Introduction

Protein kinase C (PKC) is a family of protein kinases that specifically phosphorylate serine/threonine residues. The family includes at least 11 isoforms (α, βI, βII, γ, δ, ε, η, θ, λ, and μ) in mammalian tissue. These isoforms are divided into three subgroups based on their structure and mode of activation. The first group, the classical or conventional PKCs (cPKCs), including the isoenzymes α, βI, βII and γ, with regard to their expression in adult and developing rat kidney. PKCα appeared in the ureteric bud at embryonic day (E) 16, and the proximal and distal anlage at E18. After birth, the immunoreactivity of PKCα gradually decreased. In adult, PKCα was expressed intensely in the connecting tubule (CNT), the collecting ducts (CD) and the renal corpuscle, and weakly in the proximal and distal tubules. PKCβI appeared in the ureteric bud at E16, and the proximal anlage at E18. After birth, the immunoreactivity of PKCβI gradually disappeared from the CD and proximal tubule. In adult, PKCβII was expressed in the intercalated cells of the CNT and cortical CD, the proximal straight tubule, and the renal corpuscle. PKCβIII appeared in distal anlage at E18, and increased markedly after birth. In the CD, PKCβIII immunoreactivity appeared after birth. In adult, PKCβIII was expressed in the distal tubule, the CNT and the CD. The immunoreactivity for PKCγ appeared only in the proximal anlage at E18, and increased temporally around the time of birth. However, no immunoreactivity for PKCγ was observed in adult rat kidney. These results indicate that classical PKC isoforms appear to play a role in the regulation of various renal functions and differentiation within specific functional units of the uriniferous tubule in rat kidney.

Key Words: Protein kinase C, Development, Kidney
Na\(^+\)/HCO\(_3^-\) cotransporter\(^7, 8\). According to the findings, PKC may be involved in the modulation of intracellular transporters.

There are several studies showing that various PKC isoforms that are expressed in the rat kidney. Kosaka et al.\(^9\) and Ono et al.\(^10\) showed the PKC isoenzymes \(\alpha, \beta, \gamma\); Wetsel et al.\(^11\), \(\alpha, \beta, \delta, \epsilon, \gamma\); Caterina et al.\(^12\) and Aristimuno and Good\(^13\), \(\alpha, \beta, \delta, \epsilon, \gamma\); Ostlund et al.\(^14\), \(\alpha, \beta, \gamma\); Serlachius et al.\(^15\), \(\alpha, \beta\); and Pfaff et al.\(^16\), \(\alpha, \beta, \delta, \epsilon, \gamma\); and Aristimuno and Good\(^17\), \(\alpha, \beta, \gamma\); Hashimoto et al.\(^18\) and Hirata et al.\(^19\) detected PKC in brain tissue and Pucea et al.\(^20\) and Rybin et al.\(^21\) detected it in heart tissue. Serlachius et al.\(^15\) suggested a distinct and differential expression and distribution of PKC isoenzymes depending on embryonal development in the kidney. Moreover, they reported that inhibition of PKC activation enhances apoptosis and induces impairment of nephron formation. These findings support that PKC plays a role in growth and differentiation in development\(^22-25\).

To identify the function of PKC in the kidney, we studied the differential expression and localization of the PKC isoenzymes \(\alpha, \beta, \gamma\) in the developing rat kidney using immunohistochemistry.

**Materials and Methods**

1. Animals and preservation of kidneys

Male Sprague Dawley rats weighing approximately 250 to 300 g were used in all experiments. Prenatal kidneys were obtained from 16–, 18– and 20–day-old fetuses. Postnatal kidneys were obtained from 1–, 3–, 7–, 14– and 21–day-old pups and adult. The animals were anesthetized with an intraperitoneal injection of urethane (16.5%) and perfused with periodate–lysine–paraformaldehyde (PLP) solution for 3–5 minutes through the abdominal aorta. Kidneys were removed, and cut into 2–mm-thick slices, including the renal papilla. Slices were then immersed in PLP solution for 6–12 hours at 4°C. Tissues were embedded in wax or EPON 812. For immunohistochemistry using a pre-embedding method, PLP–fixed tissues were cut on a vibratome (Lancer Vibratome Series 10 00; Technical Products International, St. Louis, MO) to a thickness of 50 μm.

2. Immunohistochemistry

1) Immunostaining of wax sections

The 50-μm-thick wax sections were dewaxed in xylene and hydrated through an ethanol series and washed for 10 minutes. Sections were incubated with 1.4% methanolic H\(_2\)O\(_2\) for 30 minutes and with 0.5% Triton X–100 (0.01 M PBS, pH 7.4) for 15 minutes. After rinsing three times in PBS, sections were incubated for 1 hour in PBS containing 10% normal goat serum (Vector Laboratories, Burlington, CA, USA). Sections were immunostained with rabbit polyclonal IgGs (Santa Cruz technology, CA, USA) against PKCs \(\alpha, \beta, \gamma\); Serlachius et al.\(^15\) suggested a distinct and differential expression and distribution of PKC isoenzymes depending on embryonal development in the kidney. Moreover, they reported that inhibition of PKC activation enhances apoptosis and induces impairment of nephron formation. These findings support that PKC plays a role in growth and differentiation in development\(^21-25\).

To identify the function of PKC in the kidney, we studied the differential expression and localization of the PKC isoenzymes \(\alpha, \beta, \gamma\) in the developing rat kidney using immunohistochemistry.

To identify the immunoreactivity of PKC in intercalated cells in adult rat kidney, 1 mm semi-thin sections, embedded in EPON 812, were cut into slices
displaying cortex, outer medulla and inner medulla. The EPON was removed using saturated sodium hydroxide. Antibodies against PKCa, βI, βII and γ were used. H\textsuperscript{+}-ATPase (1:2,000) were used on adjacent sections. Antibodies against aquaporin-1 (AQP-1; 1:2,000) were used to differentiate descending thin limbs of Henle from proximal convoluted tubules. Immunostaining was performed with avidin–biotin–peroxidase complex (ABC), and then the sections were examined after staining with the blue-gray-colored Vector SG (Vector Laboratories).

Results

1. Immunohistochemistry

The PKC isoenzymes α, βI and βII, but not γ, were expressed in the adult rat kidney in the tubules, and the distribution in the tubules was variable (Table 1, 2). PKC isoenzymes α, βI, βII and γ were expressed in the developing kidney, and distinct and differential expression patterns were shown during development.

2. PKCa

In the adult kidney, PKCa immunostaining was generally observed in the entire tubule, but was most strongly observed in the connecting tubules and the cortical collecting ducts. On staining with H\textsuperscript{+}-ATPase, PKCa-positive cells were strongly evident in the intercalated cells. Type A intercalated cells were stained in the supranuclear portion of the cytoplasm and type B intercalated cells were stained throughout the entire cytoplasm. The connecting tubule cells and principal

| Table 1. Immunoreactivity of Classical Protein Kinase (PKC) Isoforms in Rat Kidney |
|---------------------------------|-----------------|-----------------|-----------------|-----------------|
| RC    | PKCa | PKCBα | PKCBβ | PKCY |
| PE    |      | ±~+   |      |      |
| P     |      | ±     |      |      |
| MC    | ++   |      |      |      |
| PT    |      |      |      |      |
| PCT   |      |      |      |      |
| PST   | +    | ++   |      |      |
| IT    |      |      |      |      |
| ATL   |      |      |      |      |
| DT    |      |      |      |      |
| TAL   | ±    | ±~+  |      |      |
| MD    | ±    | ±~+  |      |      |
| DCT   | ±    | ±~+  |      |      |
| CNT   |      |      |      |      |
| CD    |      |      |      |      |
| CCD   |      |      |      |      |
| OMCD  |      |      |      |      |
| OS    |      |      |      |      |
| IS    |      |      |      |      |
| IMCD  |      |      |      |      |
| i     |      |      |      |      |
| t     |      |      |      |      |

Abbreviations: RC, renal corpuscle; PE, parietal epithelium; P, podocytes; MC, mesangial cells; PT, proximal tubules; PCT, proximal convoluted tubules; PST, proximal straight tubules; IT, intermediate tubules; DTL, descending thin limb; ATL, ascending thin limb; DT, distal tubules; TAL, thick ascending limb; MD, macular densa; DCT, distal convoluted tubules; CCT, connecting tubules; CD, collecting ducts; CCD, cortical CD; OMCD, outer medullary CD; OS, outer stripe of the OMCD; IS, inner stripe of the OMCD; IMCD, inner medullary CD; i, initial part of the IMCD; t, terminal part of the IMCD. Symbols designate not detectable (-), faint (+), weak (++), moderate (+++), and high (+++) levels of immunoreactivity. Values in parentheses represent the levels of immunoreactivity in the intercalated cells.
cells were stained weakly at the basolateral plasma membrane. In the medullary collecting ducts, the intercalated cells were PKCa negative and the principal cells were PKCa positive at the basolateral plasma membrane. The inner stripe of the outer medulla and the initial part of the inner medulla showed strong immu-
noreactivity. In the renal corpuscle, mesangial cells showed moderate immunoreactivity, and we observed weak immunoreactivity in parietal epithelial cells and podocytes. In the proximal tubule, the convoluted part showed weak immunoreactivity on the microvilli and faint immunoreactivity was observed in the straight portion. The cytoplasm of distal tubule cells showed faint immunoreactivity. We did not observe any PKCa immunoreactivity in the descending or ascending limbs of the Loop of Henle (Fig. 1; Table 1, 2).

Fig. 2. DIC micrographs of wax sections illustrating immunostaining for PKCa in 16− (A), 18− (C & F), and 20−day−old (G) fetal kidneys, and 3− (D & H) and 7−day−old (B, E & I) pups. Protein kinase Cα (PKCa) appeared in the ureteric buds (UB) at 16 days of gestation (A) and in the proximal and distal anlage (stars) at 18 days of gestation (C). B. Note that the PKCa−positive tubular profiles (arrows), which are newly formed proximal and distal tubules, are located only in the subcapsular region in 7−day−old pups, whereas PKCa immunoreactivity is decreased in the mature tubules such as the proximal convoluted tubule (PCT) and connecting tubule (CNT) cells located in the inner cortex. C−E. Note the PKCa immunoreactivity in the mesangial cells (arrows) and parietal epithelium (arrowheads) of the developing renal corpuscle. F−G. Immunoreactivity for PKCa in the basolateral plasma membrane of inner medullary collecting duct (IMCD) cells gradually decreased during development. Note the disappearance of apical PKC expression in the intercalated cells (arrows) of the medullary collecting duct (MCD) after birth. Magnifications: A, ×200; B, ×200; C−I, ×528.
In the developing kidney, PKCa appeared in the ureteric bud at 16 days of gestation, but there was no staining at the renal vesicle and S-shaped body (Fig. 2A). The PKCa immunoreactivity of the collecting tubule gradually decreased during development and showed a mature pattern from 14 days after birth (Fig. 2F–I). PKCa appeared strongly in the mesangial and parietal cells of the developing renal corpuscle in stage III, proximal anlage and distal anlage of the 18-day-old pups, whereas immunoreactivity for PKCa gradually decreased in mature proximal convoluted and distal convoluted tubules (Fig. 2A–E). In the intercalated cells, immunoreactivity was shown in the connecting and collecting tubules of 18-day-old pups.

3. PKCβ

In the adult kidney, there was strong positive PKCβ staining in the connecting segment and intercalated cells of the cortical collecting duct. Similar to PKCa, type A intercalated cells were positive in the supranuclear area and type B cells were positive throughout the entire cytoplasm. PKCβ staining was negative in principal cells. In the proximal tubule, the convoluted part was negative and the straight portion was moderately positive. In the renal corpuscle, the mesangial cells were weakly positive, parietal cells faintly positive, and the
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Podocytes negative. The thick ascending limb of the Loop of Henle and the distal convoluted tubule were also negative. We did not observe immunoreactivity in the outer and inner medullary collecting tubules. However, we did detect moderately positive staining in the inner medulla, at the apical plasma membrane of the descending thin limb (Fig. 3; Table 1, 2).

In the developing kidney, PKCβI immunoreactivity appeared from 16 days of gestation and was strongly positive in the ureteric bud (Fig. 4A). The immunoreactivity of the collecting tubule was strong in the fetus, but decreased markedly after birth, and the principal cells were negative 3 days after birth (Fig. 4E–G). In the renal corpuscle, mesangial cells, parietal cells, and the proximal anlage were strongly positive in 18-day-old pups (Fig. 4B, C). The renal vesicles and S-shaped bodies were negative (Fig. 4A). During development, the straight portion of the proximal anlage remained moderately immunopositive and the immunoreactivity in the convoluted portion disappeared after birth, being negative from 14 days after birth. In the distal nephron, the distal anlage was negative, with immunoreactivity only becoming positive from 21 days after birth. PKCβI immunoreactivity in the intercalated cells showed a
pattern similar to PKCa in appearance and distribution.

4. PKCβII

In the adult kidney, immunoreactivity for PKCβII was strongly positive in the thick ascending limb of the Loop of Henle, the macula densa, the distal convoluted tubule, and the basolateral membrane of the connecting tubule. In the collecting tubule, the basolateral membrane of the principal cells was moderately positive, but no immunoreactivity was seen in the intercalated cells of the connecting and collecting tubules (Fig. 5B–D). There was weak basolateral labeling in the proximal convoluted tubule. There was no immunoreactivity in the renal corpuscle or intermediate tubule (Fig. 5A; Table 1, 2).

In the developing kidney, PKCβII immunoreactivity appeared in the basolateral membrane of the distal anlage at 18 days of gestation. Immunoreactivity increased markedly in the distal tubule, including the thick ascending limb of the Loop of Henle, the distal convoluted tubule and the connecting tubule from 1 day after birth. PKCβII immunoreactivity gradually de-
increased from 7 days after birth and had a similar pattern to the adult rat from 21 days. The intercalated cells in the connecting and collecting tubules were negative for PKCβII immunostaining. The principal cells were negative during the initial stages of development, but immunoreactivity gradually increased after birth and showed a similar pattern to adult rats from 14 days after birth (Fig. 6).

5. PKCγ

There was very weak immunoreactivity for PKCγ only in the proximal tubule, whereas no immunoreactivity in other uriniferous tubules (Fig. 7; Table 1, 2). In the developing kidney, PKCγ immunoreactivity was strong in the proximal tubule. Immunoreactivity appeared in the proximal anlage at 18 days of gestation. There was strong positive staining in the entire proximal tubule at 1, 3, and
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20 days after birth. Subsequently, immunoreactivity decreased and had a similar pattern to adult rats from day 21 after birth (Fig. 8).

Discussion

PKC plays a central role in intracellular signal transduction. The various PKC isoforms are expressed in the rat kidney with distinct and differential expression patterns (Fig. 9). As a member of the cPKC group, PKCa expression was predominant in the adult kidney. PKCB was localized in the tubules. PKCy is known to be detected in the central nervous system. Our study, using immunohistochemistry and immuno-
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blotting, demonstrates that the expression of PKCa, βI and βII, but not PKCy, is evident in the tubules of the rat kidney and PKCa, βI, βII, and γ are expressed in the developing kidney.

Wetsel et al.\textsuperscript{11}, La Porta et al.\textsuperscript{12}, Dong et al.\textsuperscript{22}, Pfaff et al.\textsuperscript{20}, Saxena et al.\textsuperscript{27}, Ostlund et al.\textsuperscript{14}, Aristimuno and Good\textsuperscript{13}, and Serlachius et al.\textsuperscript{15} have reported the expression of PKC in the kidney. In our study, the expression of PKCa was detected in the cortex, outer stripe of the outer medulla, inner stripe of the outer medulla and, using immunoblotting, in cytosolic and membrane fractions from the inner medulla. Using immunohistochemistry, Dong et al.\textsuperscript{28} and Fukuzaki et al.\textsuperscript{29} reported PKCa expression in the renal corpuscle, proximal straight tubule and collecting duct of the inner medulla of rat and human kidneys. However, our study demonstrates that PKCa staining was diffusely positive, with the exception of the intermediate tubule. Especially, strong positive staining was observed in the connecting tubule, intercalated cells of the cortical collecting tubule, mesangial cells of the renal corpuscle, outer and inner stripes of the outer medulla, and the principal cells in the collecting duct of the inner medulla.

Wetsel et al.\textsuperscript{11}, Ostlund et al.\textsuperscript{14} and Aristimuno and Good\textsuperscript{13} reported the expression of PKCB in the kidney, but not PKCBII. La Porta et al.\textsuperscript{12} identified PKCB in renal corpuscles using immunohistochemistry. Our study demonstrates the expression of PKCBI and βII. On immunoblotting, PKCBI was faintly detected in the cortex and outer stripe of the outer medulla in the cytosolic fractions. Expression was generally weakly detected in the membrane fraction. Using immunohistochemistry, PKCBI immunoreactivity was detected in the connecting segment, cortical collecting tubules, proximal straight tubules, mesangial cells of the renal corpuscle and the parietal epithelium. The connecting tubules and intercalated cells of the cortical collecting tubules were strongly positive. The apical plasma membrane of the descending thin limb of Henle was positive. These results were consistent with the immunoblotting findings.

A PKCBII band was observed in the membrane fractions. On immunohistochemistry, PKCBII was ex-
pressed in the proximal convoluted tubules, distal convoluted tubules, and the basolateral plasma membrane of the connecting and collecting tubules. The distal convoluted tubules, including the thick ascending limb of the Loop of Henle, and the connecting tubules were strongly positive. The intercalated cells in the distal nephron showed distinct and different expression patterns for PKC isoenzymes. In the connecting segment and cortical collecting tubules, the intercalated A cells play a role in H⁺ secretion and the intercalated B cells are involved in HCO₃⁻ secretion. In the medullary collecting tubules, intercalated cells play a role in H⁺ secretion. Our study shows that the type A intercalated cells in the connecting and cortical collecting ducts strongly positive for PKCa and β in their supranuclear cytoplasm. Type B intercalated cells were moderately positive in their cytoplasm and basolateral plasma membranes. Those findings were consistent with the results of mouse kidneys and the location of the H⁺-ATPase, so we suggest that PKCa and β may contribute to secretion of protons. However, PKCa, and β were not expressed in the intercalated cells in the medullary collecting ducts, so we speculate that different control mechanisms exist between intercalated cells in the connecting segment and cortical and medullary collecting tubules.

Several studies have reported that PKCy is not detected in the adult rat kidney. Recently, studies have shown that PKC plays a role in growth and differentiation during development. Serlachius et al. suggested that there is distinct and differential expression and distribution of PKC isoenzymes depending on embryonal kidney development. We also observed distinct and differential expression and distribution of PKC isoenzymes depending on kidney development. PKCa, β, and βII were expressed and, interestingly, PKCy was detected temporarily in the developing kidney. Positive staining for PKCa and β appeared in the ureteric bud at 16 days of gestation and was strongly positive before birth, and then gradually decreased after birth. Therefore, we suggest that PKCa and β play a role in differentiation of the collecting tubule. PKCa expression was positive in the proximal and distal anlage of pups up to 7 days of age. Therefore, we believe that PKCa expression is correlated with differentiation of the proximal and distal tubules. Moreover, PKCβII expression is correlated with differentiation of the proximal tubule because it was temporarily expressed in the proximal anlage in early stages of development. PKCβII appeared in the basolateral membrane of the distal and connecting tubules at 18 days of gestation, and gradually increased during development. Therefore, we suggest that PKCβII expression is correlated with growth and differentiation. PKCγ immunoreactivity appeared and was highly expressed in the proximal anlage of the 18–day–old fetus, then decreased markedly, and disappeared soon after birth. Therefore, PKCγ appears to be correlated with differentiation of the proximal tubule.

In summary, our study demonstrates that the classical PKC isoforms, PKCa, β, and βII, but not PKCy, are expressed in the tubules of adult rat kidneys, and PKCa, β, βII, and γ are expressed in the developing kidney, and there are distinct and differential expression patterns for the isoforms according to location and stage of development.

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References

1) Nishizuka Y: Studies and perspectives of protein kinase C. Science 233:305–312, 1986
2) Nishizuka Y: Intracellular signaling by hydrolysis of phospholipids and activation of protein kinase C. Science 258:607–614, 1992
3) Nishizuka Y: Protein kinase C and lipid signaling for sustained cellular responses. FASEB J 9:484–496,
Bertorello AM, Aperia A, Walaas SI, Nairn AC, Greengard P: Phosphorylation of the catalytic subunit of Na+, K(+)-ATPase inhibits the activity of the enzyme. Proc Natl Acad Sci U S A 88:11359–11362, 1991

Nowicki S, Kruse MS, Brismar H, Aperia A: Dopamine-induced translocation of protein kinase C isoforms visualized in renal epithelial cells. Am J Physiol Cell Physiol 279:C1812–C1818, 2000

Middleton JP, Khan WA, Collinsonworth G, Hannun YA, Medford RM: Heterogeneity of protein kinase C-mediated rapid regulation of Na/K-ATPase in kidney epithelial cells. J Biol Chem 268:15958–15964, 1993

Horie S, Moe O, Miller RT, Alpern RJ: Long-term activation of protein kinase c causes chronic Na/H antiporter stimulation in cultured proximal tubule cells. J Clin Invest 89:365–372, 1992

Ruíz OS, Arruda JA: Regulation of the renal Na–HCO3 cotransporter by cAMP and Ca-dependent protein kinases. Am J Physiol 282:F550–F565, 1992

Kosaka Y, Ogita K, Ase K, Nomura H, Kikkawa U, Nishizuka Y: The heterogeneity of protein kinase C in various rat tissues. Biochem Biophys Res Commun 151:973–981, 1988

Ono Y, Fujiy T, Ogita K, Kikkawa U, Igarashi K, Nishizuka Y: The structure, expression, and properties of additional members of the protein kinase C family. J Biol Chem 263:8927–8932, 1988

Wetsel WC, Khan WA, Merchenthaler I, Rivera H, Halpern AE, Phung HM, Negro-Vilar A, Hannun YA: Tissue and cellular distribution of the extended family of protein kinase C isoenzymes. J Cell Biol 117:121–133, 1992

La Porta CA, Comolli R: Biochemical and immunological characterization of calcimi-dependent and -independent PKC isoenzymes in renal ischemia. Biochem Biophys Res Commun 191:1124–1130, 1993

Aristimu o PC, Good DW: PKC isoforms in rat medullary thick ascending limb: selective activation of the delta-isoform by PGE2. Am J Physiol 272:F624–F631, 1997

Ostlund E, Mendez CF, Jacobsson G, Fryckstedt J, Meister B, Aperia A: Expression of protein kinase C isoforms in renal tissue. Kidney Int 47:766–773, 1995

Serlachius E, Svennilson J, Schalling M, Aperia A: Protein kinase C in the developing kidney: isoform expression and effects of ceramide and PKC inhibitors. Kidney Int 52:901–910, 1997

Redling S, Pfaff IL, Leitges M, Vallon V: Immunolocalization of protein kinase C isoenzymes alpha, beta I, beta II, delta, and epsilon in mouse kidney. Am J Physiol Renal Physiol 287:F289–F298, 2004

Hashimoto T, Ase K, Sawamura S, Kikkawa U, Saito N, Tanaka C, Nishizuka Y: Postnatal development of a brain–specific subspecies of protein kinase C in rat. J Neurosci 8:1678–1683, 1988

Hirata M, Saito N, Kono M, Tanaka C: Differential expression of the beta I– and beta II–PKC subspecies in the postnatal developing rat brain: an immuno–cytochemical study. Brain Res Dev Brain Res 62:229–238, 1991

Puc M, Hilal–Dandar R, Strulovici B, Brunton LL, Brown JH: Differential regulation of protein kinase C isoforms in isolated neonatal and adult rat cardiomyocytes. J Biol Chem 269:16938–16944, 1994

Rybin VO, Steinberg SF: Protein kinase C isoform expression and regulation in the developing rat heart. Circ Res 74:299–309, 1994

Housey GM, Johnson MD, Hsiao WL, O’Brien CA, Murphy JP, Kirschmeier P, Weinstein IB: Overproduction of protein kinase C causes disordered growth control in rat fibroblasts. Cell 52:343–354, 1988

Dong L, Stevens JL, Fabbro D, Jaken S: Regulation of protein kinase C isoform expression in kidney regeneration. Cancer Res 53:4542–4549, 1993

Mischak H, Goodnight JA, Kolch W, Martiny–Baron G, Schaeuchte C, Kazanietz MG, Blumberg PM, Pierce JH, Mushinski JF: Overexpression of protein kinase C–delta and –epsilon in NIH 3T3 cells induces opposite effects on growth, morphology, anchorage dependence, and tumorigenicity. J Biol Chem 268:6090–6096, 1993

Berra E, Diaz–Meco MT, Dominguez I, Municio MM, Sanz L, Lozano J, chapkin RS, Moscat J: Protein kinase C zeta isoform is critical for mitogenic signal transduction. Cell 74:555–563, 1993

Szallas Z, Kosa K, Smith CB, Dlugosz AA, Williams EK, Yuspa SH, Blumberg PM: Differential regulation by anti–tumor–promoting 12–deoxyphorbol–13–phenylacetate reveals distinct roles of the classical and novel protein kinase C isoforms in biological responses of primary mouse keratinocytes. Mol Pharmacol 47:258–265, 1995

Pfaff IL, Wagner HJ, Vallon V: Immunolocalization of protein kinase C isoforms in the developing kidney. J Am Soc Nephrol 10:1861–1873, 1999

Saxena R, Saksa BA, Hawkins KS, Ganz MB: Protein kinase C beta I and beta II are differentially expressed in the developing glomerulus. FASEB J 8:646–653, 1994

Dong LQ, Stevens JL, Jaken S: Biochemical and immunological characterization of renal protein kinase C. Am J Physiol 261:F679–687, 1991

Fukuizaki A, Kaneto H, Ikeda S, Orikasa S: Characterization of protein kinase C expression in human kidney. Tohoku J Exp Med 178:263–269, 1996

Madsen KM, Tisher CC: Structural–functional relationships along the distal nephron. Am J Physiol 250:F1–F15, 1986

Kim WY, Jung JH, Park BY, Yang CW, Kim H, Nielsen 1995.
S, Madsen KM, Kim J: Expression of protein kinase C isoenzymes alpha, betaI, and delta in subtypes of intercalated cells of mouse kidney. *Am J Physiol Renal Physiol* 291:F1052–F1060, 2006

32) Madsen KM, Verlander JW, Kim J, Tisher CC: Morphological adaptation of the collecting duct to acid–base disturbances. *Kidney Int* 40(Suppl 33): S57–S63, 1991