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# Evidence for a role of protein kinase C-α in urine concentration

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[1]*Institute of Pharmacology and Toxicology, University of Tübingen, 72074 Tübingen;* [2]*Max-Planck-Institute for Experimental Endocrinology, 30625 Hannover, Germany; and* [3]*Departments of Medicine and Pharmacology, University of California, and Veterans Affairs Medical Center, San Diego, California 92161*

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**Yao, Lijun, Dan-Yang Huang, Imke L. Pfaff, Xin Nie, Michael Leitges, and Volker Vallon.** Evidence for a role of protein kinase C-α in urine concentration. *Am J Physiol Renal Physiol* 287: F299–F304, 2004. First published March 23, 2004; 10.1152/ajprenal.00274.2003.—In mouse kidney, the conventional protein kinase C (PKC) isoenzyme α is expressed in glomeruli, the cortical collecting duct (intercalated cells only), and medullary collecting duct. To get insights on its function, PKC-α knockout (−/−) and wild-type (+/+) mice were studied. When provided free access to water, PKC-α −/− mice showed ~50% greater urine flow rate and lower urinary osmolality in 24-h metabolic cage experiments despite a greater urinary vasopressin-to-creatinine ratio vs. PKC-α +/+ mice. Renal albumin excretion was not different. Clearance experiments under inactin/ketamine anesthesia revealed a modestly reduced glomerular filtration rate and showed a reduced absolute and fractional renal fluid reabsorption in PKC-α −/− mice. The sodium-restricting response to a low-sodium diet was unaffected in PKC-α −/− mice. Urinary osmolality was reduced to similar hypotonic levels in PKC-α −/− and +/+ mice during acute oral water loading or application of the vasopressin $V_2$-receptor antagonist SR-121463. In comparison, the lower urinary osmolality observed in PKC-α −/− mice vs. wild-type mice under basal conditions persisted during water restriction for 36 h. In conclusion, PKC-α appears not to play a major role in renal sodium reabsorption but, consistent with its expression in the medullary collecting duct, contributes to urinary concentration in mice. Considering that PKC-βI and -βII are coexpressed with PKC-α in mouse medullary collecting duct, the present results indicate that conventional PKC isoenzymes cannot fully compensate for each other.

mouse; collecting duct; knockout

PROTEIN KINASE C (PKC) REPRESENTS a family of closely related enzymes that phosphorylate serine or threonine residues of various intracellular proteins and is thus involved in a wide range of cellular functions such as ion transport, release of hormones or neurotransmitters, downregulation of receptors, or expression of various proteins (26). PKC has been proposed to play a central role in the mammalian kidney including vascular and glomerular function (21, 23, 37, 38), renin gene transcription (25), and transport in the tubular system (3, 4, 5, 10, 12, 15, 24, 28, 31, 33) and collecting duct (1, 8, 12, 16). Based on their primary structure, the 10 isoenzymes of the mammalian PKC family have been classified into three classes: conventional (α, γ, and the alternatively spliced βI and βII); novel (δ, ϵ, η/L, θ); and atypical (ζ, ι/λ). Evidence for the expression in both glomeruli and the tubular system has been provided for PKC isoenzymes α, βI, βII, δ, ϵ, and ζ (9, 17, 18, 25, 29, 38).

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PKC-α, which is a member of the "conventional" PKC class, appears to be one of the predominant isoenzymes in the kidney (9, 11, 27, 29). Employing immunohistochemistry and confocal laser-scanning microscopy in the mouse kidney, PKC-α was localized to glomeruli, intercalated cells of the cortical collecting duct, as well as the medullary collecting duct (31a). Gene knockout can be a powerful approach to delineate respective gene function, and therefore, using homologous recombination, we recently generated a mouse that is deficient for PKC-α (22). Despite the fact that PKC-α is presumed to be the most ubiquitously expressed PKC isoenzyme, the PKC-α knockout mouse appears normal with regard to external characteristics, viability, and fertility. A preliminary closer look revealed that insulin signaling to insulin receptor substrate 1-dependent phosphoinositide 3-kinase, PKB, and PKC-λ and downstream processes like glucose transport and activation of extracellular signal-regulated kinases (ERK) are enhanced in skeletal muscles and adipocytes from PKC-α knockout mice. Serum glucose or insulin levels, however, were not significantly different in fed PKC-α knockout mice compared with fed control PKC-α wild-type mice (22). Here, we used PKC-α knockout mice to gain insights into the role of PKC-α in kidney function.

## METHODS

*Targeted disruption of the PKC-α gene.* The generation of a targeted PKC-α mutation in the mouse by homologous recombination was described before (22). In the process, germ line-transmitting male chimeras were crossed to 129/Sv females and gave rise to F1 heterozygous offspring on a pure 129 background. Intercrosses of these were used to establish a homozygote PKC-α-deficient (−/−) mouse line. Male mice from this line were compared with age-matched male 129/Sv PKC-α control (+/+) mice.

*Metabolic cage experiments in conscious mice.* To assess basal renal function, mice had free access to tap water and a standard diet (2.5 g $Na^+$/kg). Mice were placed in metabolic cages (Tecniplast, Hohenpeissenberg, Germany) to assess urinary excretion of fluid and sodium as well as urinary osmolality. After adaptation of the mice to metabolic cages over a period of 3 days, a 24-h urine collection was performed. To ensure quantitative urine collection, metabolic cages were siliconized, and urine was collected under water-saturated oil. The concentration of $Na^+$ in urine was determined using a flame photometer (ELEX 6361, Eppendorf, Hamburg, Germany) and osmolality by freezing-point depression. The concentrations of albumin and vasopressin were determined using a commercial ELISA kit for mouse albumin (CellTrend, Luckenwalde, Germany) or a commercial RIA kit for vasopressin (IBL, Hamburg, Germany), respectively. After urine collection was completed, the mice were anesthetized with ether and blood was drawn

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from the retrobulbar plexus for determination of hematocrit and plasma osmolality.

Twenty-four-hour urine collections were performed after an adaptation period of 2 days under a standard sodium diet (2.5 g $Na^+$/kg) and, subsequently, in response to a sodium-deficient diet (0 g $Na^+$/kg) for 6 days.

Urinary flow rate and urinary osmolality were determined in conscious mice in response to *1*) 36-h water deprivation with urine collection being performed over the last 14 h of water deprivation; *2*) application of the vasopressin $V_2$-receptor antagonist SR-121463 (1 mg/kg ip), which in the rat induces diuresis through internalization of aquaporin-2 from the luminal membrane and subsequent inhibition of water transport in medullary collecting duct (14); and *3*) acute water loading (1 ml/16 g body wt) using a gastric tube. In the latter experiments, access to food had been withdrawn 4 h before to facilitate water application. To prevent contamination with the concentrated urine being present in the bladder before administration of SR-121463 or water loading, osmolality was measured in the second portion of spontaneously voided urine after drug application or water loading, respectively.

*Blood pressure measurements and clearance experiments in anesthetized mice.* As described before (34–36, 39), mice were anesthetized for terminal clearance experiments (100 mg/kg Inactin ip and 100 mg/kg ketamine im). After every hour, 20% of this dose was readministered intraperitoneally to maintain anesthesia until euthanization. The femoral artery was cannulated for blood pressure measurement and blood sample withdrawal. The jugular vein was cannulated for a continuous maintenance infusion of 2.25 g/dl BSA in 0.9% NaCl at a rate of 0.35 ml/h. For assessment of glomerular filtration rate (GFR), [$^3$H]inulin was added to deliver 20 μCi/h. After surgery, the mice were allowed to stabilize for 60 min. Then, a timed urine collection was performed for 60 min using a bladder catheter. Blood was withdrawn at the beginning and end of the collection period to determine [$^3$H]inulin. Urine was analyzed for [$^3$H]inulin. Before the mice were euthanized by intravenous application of saturated KCl at the end of the clearance experiment, the kidneys were excised and decapsulated and the wet weight was determined. After the kidneys were dried overnight at 50°C, the dry weight was determined.

*Aquaporin-2 expression in inner medulla and thickness of inner medulla.* Semiquantitative Western blot analysis was adapted from the rat (14, 29, 30) to the mouse. PKC-α +/+ and −/− mice (*n* = 5/group) were anesthetized (ketamine, 60 mg/kg ip and xylazine, 9 mg/kg ip). After opening of the abdominal cavity, the renal artery and vein of the left kidney were clamped and the kidney was rapidly excised. The inner medulla was dissected and immediately immersed in liquid nitrogen for later Western blot analysis. Subsequently, the left cardiac ventricle was exposed. The tip of the perfusion system was inserted into the left ventricle, and the arterial system was perfused for 1 min with 3–5 ml PBS to clear the kidney of blood and subsequently for 10 min with 10–15 ml of a fixation solution (2% paraformaldehyde and 3% sucrose in phosphate buffer). Both solutions were at room temperature (RT). Then, the right kidney was removed for morphological examination.

For determination of aquaporin-2 expression in the inner medulla by Western blotting, total cellular proteins of the inner medulla of the left kidney were obtained by pulverizing the tissue and dissolving the powder in lysis buffer. Homogenization was followed by centrifugation (1,000 *g*, 10 min, 4°C) to remove nuclei and unbroken cells, and the supernatant was used for further analysis. Protein content was determined by the method of Bradford using a commercial protein assay and bovine serum albumin as a standard. Samples were diluted 1:3 with Roti-Load sample buffer and boiled for 10 min at 65°C. The following steps were performed at RT. Samples of protein (10 μg) were subjected to SDS-PAGE using 12% acrylamide gels in a Mini-PROTEAN II Electrophoresis Cell. For determination of molecular mass, a prestained protein ladder was used. After gel electrophoresis (60 mA/gel, 70 min), proteins were transferred to polyvinylidene difluoride membranes of 0.45-μm pore size. Membranes were blocked for 90 min with blocking buffer and rinsed twice with TBS containing 0.1% Tween 20 (TBST). Thereafter, the aquaporin-2 antibodies (0.5 μg/ml) were incubated overnight in TBST. The secondary alkaline phosphate-conjugated antibody was incubated at a concentration of 0.1 μg/ml for 2 h at RT in TBST. Blots were rinsed twice with TBST and washed three times for 15 min with TBST, then twice in assay buffer at RT. Development was done with CDP-Star ready-to-use solution. Immunoreactive bands were visualized by chemiluminescence system with CSC1.1 program software and then quantitated by TINA program (Raytest, Straubenhardt, Germany).

For determination of inner medulla thickness and morphology, the perfusion-fixated right kidney was cut into slices (3 mm in thickness) displaying cortex and outer and inner medulla and incubated for 3 h in the fixation at 4°C. After rinsing in PBS for 15 min, kidney slices were dehydrated in 30% sucrose in PBS overnight at 4°C. Thereafter, kidney slices were frozen in isopentane precooled by liquid nitrogen and stored at −80°C until further use. Cryosections of ~10 μm were made at −20°C and transferred onto gelatin-coated glass slides. Cortex and inner medulla thicknesses were determined in the maximal cross-sectional area of frontal sections, and 10 sections/animal in all were evaluated (20).

*Statistical methods.* Results are presented as means ± SE. The statistical significance of differences between PKC-α −/− and PKC-α +/+ mice was assessed by an unpaired Student's *t*-test.

## RESULTS

*Basal kidney function in metabolic cage experiments in conscious mice.* When provided free access to water and standard diet, PKC-α −/− mice showed similar food intake and urinary albumin excretion but a higher urinary flow rate and reduced urinary osmolality compared with PKC-α +/+ mice (see Figs. 1 and 2). These changes in PKC-α −/− mice were associated with an enhanced urinary vasopressin-to-creatinine ratio (see Fig. 1), which is a surrogate parameter for mean daily plasma vasopressin concentration (2). Arterial hematocrit under these conditions was not different [51.4 ± 1.2 vs. 52.9 ± 1.0%; *n* = 8/group, not significant (NS)], and plasma osmolality tended to be higher in PKC-α −/− vs. +/+ mice (329 ± 1 vs. 322 ± 5; *n* = 8/group; *P* = 0.09).

*Response to water deprivation, water loading, and vasopressin $V_2$-receptor blockade.* Withdrawal of water for 36 h increased urinary osmolality in both groups, but osmolality remained lower in PKC-α −/− compared with PKC-α +/+ mice (see Fig. 2). In comparison, acute water loading or application of vasopressin $V_2$-receptor antagonist SR-121463 lowered urinary osmolality to comparable hypotonic levels in PKC-α −/− and +/+ mice.

*Basal kidney function in clearance experiments in anesthetized mice.* Mean arterial blood pressure (see Fig. 3) and heart rate (375 ± 13 vs. 391 ± 12 beats/min; *n* = 8/group, NS) were not different between PKC-α −/− and +/+ mice. PKC-α-deficient mice, however, exhibited a modestly lower GFR, which was associated with an enhanced absolute and fractional fluid excretion (see Fig. 3) and a modestly lower kidney wet weight (0.443 ± 0.017 vs. 0.497 ± 0.013 g for 2 kidneys; *n* = 8/group; *P* < 0.05) and kidney dry weight (see Fig. 3), such that GFR per gram kidney weight was preserved (3.1 ± 0.2 vs. 3.2 ± 0.2 $ml \cdot min^{-1} \cdot g$ kidney dry $wt^{-1}$, NS).

*Aquaporin-2 expression in the inner medulla and thickness of the inner medulla.* Expression of aquaporin-2 in the inner medulla was not significantly altered in PKC-α −/− vs. PKC-α +/+ mice as determined by semiquantitative immu-



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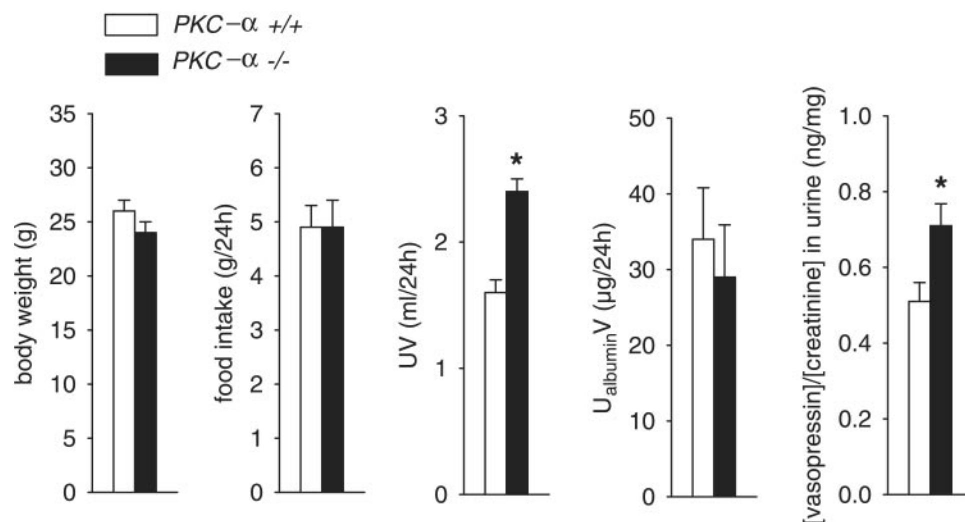


Fig. 1. Under conditions of free access to food and water in metabolic cage experiments, PKC-α knockout mice (−/−) exhibited modestly greater urinary flow rate (UV) despite a greater urinary vasopressin-to-creatinine ratio compared with PKC-α wild-type mice (+/+). The urinary vasopressin-to-creatinine ratio is used as a surrogate parameter for mean daily plasma vasopressin concentration (2). Urinary albumin excretion ($U_{albumin}V$) was not different between groups ($n$ = 8/group). $^*P < 0.05$ vs. PKC-α +/+.

noblotting under basal conditions (i.e., free access to water; 86 ± 18% in PKC-α −/− vs. 100% in PKC-α +/+ mice, $n$ = 5/group, NS). Furthermore, PKC-α −/− mice did not present significant differences in the ratio of the thickness of the inner medulla vs. the thickness of the cortex compared with PKC-α +/+ mice (1.58 ± 0.15 vs. 1.67 ± 0.07, $n$ = 9 each group, NS) or apparent alterations in the morphology of the inner medulla ($n$ = 5/group, not shown).

*Response to a sodium-deficient diet.* As illustrated in Fig. 4, urinary sodium excretion was not different under a standard diet (*day 0*). Dietary sodium restriction for 6 days also did not reveal significant differences in urinary sodium excretion or in body weight change between groups. The greater urine flow rate and lower urinary osmolality, however, persisted in PKC-α −/− mice over the 6 days of sodium restriction (data not shown).

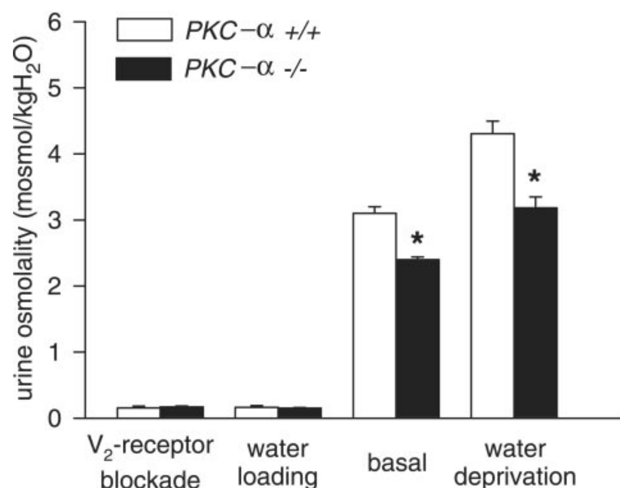


Fig. 2. Under conditions of free access to food and water (basal) in metabolic cage experiments, PKC-α −/− mice exhibited modestly lower urinary osmolality compared with PKC-α +/+ mice. The difference persisted in response to water deprivation for 36 h but was absent during acute water loading (1 ml/16 g body wt, using a gastric tube) or application of the vasopressin $V_2$-receptor antagonist SR-121463 (1 mg/kg ip), which induced hypotonic urine in both groups ($n$ = 6–8/group). $^*P < 0.05$ vs. PKC-α +/+.

## DISCUSSION

The present experiments show that mice deficient for PKC-α exhibit a modestly greater urinary flow rate and modestly lower urinary osmolality under basal conditions, i.e., under conditions of free access to water. This phenotype could be secondary to defects that do not reside within the kidney, such as alterations in thirst and water intake or vasopressin release. Further observations, however, supported the notion that the observed phenotype is due to intrarenal alterations that most likely reside within the medullary collecting duct, as discussed below.

The greater urinary flow rate and lower urinary osmolality observed under basal conditions in PKC-α −/− mice were associated with a greater urinary vasopressin-to-creatinine ratio as determined from 24-h urine collection in metabolic cages and a tendency for greater plasma osmolality. The urinary vasopressin-to-creatinine ratio has been used before as a surrogate parameter for mean daily plasma vasopressin concentration in mice (2) and was also used in the present study because precise measurements of plasma vasopressin in mice were not possible due to the relatively large volume of plasma required for commercial assays and that could not be obtained by decapitation of the mice. A tendency for greater plasma osmolality and a greater urinary vasopressin-to-creatinine ratio, however, are not compatible with a greater intake of water or a reduced central release of vasopressin as the primary cause of greater renal water excretion in PKC-α −/− mice. In accordance, a significantly lower urinary osmolality persisted in PKC-α −/− mice compared with PKC-α +/+ mice during 36-h water deprivation. This finding shows that the phenotype is independent of water intake and also indicates a lower urinary concentrating ability in PKC-α −/− mice.

Clearance experiments established that the modestly greater urine flow rate in PKC-α −/− mice was in fact the consequence of reduced fluid reabsorption in the kidney. The question remained of which segment of the tubular and collecting duct system contributed to this phenotype. Notably, the dilution of urine was not impaired in PKC-α −/− mice as indicated by experiments with acute water loading and application of a vasopressin $V_2$-receptor antagonist. Because intact



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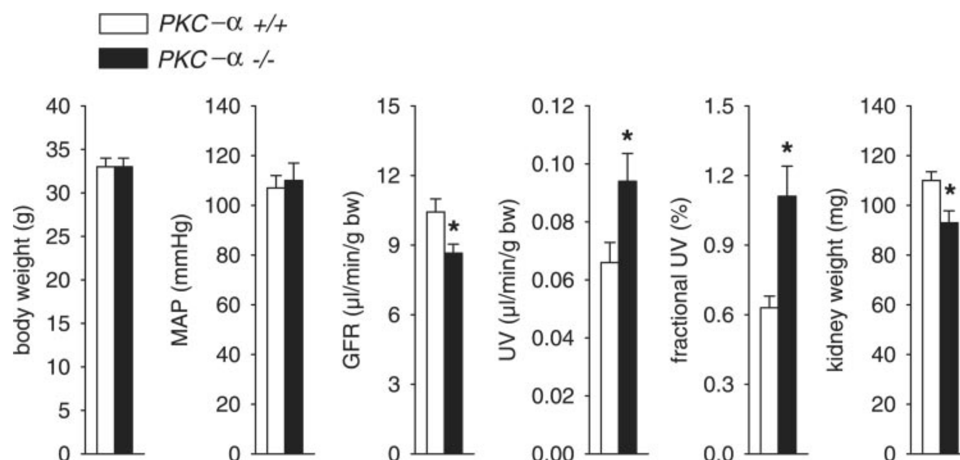


Fig. 3. In clearance experiments under anesthesia, PKC-α −/− mice showed a modestly lower glomerular filtration rate (GFR) and kidney weight and greater UV and fractional urinary water excretion (fractional $U_V$) compared with PKC-α +/+ mice ($n$ = 8/group). MAP, mean arterial blood pressure. $*P < 0.05$ vs. PKC-α +/+.

function of the thick ascending limb is required for not only urine concentration but also hypotonic dilution, these findings argue against a significant primary defect in PKC-α −/− mice in this nephron segment. Furthermore, the ability of the kidney to retain sodium in response to dietary sodium restriction was unaffected in PKC-α −/− mice, arguing against a significant defect in the major sodium-retaining segments of the nephron, including the proximal tubule, thick ascending limb, distal tubule, and cortical collecting duct. This leaves the medullary collecting duct as the most likely candidate for the defective nephron segment in PKC-α −/− mice, and this notion matches perfectly the renal localization of the isoenzyme in PKC +/+ mice: PKC-α is highly expressed in the medullary collecting duct, whereas it is not detectable in the proximal tubule, thick ascending limb, distal tubule, or principal cells of the cortical collecting duct (31a).

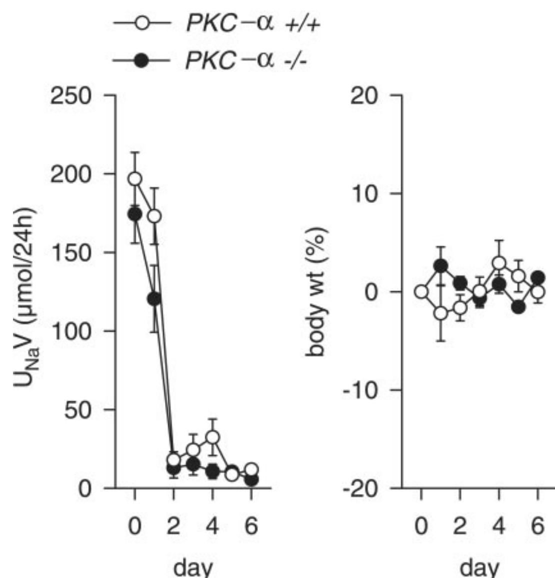


Fig. 4. Under conditions of free access to food and water in metabolic cage experiments, PKC-α −/− mice showed no difference in renal sodium excretion ($U_{Na}V$) compared with PKC-α +/+ mice (at *day 0*). The ability to reduce renal sodium excretion in response to dietary sodium restriction for 6 days was also comparable between groups. In accordance, body weight change (Δbody wt) was not different between groups ($n$ = 8/group).

The observed modestly lower GFR may be part of a water-conserving/compensating mechanism in PKC-α −/− mice. Alternatively, Bankir and colleagues (6) have proposed before that vasopressin-mediated urinary concentrating mechanisms are related to GFR, with high concentrating activity being related to high GFR and vice versa. Thus the lower GFR observed in PKC-α −/− mice may be related to an impaired urinary concentrating ability independent of body fluid loss. The modestly lower kidney wet and dry weight, on the other hand, may be the consequence of the lower GFR, which requires less absolute salt reabsorption and is in accordance with the preserved GFR-to-kidney weight ratio. Furthermore, PKC-α −/− mice did not present significant differences in the ratio of the thickness of the inner medulla vs. the cortex compared with PKC-α +/+ mice or alterations in the morphology of the inner medulla, which could have explained differences in urinary concentration.

The mechanism of how PKC-α can potentially contribute to urinary concentration in the medullary collecting duct, i.e., what target molecules are phosphorylated and what activates PKC-α, remains to be determined. Using semiquantitative immunoblotting, we observed no significant differences in the expression of aquaporin-2 in the inner medulla between PKC-α −/− vs. PKC-α +/+ mice. Interestingly, there are reports on rabbit and rat collecting duct that PKC in this nephron segment can mediate an inhibitory influence on water channels and transport (1, 13, 32), i.e., an influence that is opposite of the one proposed here for PKC-α in mouse medullary collecting duct. On the other hand, a study by Kato et al. (19) in rat terminal inner medullary collecting duct showed that angiotensin II increases vasopressin-stimulated facilitated urea permeability via a PKC-mediated signaling pathway, and the authors proposed that this may play a physiological role in the urinary concentrating mechanism by augmenting the maximal response to vasopressin. It remains to be determined whether vasopressin-stimulated facilitated urea permeability is affected in PKC-α −/− mice.

Notably, neither PKC-α, -βI, -βII, -δ, nor -ϵ was detected in principal cells of the cortical collecting duct in mice (31a), and therefore the identification and functional characterization of the PKC isoenzyme expressed at this site will be of significant interest. Furthermore, it is of interest that besides PKC-α,



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PKC-βI and -βII, which also belong to the group of conventional PKC isoenzymes, are similarly expressed in the medullary collecting duct of the mouse (31a). The fact that PKC-α knockout mice show a phenotype that presumably is localized to the medullary collecting duct indicates that classic PKC isoenzymes cannot fully compensate for each other in vivo even though they are expressed within the same cell.

Besides the medullary collecting duct, PKC-α in mouse kidney is expressed in intercalated cells of the cortical collecting duct and in glomeruli. In the present studies, we did not follow up on the potential role of PKC-α in intercalated cells. Because PKC-α expression is enhanced in mesangial cells by high glucose and in whole rat kidney in response to streptozotocin-induced diabetes mellitus and because there is evidence for a link between glomerular PKC and albuminuria (7, 17, 30), we determined urinary albumin excretion under basal conditions but could not detect differences between PKC-α knockout and wild-type mice. Whether glomerular PKC-α plays a role in albuminuria under pathophysiological conditions remains to be determined.

In summary, evidence is provided that PKC-α, which is expressed in the medullary collecting duct, is involved in urinary concentration in mice. Considering that PKC-βI and -βII are coexpressed with PKC-α in mouse medullary collecting duct, the observed phenotype of PKC-α knockout mice in urinary concentration indicates that conventional PKC isoenzymes cannot fully compensate for each other.

## GRANTS

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## REFERENCES


1. **Ando Y, Jacobson HR, and Breyer MD.** Phorbol myristate acetate, dioctanoglycerol, and phosphatidic acid inhibit the hydroosmotic effect of vasopressin on rabbit cortical collecting tubule. *J Clin Invest* 80: 590–593, 1987.
2. **Akizuki N, Uchida S, Sasaki S, and Marumo F.** Impaired solute accumulation in inner medulla of Clcnk1−/− mice kidney. *Am J Physiol Renal Physiol* 280: F79–F87, 2001.
3. **Baum M and Hays SR.** Phorbol myristate acetate and dioctanoylglycerol inhibit transport in rabbit proximal convoluted tubule. *Am J Physiol Renal Fluid Electrolyte Physiol* 254: F9–F14, 1988.
4. **Bertorello A and Aperia A.** $Na^+$-$K^+$-ATPase is an effector protein for protein kinase C in renal proximal tubule cells. *Am J Physiol Renal Fluid Electrolyte Physiol* 256: F370–F373, 1989.
5. **Boesch DM and Garvin JL.** Age-dependent activation of PKC isoforms by angiotensin II in the proximal nephron. *Am J Physiol Regul Integr Comp Physiol* 281: R861–R867, 2001.
6. **Bouby N, Ahloulay M, Nsegbe E, Dechaux M, Schmitt F, and Bankir L.** Vasopressin increases glomerular filtration rate in conscious rats through its antidiuretic action. *J Am Soc Nephrol* 7: 842–851, 1996.
7. **Ceol M, Gambaro G, Sauer U, Baggio B, Anglani F, Forino M, Facchin S, Bordin L, Weigert C, Nerlich A, and Schleicher ED.** Glycosaminoglycan therapy prevents TGF-beta1 overexpression and pathologic changes in renal tissue of long-term diabetic rats. *J Am Soc Nephrol* 11: 2324–2336, 2000.
8. **Chou CL, Rapko SI, and Knepper MA.** Phosphoinositide signaling in rat inner medullary collecting duct. *Am J Physiol Renal Physiol* 274: F564–F572, 1998.
9. **Dong L, Stevens JL, and Jaken S.** Biochemical and immunological characterization of renal protein kinase C. *Am J Physiol Renal Fluid Electrolyte Physiol* 261: F679–F687, 1991.
10. **Du Z, Ferguson W, and Wang T.** Role of PKC and calcium in modulation of effects of angiotensin II on sodium transport in proximal tubule. *Am J Physiol Renal Physiol* 284: F688–F692, 2003.
11. **Erdbrügger W, Keffel J, Knocks M, Otto T, Philipp T, and Michel MC.** Protein kinase C isoenzymes in rat and human cardiovascular tissues. *Br J Pharmacol* 120: 177–186, 1997.
12. **Gagnon F, Hamet P, and Orlov SN.** $Na^+$,$K^+$ pump and $Na^+$-coupled ion carriers in isolated mammalian kidney epithelial cells: regulation by protein kinase C. *Can J Physiol Pharmacol* 77: 305–319, 1999.
13. **Hebert RL, Jacobson HR, and Breyer MD.** $PGE_2$ inhibits AVP-induced water flow in cortical collecting ducts by protein kinase C activation. *Am J Physiol Renal Fluid Electrolyte Physiol* 259: F318–F325, 1990.
14. **Huang DY, Pfaff I, Serradeil-Le Gal C, and Vallon V.** Acute renal response to the non-peptide vasopressin $V_2$-receptor antagonist SR 121463B in anesthetized rats. *Naunyn-Schmiedebergs Arch Pharmacol* 362: 201–207, 2000.
15. **Ibarra FR, Cheng SX, Agren M, Svensson LB, Aizman O, and Aperia A.** Intracellular sodium modulates the state of protein kinase C phosphorylation of rat proximal tubule $Na^+$,$K^+$-ATPase. *Acta Physiol Scand* 175: 165–171, 2002.
16. **Jo I, Ward DT, Baum MA, Scott JD, Coghlan VM, Hammond TG, and Harris HW.** AQP2 is a substrate for endogenous PP2B activity within an inner medullary AKAP-signaling complex. *Am J Physiol Renal Physiol* 281: F958–F965, 2001.
17. **Kang N, Alexander G, Park JK, Maasch C, Buchwalow I, Luft FC, and Haller H.** Differential expression of protein kinase C isoforms in streptozotocin-induced diabetic rats. *Kidney Int* 56: 1737–1750, 1999.
18. **Karim Z, Defontaine N, Paillard M, and Poggioli J.** Protein kinase C isoforms in rat kidney proximal tubule: acute effect of angiotensin II. *Am J Physiol Cell Physiol* 269: C134–C140, 1995.
19. **Kato A, Klein JD, Zhang C, and Sands JM.** Angiotensin II increases vasopressin-stimulated facilitated urea permeability in rat terminal IMCDs. *Am J Physiol Renal Physiol* 279: F835–F840, 2000.
20. **Kihara M, Umemura S, Sumida Y, Yokoyama N, Yabana M, Nyui N, Tamura K, Murakami K, Fukamizu A, and Ishii M.** Genetic deficiency of angiotensinogen produces an impaired urine concentrating ability in mice. *Kidney Int* 53: 548–555, 1998.
21. **Kirton SA and Loutzenhiser R.** Alterations in basal protein kinase C activity modulate renal afferent arteriolar myogenic reactivity. *Am J Physiol Heart Circ Physiol* 275: H467–H475, 1998.
22. **Leitges M, Plomann M, Standaert ML, Bandyopadhyay G, Sajan MP, Kanoh Y, and Farese RV.** Knockout of PKC alpha enhances insulin signaling through PI3K. *Mol Endocrinol* 16: 847–858, 2002.
23. **Ma R, Kudlacek PE, and Sansom SC.** Protein kinase Cα participates in activation of store-operated $Ca^{2+}$ channels in human glomerular mesangial cells. *Am J Physiol Cell Physiol* 283: C1390–C1398, 2002.
24. **Miyata Y, Okada K, Ishibashi S, Asano Y, and Muto S.** P-gp-induced modulation of regulatory volume increase occurs via PKC in mouse proximal tubule. *Am J Physiol Renal Physiol* 282: F65–F76, 2002.
25. **Muller MW, Todorov V, Kramer BK, and Kurtz A.** Angiotensin II inhibits renin gene transcription via the protein kinase C pathway. *Pflügers Arch* 444: 499–505, 2002.
26. **Newton AC.** Protein kinase C: structure, function and regulation. *J Biol Chem* 270: 28495–28498, 1995.
27. **Östlund E, Mendez CF, Jacobsson G, Fryckstedt J, Meister B, and Aperia A.** Expression of protein kinase C isoforms in renal tissue. *Kidney Int* 47: 766–773, 1995.
28. **Pedemont CH and Bertorello AM.** Short-term regulation of the proximal tubule Na, K-ATPase activity mediated by protein kinase C isoforms. *J Bioenerg Biomembr* 33: 439–447, 2001.
29. **Pfaff IL, Wagner HJ, and Vallon V.** Immunolocalization of protein kinase C isoenzymes α, β I and β II in rat kidney. *J Am Soc Nephrol* 10: 1861–1873, 1999.
30. **Pfaff IL and Vallon V.** Protein kinase C β isoenzymes in experimental diabetes mellitus and their relation to nephroprotective actions of the ACE inhibitor lisinopril. *Kidney Blood Press Res* 25: 329–340, 2002.
31. **Rangel LB, Caruso-Neves C, Lara LS, and Lopes AG.** Angiotensin II stimulates renal proximal tubule $Na^+$-ATPase activity through the activation of protein kinase C. *Biochim Biophys Acta* 1564: 310–316, 2002.

31a. **Redling S, Pfaff IL, Leitges M, and Vallon V.** Immunolocalization of protein kinase C isoenzymes α, βI, βII, δ, and ϵ in mouse kidney. *Am J Physiol Renal Physiol* 287: F289–F298, 2004.

32. **Tomita K, Nonoguchi H, and Marumo F.** Effects of endothelin on peptide-dependent cyclic adenosine monophosphate accumulation




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along the nephron segments of the rat. *J Clin Invest* 85: 2014–2018, 1990.

33. **Tsimaratos M, Roger F, Chabardes D, Mordasini D, Hasler U, Doucet A, Martin PY, and Feraille E.** C-peptide stimulates $Na^+$,$K^+$-ATPase activity via PKC α in rat medullary thick ascending limb. *Diabetologia* 46: 124–131, 2003.
34. **Vallon V, Grahammer F, Richter K, Bleich M, Lang F, Barhanin J, Völkl H, and Warth R.** Role of KCNE1-dependent $K^+$ fluxes in mouse proximal tubule. *J Am Soc Nephrol* 12: 2003–2011, 2001.
35. **Vallon V, Traynor T, Barajas L, Huang YG, Briggs JP, and Schnermann J.** Feedback control of glomerular vascular tone in nNOS knockout mice. *J Am Soc Nephrol* 12: 1599–1606, 2001.
36. **Vallon V, Verkman AS, and Schnermann J.** Luminal hypotonicity in proximal tubules of aquaporin-1 knockout mice. *Am J Physiol Renal Physiol* 278: F1030–F1033, 2000.
37. **Wang SX, Mene P, and Holthofer H.** Nephrin mRNA regulation by protein kinase C. *J Nephrol* 14: 98–103, 2001.
38. **Whiteside CI and Dlugosz JA.** Mesangial cell protein kinase C isozyme activation in the diabetic milieu. *Am J Physiol Renal Physiol* 282: F975–F980, 2002.
39. **Wulff P, Vallon V, Huang DY, Völkl H, Yu F, Richter K, Jansen M, Schlünz M, Klingel K, Loffing J, Kauselmann G, Bösl MR, Lang F, and Kuhl D.** Impaired renal $Na^+$ retention in the sgk1-knockout mouse. *J Clin Invest* 110: 1263–1268, 2002.

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