Original article

Immunolocalization of Protein Kinase C Isoenzymes α, βI, βII and γ in Adult and Developing Rat Kidney

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Protein kinase C (PKC) plays an important role not only in signal transduction mechanisms in various biological processes, but also in the regulation of growth and differentiation during development. We studied the classical PKC 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, PKCβI was expressed in the intercalated cells of the CNT and cortical CD, the proximal straight tubule, and the renal corpuscle. PKCβII appeared in distal anlage at E18, and increased markedly after birth. In the CD, PKCβII immunoreactivity appeared after birth. In adult, PKCβII 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

Introduction

Protein kinase C (PKC) is a family of protein kinases that specifically phospholylate serine/threonine residues. The family includes at least 11 isoforms (α, βI, βII, γ, δ, ε, ζ, 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, are dependent on activation through diacylglycerol (DAG) and Ca^{2+}. The second group, the new or novel PKCs (nPKCs), including δ, ε, η θ and μ, are activated by DAG. The third group, the atypical PKCs (aPKCs), including ζ and λ, are not activated by DAG or Ca^{2+} [1-3].

PKC plays a central role in intracellular signal transduction pathways for hormones, neurotransmitters and growth factors, and significantly contributes to the control of various renal functions, including cellular proliferation, differentiation, exocytosis, and ion and water transport[3-5].

In cultured cells, PKC inhibits activation of Na^+,K^+

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-ATPase[4, 6] and activates the Na^+/H^+-exchanger and 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 α, β, and ζ; Wetsel et al.[11], α, βII, δ, ε, and ζ; Caterina et al.[12] and Aristimuno and Good[13], α, β, δ, ε, and ζ; Ostlund et al.[14] and Serlachius et al.[15], α, δ, ε, and λ; and Pfaff et al.[16], α, βI, and βII. Although most of these studies identified the PKC isoenzymes using molecular biologic approaches, little is known about their localization along the nephron.

Hashimoto et al.[17] and Hirataet al.[18] detected PKC in brain tissue and Puceat et al.[19] and Rybin et al.[20] 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[21-25].

To identify the function of PKC in the kidney, we studied the differential expression and localization of the PKC isoenzymes α, βI, βII and γ 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℃. Tissues were embedded in wax or EPON 812. For immunohistochemistry using a pre-embedding method, PLP-fixed tissues were cut on a vibratome (Lancer Vibratomes 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_2O_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 α, βI, βII and γ as primary antibodies, using a Vectastain ABC kit (Vector Laboratories) according to the manufacturer's instructions. The sections were incubated overnight in PBS solutions containing antibodies diluted 1:2000 for PKCα, 1:2000 for PKCβI, 1:1500 for PKCβII and 1:5000 for PKCγ. Tissue sections were washed three times for 10 minutes and then incubated for 2 hours at room temperature with PBS containing biotin-conjugated goat anti-rabbit IgG (Vector Laboratories), diluted 1:500, as the secondary antibody. Avidin-biotin-peroxidase complex (Vector Laboratories) diluted 1:100 was used as the tertiary reagent. After the sections were washed three times for 10 minutes with 0.05 M Tris-HCl buffer (pH 7.6), the 0.05% diaminobenzidine and 0.0033% H_2O_2 were used as chromogen. After the immunostaining, sections were counterstained with hematoxylin.

2) Immunostaining of 1-mm-thick EPON sections

To identify the immunoreactivity of PKC in inter-

calated 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 PKCα, βI, βII and γ were used. H^+-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. PKCα

In the adult kidney, PKCα 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^+-ATPase,

Table 1. Immunoreactivity of Classical Protein Kinase (PKC) Isoforms in Rat Kidney

	PKCα		PKCβI		PKCβII		PKCγ	
RC								
PE	+		±~+		-		-	
P	±		-		-		-	
MC	++		+		-		-	
PT								
PCT	-		-		+		+	
PST	+		+~++		-		+	
IT								
DTL	-		++		-		-	
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; CNT, 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 immunore activity in the intercalated cells.

Table 2. Immunoreactivity of Conventional Proten Kinase C (PKC) Isoforms in the Intercalated Cells of Rat Kidney

	PKCα			PKCβI			PKCβII			PKCγ		
	A-IC	B-IC	PC	A-IC	B-IC	PC	A-IC	B-IC	PC	A-IC	B-IC	PC
CNT	+++	++	+	+++	++	-	-	-	++	-	-	-
CCD	+++	++	+	+++	+	-	-	-	++	-	-	-
OMCDo	+	+	+~++	-	-	-	-	-	+	-	-	-
OMCDi	-~+		+++	-		-	-		+	-		-
IMCDi	-		+++	-		-	-		+	-		-
IMCDt			++			-			++			-

A-IC, type A intercalated cells; B-IC, type B intercalated cells; PC, principal cells; CNT, connecting tubules; CCD, cortical collecting duct; OMCDo, outer stripe part of outer medullary collecting duct; OMCDi; inner stripe part of OMCD; IMCDi, initial part of the inner medullary collecting duct; IMCDt, terminal part of the IMCD. Symbols designate not detectable (-), faint (+), weak (+), moderate (++), and high (+++) levels of immunoreactivity.

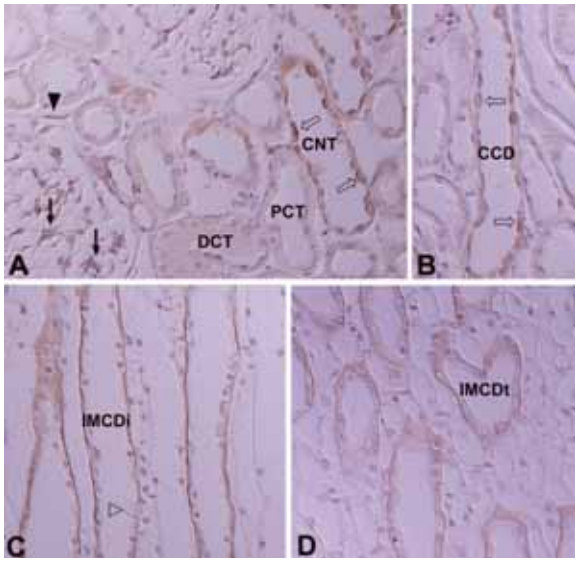

Fig. 1. *Differential interference contrast (DIC) micrographs of wax sections illustrating immunostaining for protein kinase C α (PKCα) in the cortical labyrinth (A), medullary ray (B), initial part of the inner medulla (C), and the terminal part of the inner medulla (D) of adult rat kidneys. PKCα immunostaining is observed in the cytoplasm of mesangial cells (arrows) and the parietal epithelium (arrowhead) of renal corpuscles, intercalated cells (open arrows) of the connecting tubule (CNT) and cortical collecting duct (CCD), and on the basolateral plasma membrane of the CNT cells and the principal cells of the collecting duct. Note the PKCα-negative intercalated cells (open arrowheads) in the initial part of the inner medullary collecting duct (IMCDi). IMCDt, terminal part of the IMCD. Magnifications: A-D, ×400.*

PKCα-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 cells were stained weakly at the basolateral plasma membrane. In the medullary collecting ducts, the intercalated cells were PKCα negative and the principal cells

were PKCα positive at the basolateral plasma membrane. The inner stripe of the outer medulla and the initial part of the inner medulla showed strong immunoreactivity. 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

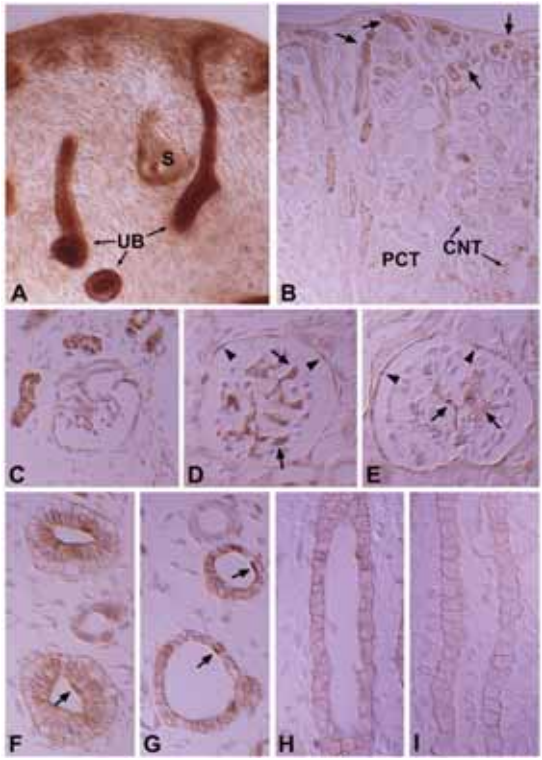

Fig. 2. *DIC micrographs of wax sections illustrating immunostaining for PKCα in 16- (A), 18- (C & F), and 20-day-old (G) fetal kidneys, and 3- (D & H) and 7-day-old (B, E & I) pups. protein kinase C α (PKCα) appeared in the ureteric buds (UB) at 16 days of gestation (A) and in the proximal and distal anlage (stars) at 18 days of gestation (C). B. Note that the PKCα-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 PKCα 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 PKCα immunoreactivity in the mesangial cells (arrows) and parietal epithelium (arrowheads) of the developing renal corpuscle. F-G. Immunoreactivity for PKCα 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.*

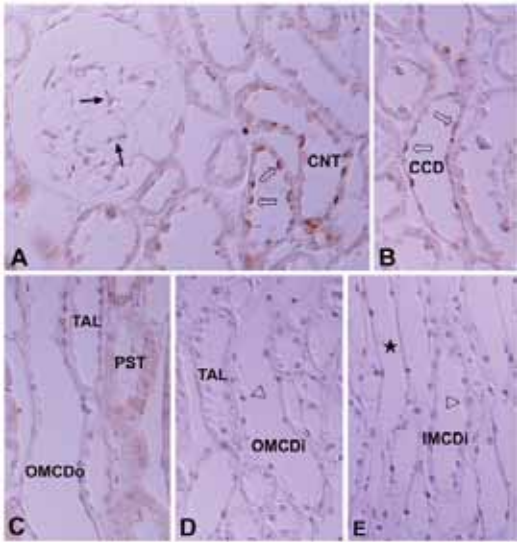

Fig. 3. *DIC micrographs of wax sections illustrating protein kinase C βI (PKCβI) immunostaining in the cortical labyrinth (A), medullary rays (B), outer stripe of the outer medulla (C), inner stripe of the outer medulla (D), and initial part of the inner medulla (E) of the adult rat kidney. PKCβI immunoreactivity is strong in the intercalated cells (open arrows) of the connecting tubules (CNT) and cortical collecting ducts (CCD), moderate in the proximal straight tubules (PST), and weak in the mesangial cells (arrows) and parietal epithelium of the renal corpuscle. In the inner medulla, PKCβI immunostaining is observed only in the descending thin limb (stars) of the Loop of Henle. There is no immunoreactivity in the CNT cells, principal cells of the CCD, outer (OMCDo) and inner stripe of the outer medullary collecting duct (OMCDi), and initial inner medullary collecting duct (IMCDi). Note that there is no immunoreactivity in the intercalated cells (open arrowheads) of the OMCD and IMCDi. Magnifications: A-E, ×400.*

faint immunoreactivity. We did not observe any PKCα immunoreactivity in the descending or ascending limbs of the Loop of Henle (Fig. 1; Table 1, 2).

In the developing kidney, PKCα appeared in the ureteric bud at 16 days of gestation, but there was no staining of the renal vesicle and S-shaped body (Fig. 2A). The PKCα immunoreactivity of the collecting tubule gradually decreased during development and showed a mature pattern from 14 days after birth (Fig. 2F-I). PKCα 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 PKCα 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. PKCβI

In the adult kidney, there was strong positive PKCβI staining in the connecting segment and intercalated cells of the cortical collecting duct. Similar to PKCα, type A intercalated cells were positive in the supranuclear area and type B cells were positive throughout the entire cytoplasm. PKCβI staining was negative in principal cells. In the proximal tubule, the convoluted

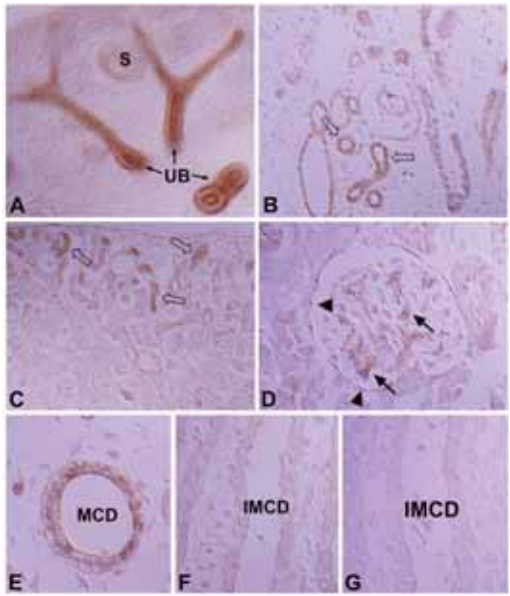

Fig. 4. *DIC micrographs of wax sections illustrating immunostaining for protein kinase C βI (PKCβI) in 16- (A), 18- (B) and 20-day-old (E) fetal kidneys, and 3-(F) and 7-day-old (C, D & G) pups. PKCβI appeared in the ureteric buds (UB) at 16 days of gestation and in the proximal anlage (open arrows) at 18 days of gestation. PKCβI immunostaining appeared in the differentiating proximal tubules (open arrows) in the fetal stage and disappeared from the mature proximal tubule after birth. PKCβI-positive proximal anlage are observed in the subcapsular region until 7 days after birth. Note the PKCβI immunostaining in the mesangial cells (arrows) and parietal epithelium (arrowheads) of the developing renal corpuscle in D. Immunoreactivity for PKCβI in the medullary collecting duct (MCD) cells gradually disappeared after birth (E-G). IMCD, inner medullary collecting duct. Magnifications: A, ×200; B, ×264; C & D-G, ×528.*

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 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

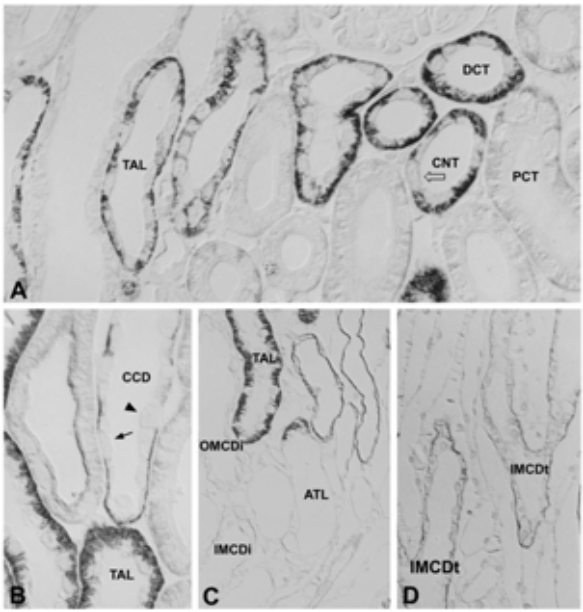

Fig. 5. DIC micrographs of 1-mm-thick plastic sections illustrating immunostaining for protein kinase C βII (PKCβII) in the cortex (A), medullary rays (B), the border between the outer and inner medulla (C), and the terminal part of the inner medulla (D) of adult rat kidney. Immunoreactivity for PKCβII is well localized on the basolateral plasma membrane of distal convoluted tubule (DCT) cells, connecting tubule (CNT) cells, thick ascending limb (TAL) cells, and principal cells throughout the collecting duct. Note that there is no immunoreactivity for PKCβII in both the type A intercalated cells (arrow) and type B intercalated cells (arrowhead) in the cortical collecting duct (CCD). There is weak basolateral PKCβII labeling in the proximal convoluted tubule (PCT). The open arrow indicates a PKCβ II-negative intercalated cell in the CNT. ATL, ascending thin limb; G, glomerulus; OMCDi, inner stripe of the outer medullary collecting duct; IMCDi, initial part of the inner medullary collecting duct; IMCDt, terminal part of the IMCD. Magnifications: A, ×368; B, ×640; C & D, ×320.

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 PKCα 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

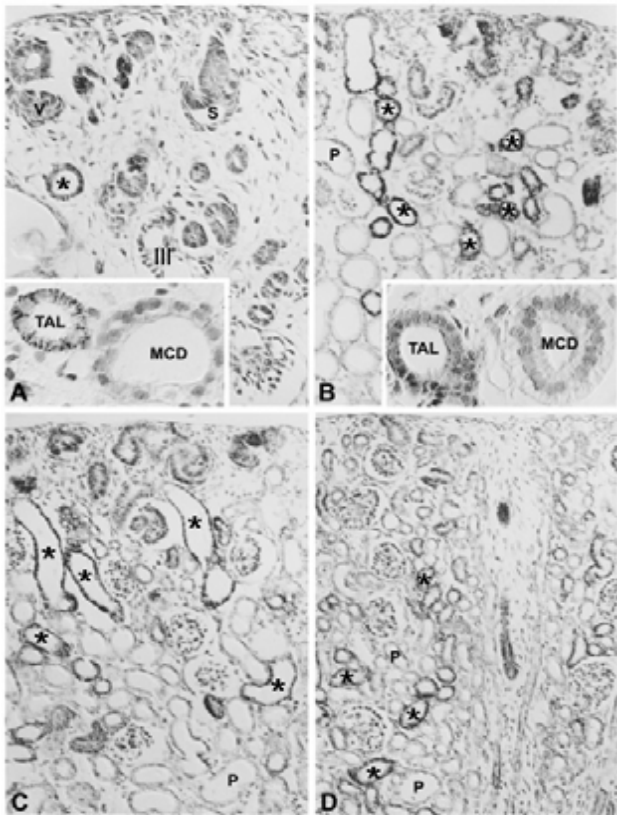

Fig. 6. DIC micrographs of wax sections illustrating immunostaining for protein kinase C βII (PKCβII) in 18-day-old fetuses (A), and 1- (B), 3- (C), and 7-day-old (D) pups. A. PKCβII immunoreactivity appeared in the distal tubules (asterisks) at 18 days of gestation. B-C. Note a marked increase in PKCβII immunostaining in the distal tubules (asterisks) after birth. Insets demonstrating PKCβII-negative medullary collecting ducts (MCD) from an 18-day-old fetus (A) and a 1-day-old pup (B). In the thick ascending limb (TAL), PKCβII immunoreactivity appeared around the time of birth. P, proximal tubule; S, S-shaped body; III, stage III renal corpuscle; V, renal vesicle. Magnifications: A-D, ×200; insets, ×528.

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 decreased 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

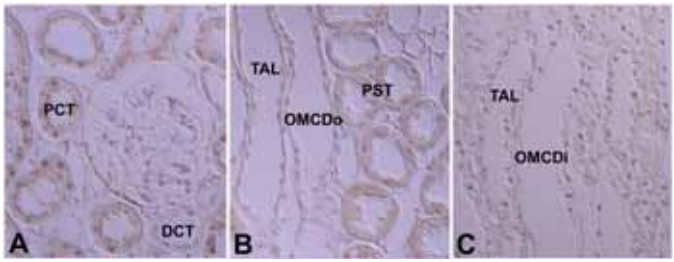

Fig. 7. Light micrographs of wax sections illustrating immunostaining for protein kinase C γ (PKCγ) in adult rat kidneys. There is very weak immunostaining for PKCγ only in the cytoplasm of proximal convoluted tubules (PCT) and proximal straight tubules (PST). Co, cortex; DCT, distal convoluted tubule; OMCDi, inner stripe part of outer medullary collecting duct; OMCDo, outer stripe part of OMCD; OSOM, outer stripe of outer medulla; ISOM, inner stripe of outer medulla; TAL, thick ascending limb. Magnifications: A-C, ×330.

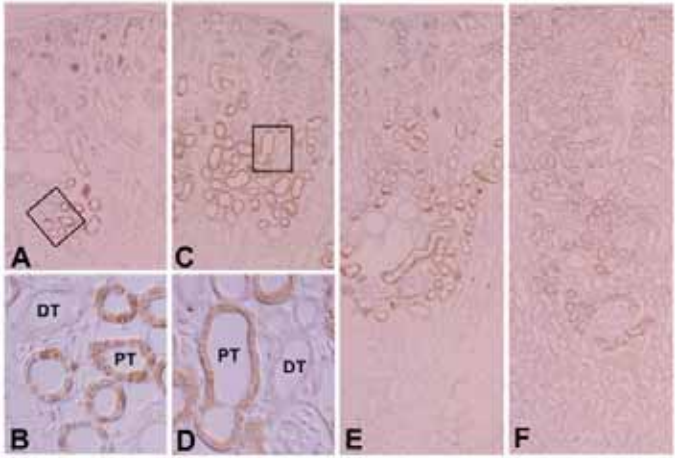

Fig. 8. Light micrographs of wax sections illustrating immunostaining for protein kinase C γ (PKCγ) in kidneys from 20-day-old fetuses (A & B), and 1- (C & D), 3- (E), and 21-day-old (F) pups. PKCγ immunoreactivity is highly expressed in the proximal tubules (PT) during development and markedly decreased in the PT from 21 days after birth. B and D are higer magnification area indicate by rectangular in A and C, respectively. DT, distal tubule; PCT, proximal convoluted tubule; PST, proximal straight tubule. Magnifications: A, C, E & F, ×83; B & D, ×330.

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 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

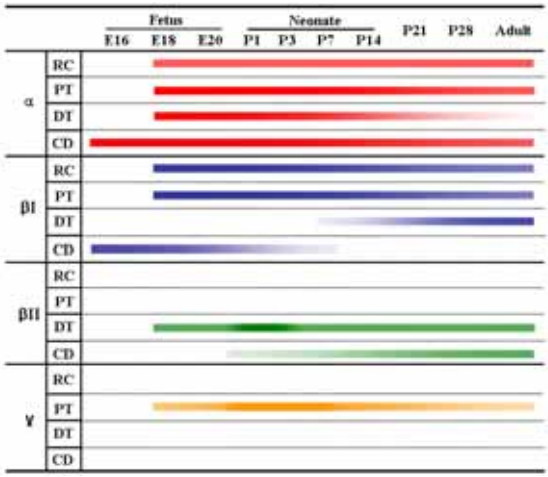

Fig. 9. Changes in immunoreactivity of classical protein kinase C isoforms in uriniferous tubules during renal development in the rat. RC, renal corpuscle; PT, proximal tubule; DT, distal tubule; CD, collecting duct.

in the rat kidney with distinct and differential expression patterns (Fig. 9). As a member of the cPKC group, PKCα expression was predominant in the adult kidney. PKCβ was localized in the tubules. PKCγ is known to be detected in the central nervous system[10]. Our study, using immunohistochemistry and immunoblotting, demonstrates that the expression of PKCα, βI and βII, but not PKCγ, is evident in in the tubules of the rat kidney and PKCα, βI, βII, and γ are expressed in the developing kidney.

Wetsel et al.[11], La Porta et al.[12], Dong et al.[22], Pfaff et al.[26], Saxena et al.[27], Ostlund et al.[14], Aristimuno and Good[13], and Serlachius et al.[15] have reported the expression of PKCα in the kidney. In our study, the expression of PKCα 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.[28] and Fukuzaki et al.[29] reported PKCα 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 PKCα 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.[11], Ostlund et al.[14] and Aristimuno and Good[13] reported the expression of PKCβI in the kidney, but not PKCβII. La Porta et al.[12] identified PKCβ in renal corpuscles using immunohistochemistry. Our study demonstrates the expression of PKCβI and βII. On immunoblotting, PKCβI 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, PKCβI 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 PKCβII band was observed in the membrane fractions. On immunohistochemistry, PKCβII was expressed 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 HCO_3^- secretion. In the medullary collecting tubules, intercalated cells play a role in H^+ secretion[30]. Our study shows that the type A intercalated cells in the connecting and cortical collecting duc tare strongly positive for PKCα 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[31] and the location of the H^+-ATPase[32], so we suggest that PKCα and βI may contribute to secretion of protons. However, PKCα, and βI 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 PKCγ is not detected in the adult rat kidney[11, 13, 14, 22]. Recently, studies have shown that PKC plays a role in growth and differentiation during development[21-24]. Serlachius et al.[15] suggested that there is distinctand differential expression and distribution of PKC isoenzymes depending on embryonal kidney development. We also observed distinctand differential expression and distribution of PKC isoenzymes depending on kidney development. PKCα, βI, and βII were expressed and, interestingly, PKCγ was detected temporarily in the developing kidney. Positive staining for PKCα and βI 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 PKCα and βI play a role in differentiation of the collecting tubule. PKCα expression was positive in the proximal and distal anlage of pups up to 7 days of age. Therefore, we believe that PKCα expression is correlated with differentiation of the proximal and distal tubules. Moreover, PKCβI 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 not 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, PKCα, βI and βII, but not PKCγ, are expressed in the tubules of adult rat kidneys, and PKCα, βI, βII, and g 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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