was detected in intercalated cells (filled arrows) but not in principal cells (open arrows). *Right*: PKC-α was detected in principal cells of the MCD (filled arrows).

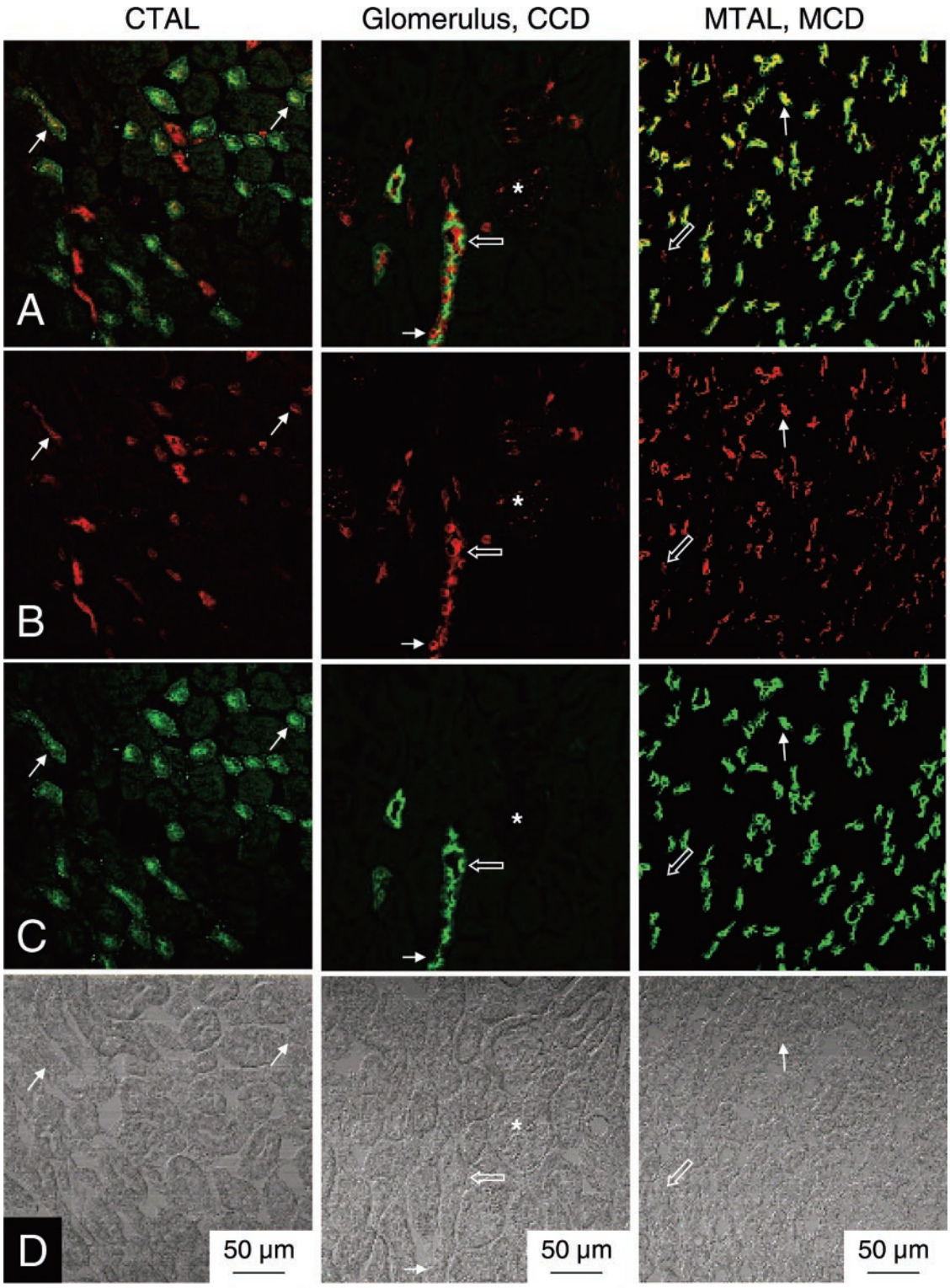

Fig. 3. Immunolocalization of PKC-βI. *Row A*: superimposition. *Row B*: PKC-βI (red fluorescence). *Row C*: Tamm-Horsfall protein expressed in cortical thick ascending limb (CTAL; *left*, green fluorescence); principal cells of collecting duct expressing AQP2 (*middle* and *right*, green fluorescence). *Row D*: Numarski. *Left*: PKC-βI was detected in CTAL (filled arrows). *Middle*: in CCD, PKC-βI was detected in intercalated cells (filled arrows), whereas principal cells were negative (open arrows). In addition, PKC-βI was detected in glomeruli (asterisks). *Right*: PKC-βI was detected in principal cells of MCD (filled arrows) and was less intensive in medullary thick ascending limb (MTAL; open arrows).

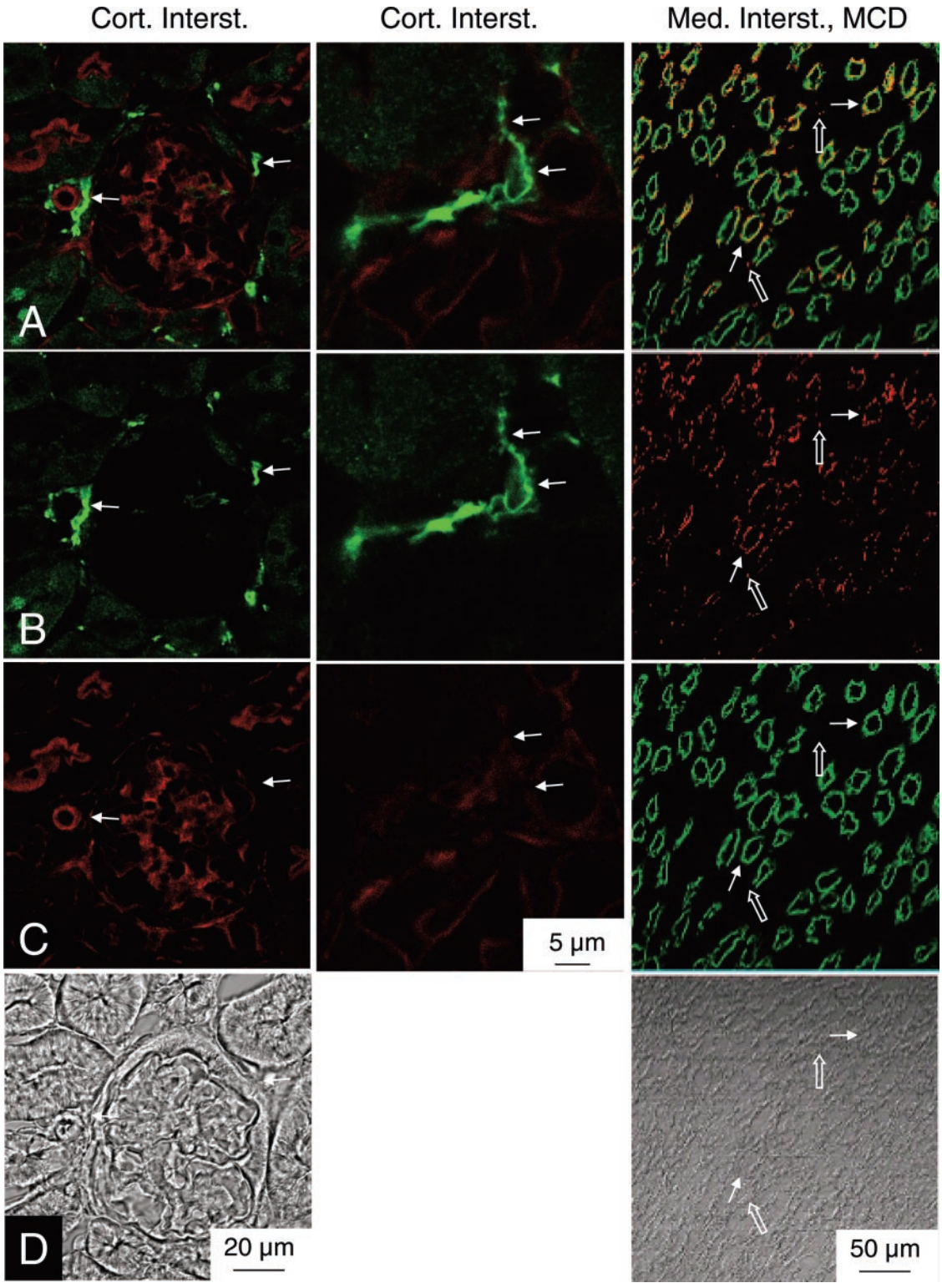

Fig. 4. Immunolocalization of PKC-βII. *Row A*: superimposition. *Row B*: PKC-βII (*left* and *middle*, green fluorescence; *right*, red fluorescence). *Row C*: filamentous actin in foot processes of podocytes or proximal tubular brush border labeled by phalloidin (*left* and *middle*, red fluorescence) or principal cells of collecting duct expressing AQP2 (*right*, green fluorescence). *Row D*: Numarski (except *middle*). *Left* and *middle*: PKC-βII was detected in cortical interstitial cells (Cort. Interst.; filled arrows). *Right*: PKC-βII was expressed in medullary interstitial cells (Med. Interst.; open arrows) as well as in MCD (filled arrows).

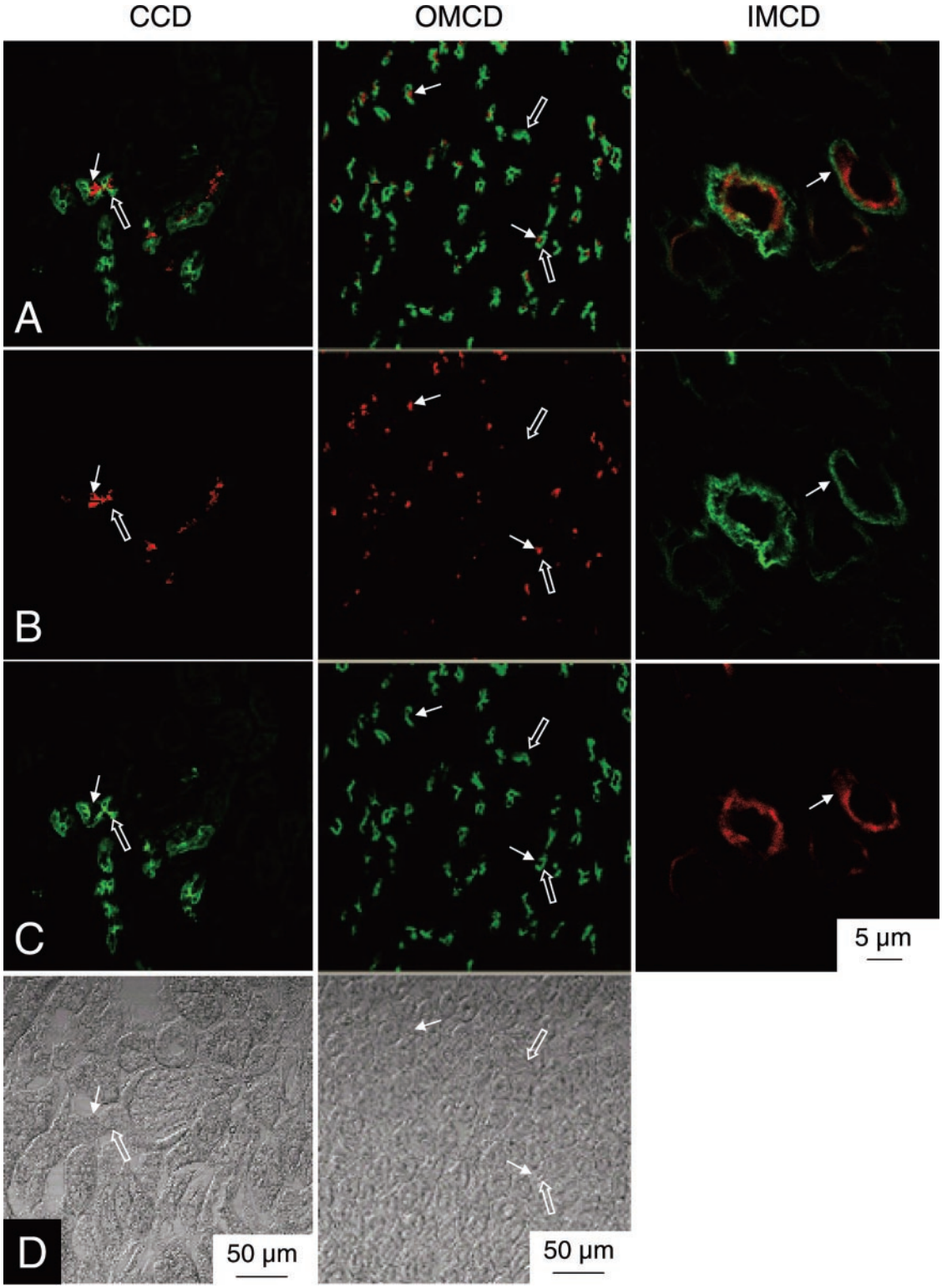

Fig. 5. Immunolocalization of PKC-δ (*left* and *middle*) and PKC-α (*right*). *Row A*: superimposition. *Row B*: PKC-δ (*left* and *middle*, red fluorescence) or PKC-α (*right*, green fluorescence). *Row C*: principal cells of collecting duct expressing AQP2 (*left* and *middle*, green fluorescence; *right*, red fluorescence). *Row D*: Numarski (except *right*). *Left*: PKC-δ was detected in intercalated cells of CCD (filled arrows), whereas principal cells were negative (open arrows). *Middle*: PKC-δ was detected in intercalated cells of outer MCD (OMCD; filled arrows), whereas principal cells were negative (open arrows). *Right*: PKC-α was expressed in basolateral aspects of IMCD (filled arrows).

With regard to the loop of Henle, the present immunohistochemical experiments indicate that PKC-βI, as observed before in the rat (30), is expressed in MTAL and CTAL. Similarly confirming previous studies in the rat (2), PKC-ε was found in MTAL. In contrast to the rat (2, 23), however, PKC-α and PKC-δ could not be detected in TAL of either mouse strain, providing further evidence for potential species differences. A recent study performed in the rat indicated that activation of

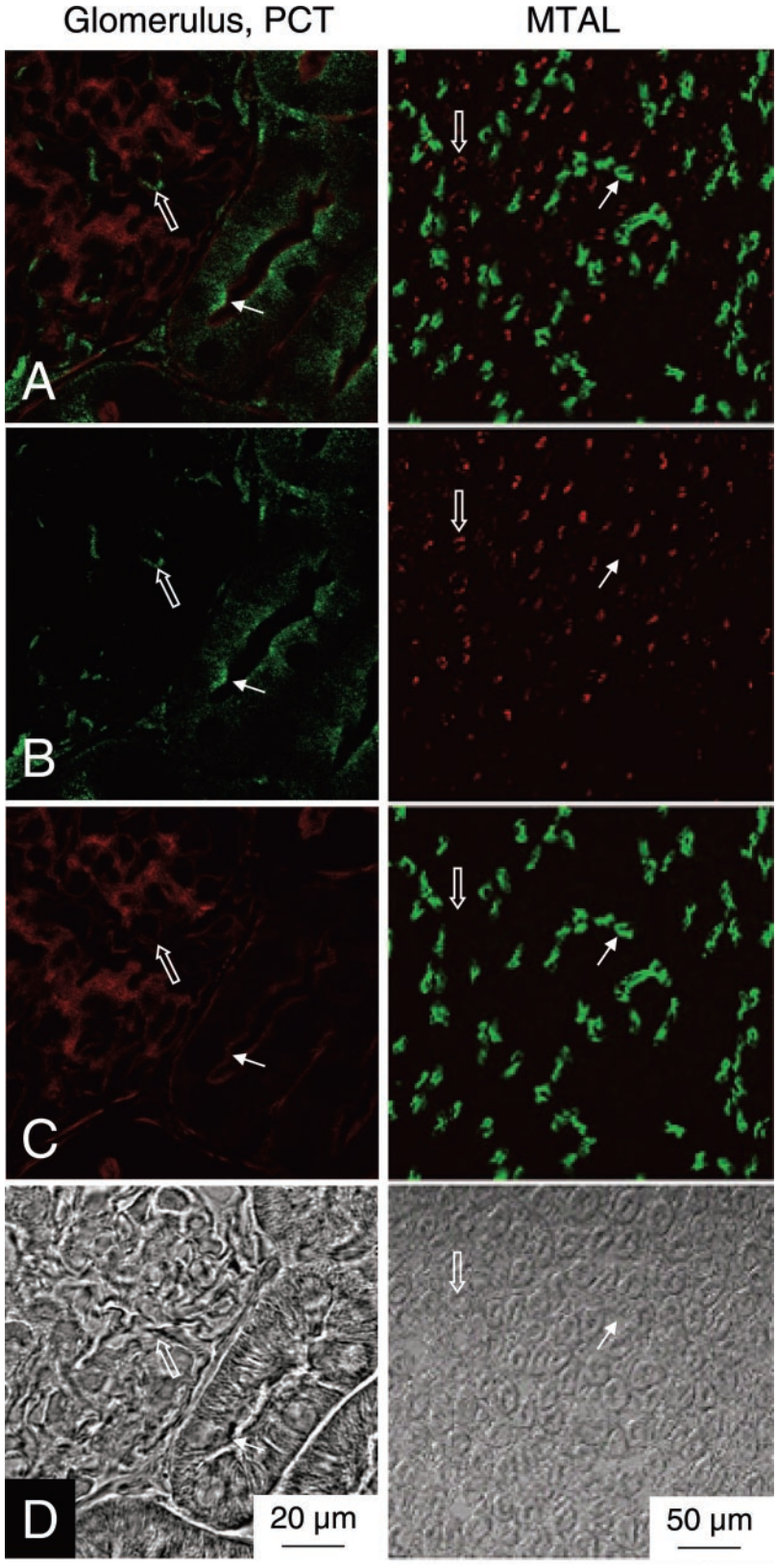

Fig. 6. Immunolocalization of PKC-ε. *Row A*: superimposition. *Row B*: PKC-ε (*left*, green fluorescence; *right*, red fluorescence). *Row C*: filamentous actin in foot processes of podocytes or proximal tubular brush border labeled by phalloidin (*left*, red fluorescence) or principal cells of collecting duct expressing AQP2 (*right*; green fluorescence). *Row D*: Numarski. *Left*: PKC-ε was detected in apical aspects of convoluted proximal tubule (filled arrows) as well as in glomeruli (open arrows). *Right*: PKC-ε was detected in (AQP2-negative) MTAL (open arrows) but not in AQP2-positive OMCD (filled arrows).

PKC-α can stimulate Na^+-K^+-ATPase activity and can thus contribute to regulation of NaCl reabsorption in MTAL (34). Studies in PKC-α knockout mice revealed a normal ability to dilute the urine (38). Because intact NaCl reabsorption in MTAL is a prerequisite for a normal ability to dilute urine, the latter observation does not prove, but is consistent with, the lack of influence of PKC-α on NaCl reabsorption in mouse TAL. This would be consistent with the fact that PKC-α was not detectable in mouse TAL by immunohistochemistry.

PKC has been shown to mediate the inhibitory influence of various hormones on water channels and sodium transport as well as inhibition of the secretory K^+ channel in the CCD (1, 7, 13, 35). In the present study, PKC-α and PKC-βI were detected in mouse CCD. As observed in the rat (30), PKC-βI was found in intercalated cells of the CCD identified by the absence of AQP2. Whereas PKC-α was described in principal cells and part of intercalated cells in the rat CCD (30), the present studies revealed a restriction to intercalated CCD cells in the mouse. Besides PKC-α and PKC-βI, PKC-δ was also detected in AQP2-negative cells in the CCD, i.e., intercalated cells. Thus none of the studied isoenzymes was detectable in principal cells of the CCD in the two mouse strains, indicating that in the mouse regulation of transport in principal cells of the CCD may be mediated by other isoenzymes.

Consistent with previous findings in the rat, PKC-α (9, 30), PKC-βI (30), and PKC-βII (30) were detected in AQP2-positive cells of mouse MCD with the expression of PKC-βII being restricted to the IMCD. In the latter segment, PKC-α expression was restricted to basolateral aspects. In contrast to these isoenzymes, PKC-δ was found in AQP2-negative cells of the OMCD, indicating a functional role in intercalated cells of the outer medulla. PKC-ε was the only isoenzyme studied not detectable in the collecting duct of the two mouse strains. Providing further evidence for species differences, a previous study had shown the expression of PKC-ε in rabbit CCD (37).

The observation that PKC-α expression along the tubular and collecting duct system of the mouse appears to be restricted to intercalated cells of the CCD and basolateral aspects of the IMCD indicates potential distinct functions of PKC-α that may relate to acid-base balance and/or urinary concentration mechanisms, respectively. Whereas the role of PKC-α in intercalated cells has not yet been addressed, experiments in PKC-α knockout mice in fact revealed evidence for impaired urinary concentration, which is due to intrarenal alterations that most likely reside within the MCD (38).

Unlike PKC-α, -βI, -δ, or -ε, PKC-βII was detected in interstitial cells of both the cortex and medulla, which is consistent with previous observations in the rat (30). The studies in the rat indicated that a substantial part of cortical and medullary interstitial cells expressing PKC-βII are dendritic, antigen-presenting cells. Moreover, a very recent study showed enhanced renal cortical expression of PKC-βII associated with an increased number of PKC-βII-expressing interstitial cells in the renal cortex of early diabetic rats (29). This is of interest because selective pharmacological inhibition of PKC-β has been reported to ameliorate early changes in the diabetic kidney (15). Furthermore, nephroprotective effects of angiotensin-converting enzyme inhibition in diabetic rats were associated with, and thus may be mediated in part by, inhibiting diabetes-induced activation of PKC-βII in the renal cortex (29). Clearly, more insights into the role of PKC-βII in renal interstitial cells are required and studies in respective knockout mice may be very helpful.

In conclusion, the present study shows a distinct and differential expression pattern for PKC isoenzymes α, βI, βII, δ, and ε in the mouse kidney, which in combination with experiments in respective knockout mice, can contribute to a greater understanding of the specific role of these isoenzymes in the control of renal function. The present study further indicates that whereas many expression sites of PKC isoenzymes appear to be the same in rat and mouse kidney, some significant species differences that deserve consideration do exist. As a consequence, to consider PKC isoenzyme-selective inhibitors for therapy, a detailed localization of PKC isoenzymes in the human kidney seems to be required.

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