NBC3 expression in rabbit collecting duct: colocalization with vacuolar H\(^+\)-ATPase

ALEXANDER PUSHKIN,\(^1\) KAY-PONG YIP,\(^2\) IMRAN CLARK,\(^2\) NATALIA ABULADZE,\(^3\) TAE-HWAN KWON,\(^6\) SHUICHI TSURUOKA,\(^4\) GEORGE J. SCHWARTZ,\(^2\) SØREN NIELSEN,\(^6\) AND IRA KURTZ\(^1\)

\(^1\)Division of Nephrology and \(^2\)Department of Biological Chemistry, University of California at Los Angeles, School of Medicine, Los Angeles, California 90095; \(^3\)Department of Physiology and Biophysics, School of Medicine, University of South Florida, Tampa, Florida 33612; \(^4\)Department of Pharmacology, Jichi Medical School, Tochigi 329-04, Japan; \(^5\)Departments of Pediatrics and Medicine, University of Rochester School of Medicine, Rochester, New York 14642; and \(^6\)Department of Cell Biology, Institute of Anatomy, University of Aarhus, Aarhus DK-8000, Denmark

Pushkin, Alexander, Kay-Pong Yip, Imran Clark, Natalia Abuladze, Tae-Hwan Kwon, Shuichi Tsuruoka, George J. Schwartz, Søren Nielsen, and Ira Kurtz. NBC3 expression in rabbit collecting duct: colocalization with vacuolar H\(^+\)-ATPase. Am. J. Physiol. 277 (Renal Physiol. 46): F974–F981, 1999.—We have recently cloned and characterized a unique sodium bicarbonate cotransporter, NBC3, which unlike other members of the NBC family, is ethylisopropylamiloride (EIPA) inhibitable, DIDS insensitive, and electroneutral (A. Pushkin, N. Abuladze, I. Lee, D. Newman, J. Hwang, and I. Kurtz. J. Biol. Chem. 274: 16569–16575, 1999). In the present study, a specific polyclonal antipeptide COOH-terminal antibody, NBC3-C1, was generated and used to determine the pattern of NBC3 protein expression in rabbit kidney. A major band of ~200 kDa was detected on immunoblots of rabbit kidney. Immunocytochemistry of rabbit kidney frozen sections revealed specific staining of the apical membrane of intercalated cells in both the cortical and outer medullary collecting ducts. The pattern of NBC3 protein expression in the collecting duct was nearly identical to the same sections stained with an antibody against the vacuolar H\(^+\)-ATPase 31-kDa subunit. In addition, the NBC3-C1 antibody immunoprecipitated the vacuolar H\(^+\)-ATPase 31-kDa subunit. Functional studies in outer medullary collecting ducts (inner stripe) showed that type A intercalated cells have an apical Na\(^+\)-dependent base transporter that is EIPA inhibitable and DIDS insensitive. The data suggest that NBC3 participates in H\(^+\)/base transport in the collecting duct. The close association of NBC3 and the vacuolar H\(^+\)-ATPase in type A intercalated cells suggests a potential structural/functional interaction between the two transporters.

bicarbonate; sodium; transport; kidney

SINCE ITS INITIAL CHARACTERIZATION in the salamander Ambystoma tigrinum kidney (9), Na(HCO\(_3\))\(_n\) cotransport has been reported in several cell types (1, 2, 4–6, 14, 15, 18, 27, 28, 30, 31, 44). The transport properties and sensitivity to inhibitors have differed from the renal transporter in several tissues. The molecular mechanisms responsible for these differences are partially understood with the recent characterization of several sodium bicarbonate cotransport proteins. Following the initial cloning and functional characterization of an electrogenic sodium bicarbonate cotransporter (NBC) from salamander kidney (35), sodium bicarbonate cotransporters were cloned and functionally characterized from mammalian kidney (knBC1) and pancreas (pNBC1) (3, 12, 13, 36). The renal proximal tubule uniquely expresses the knBC1 isoform, whereas expression of pNBC1 is more widespread (3). An additional NBC-like clone (NBC2), whose function is unknown, has been isolated from a human retina cDNA library (23). A new member of the sodium bicarbonate cotransporter family, NBC3, has been recently cloned from human skeletal muscle and functionally characterized (33, 34). Unlike NBC1, NBC3 is electroneutral, ethylisopropylamiloride (EIPA) inhibitable, and DIDS insensitive.

In the present study we demonstrate that NBC3 is expressed in the rabbit kidney. NBC3 was found to colocalize with the vacuolar H\(^+\)-ATPase in the cortical and outer medullary collecting duct type A intercalated cells. Immunoprecipitation experiments confirmed the close association of NBC3 and the vacuolar H\(^+\)-ATPase. Finally, functional studies of the outer medullary collecting duct type A intercalated cells revealed a novel apical stilbene-insensitive, EIPA-inhibitable sodium bicarbonate cotransporter. The properties of this transporter in the outer medullary collecting duct were indistinguishable from the functional characteristics of NBC3 cRNA, expressed in Xenopus laevis oocytes (34).

MATERIAL AND METHODS

Generation of NBC3 polyclonal antibodies and immunoblotting. A synthetic peptide corresponding to amino acids 1197–1214 was used to generate a polyclonal antibody specific for
human NBC3 (NBC3-C1) (34). The purified peptide was coupled to keyhole limpet hemocyanin for immunization in rabbits. Rabbit kidney samples were analyzed by SDS-PAGE. The primary antibody (NBC3-C1) was diluted 1:500 in TBS (20 mM Tris·HCl, pH 7.5, 137 mM NaCl). For peptide blocking, 10 µg/ml of peptide was used. A biotinylated goat anti-rabbit secondary antibody and streptavidin-alkaline phosphatase conjugate were used at 1:10,000 and 1:10,000 dilutions, respectively. A monoclonal antibody E11 against the 31-kDa subunit of the vacuolar H^+-ATPase (gift from Dr. S. Glick) was used undiluted. An alkaline phosphatase-conjugated sheep anti-mouse secondary antibody was used at a dilution of 1:10,000.

Immunoprecipitation. Five grams of rabbit kidney was disrupted at 0°C in a glass homogenizer with 100 ml of TBS, containing protease inhibitors: 1 mM EDTA, 1 mM phenylmethylsulfonfyl fluoride, 1 µg/ml pepstatin, 1 µg/ml bestatin, 1 µg/ml leupeptin, and 1 µg/ml aprotinin (buffer A). The homogenate was centrifuged at 300 g for 5 min and then at 4,000 g for 10 min. The supernatant was centrifuged at 110,000 g for 2 h. The pellet was solubilized in buffer A and centrifuged at 110,000 g for 2 h. The final membrane pellet was solubilized in buffer A containing 0.1% Triton X-100 and centrifuged at 110,000 g for 1 h. One milliliter of the supernatant was mixed with 0.15 ml of NBC3-C1 or preimmune sera. After incubation for 1 h, 0.2 ml of protein A Sepharose was added. The mixture was incubated for 1 h and then centrifuged at 10,000 g for 10 s. The pellet was washed 10 times with the buffer A containing 0.1% Triton X-100. The protein A Sepharose was mixed with 0.2 ml of 0.2 M glycine (pH 2.5). After 20 min incubation, the mixture was spun at 10,000 g for 10 s and the supernatant was analyzed by SDS-PAGE and immunoblotting.

Immunocytochemistry. The kidney was removed and cut into thin slices. The slices were immediately frozen in liquid nitrogen. The primary antibody, NBC3-C1 (1:100 dilution) was applied for 1 h at 37°C to cryostat (5 µm) sections attached to ProbeOn Plus slides (Fisher Scientific). Following several washes in PBS, goat anti-rabbit IgG conjugated with Alexa 488 (1/500 dilution, Molecular Probes) was applied for 1 h at 37°C. The sections were labeled for 1 h at 37°C with a monoclonal antibody against the 31-kDa subunit of the vacuolar H^+-ATPase, E11, undiluted. Following several washes in PBS, goat anti-mouse IgG conjugated with Alexa 594 (1/500 dilution, Molecular Probes) was applied for 1 h at 37°C. The slides were rinsed in PBS and mounted in Cytoseal 60 (Stephens Scientific). A liquid-cooled PXL charge-coupled device camera (model CH1, Photometrics) coupled to a Nikon Microphot-FXA epifluorescence microscope, was used to capture and digitize the fluorescence images. The images were transferred to a Silicon Graphics Indy 5000 computer using ISEE 4.0 software (Inovision), and printed on a Kodak 8650 PS color printer. The confocal images were captured with a Leica TCS SP inverted confocal Microscope (Leitz). Alexa 488 and Alexa 594 were excited simultaneously using an argon laser (model 2014, Cyonics Uniphase) and krypton laser (model 643, Melles Griot).

Measurement of intracellular pH. Outer medullary collecting ducts from the inner stripe were dissected from male New Zealand White rabbits. Intracellular pH (pHi) was measured using an MRC-1000 laser-scanning inverted confocal microscope (Bio-Rad), coupled to the tubule perfusion apparatus (17, 50, 51). The tubules were dissected in the following Na^+- and Cl^-free HEPS-buffered solution: tetramethylammonium hydroxide (140 mM), gluconic acid lactone (140 mM), K_2HPO_4 (2.5 mM), calcium gluconate (7 mM), magnesium gluconate (2 mM), and HEPS (5 mM) buffered with 100% O_2, pH 7.4. To measure pHi, in single intercalated cells, the tubule was exposed for 5–10 min to acetoxyethyl ester of 2,7'-bis(2-carboxyethyl)-(6)-carboxylfluorescein (BCECF-AM, 30 µM) or carboxy SNARF-1 acetate ester (10 µM). Only brightly staining minority (type A) intercalated cells were studied, as previously described by Weiner et al. (47). The tubules were then perfused in a dye-free solution for at least 15 min before beginning an experiment. All studies were done in Cl^-free solutions. The tubules were perfused and bathed following dye loading in the following bicarbonate-buffered Na^+- and Cl^-free solution: tetramethylammonium hydroxide (115 mM), gluconic acid lactone (115 mM), K_2HPO_4 (2.5 mM), calcium gluconate (7 mM), magnesium gluconate (2 mM), and tetramethylammonium bicarbonate (25 mM), buffered with 6.5% CO_2-93.5% O_2, pH 7.4. Confocal images were acquired from the bottom of the tubule with a zoom factor of 3.5–4.0. Pairs of images (384 × 256 pixels) were stored digitally at a rate of 1 Hz for the first 60 s after a luminal switch, and then the sampling rate was reduced to 0.2 Hz. After a baseline recording of approximately 25 pairs of images, the luminal solution was switched to the following solution containing 140 mM Na^+-: sodium gluconate (115 mM), K_2HPO_4 (2.5 mM), calcium gluconate (7 mM), magnesium gluconate (2 mM), and sodium bicarbonate (25 mM), buffered with 6.5% CO_2-93.5% O_2, pH 7.4. Similar experiments were performed with DIDS (1 mM, lumen) or EIPA (50 µM, lumen). In separate studies, the tubules were perfused and bathed in a Na^+- and Cl^-free HEPS-buffered solution containing tetramethylammonium hydroxide (140 mM), gluconic acid lactone (140 mM), K_2HPO_4 (2.5 mM), calcium gluconate (7 mM), magnesium gluconate (2 mM), and HEPS (5 mM), buffered with 100% O_2, pH 7.4. After a baseline recording of approximately 25 pairs of images, the apical solution was switched to the following solution containing 140 mM Na^+-: sodium gluconate (140 mM), K_2HPO_4 (2.5 mM), calcium gluconate (7 mM), magnesium gluconate (2 mM), and HEPS (5 mM) buffered with 100% O_2, pH 7.4. Calibration was performed at the end of each experiment using the high-potassium/nigericin technique (43). Analysis of the pHi transients was obtained retrospectively from stored image pairs using the TSCM software (Bio-Rad) as previously described (50, 51). Fluorescence ratios from each image pair were corrected by subtracting the dark current and background from each image at each wavelength. The fluorescence ratios were converted to pHi, from the calibration parameters, obtained from the same cell at the end of the experiment. In bicarbonate-containing solutions, total buffer capacity (b_t) of the intercalated cells was equal to their intrinsic buffer capacity, b_i when HEPES was used and signifies b_3 when HCO_3^- buffer solutions were used.

RESULTS

NBC3 localization in the collecting duct. As shown in Figs. 1 and 2, NBC3 is expressed in rabbit cortical and outer medullary collecting ducts. The glomeruli, proximal tubules, descending thin limbs, thick ascending limbs, distal convoluted tubules, and vascular structures were consistently unlabeled. NBC3 was detectable on the apical membrane of intercalated cells in the cortical and outer medullary collecting ducts. Labeling...
was blocked with a specific NBC3 peptide. Double labeling experiments on the same slides with antibodies against the 31-kDa subunit of the vacuolar proton pump (red) revealed that NBC3 (green) colocalizes with the proton pump. High-magnification confocal microscopic images (Fig. 3), showed that NBC3 and the vacuolar proton pump are colocalized not only on the apical membrane of type A intercalated cells but, in addition, in subapical vesicles as well. Both the NBC3-C1 antibody and the E11 antibody failed to label principal cells. The results indicate that NBC3 and the vacuolar H\textsuperscript{+}-ATPase are closely associated in type A intercalated cells.

Immunoblotting and immunoprecipitation. A major band of \( \sim 200 \) kDa was detected using the NBC3-C1 antibody in crude rabbit kidney membranes on immunoblotting (Fig. 4). This band was not detected with NBC3-C1 preincubated with a specific peptide. The predicted size of NBC3 based on the cDNA sequence is \( \sim 136 \) kDa, suggesting that NBC3 is posttranslationally modified.

The finding that in type A intercalated cells, the 31-kDa subunit of the vacuolar H\textsuperscript{+}-ATPase colocalized with NBC3 in both the apical membrane and subapical vesicles suggested that these proteins are closely associated. In immunoprecipitation experiments (Fig. 4), the NBC3-C1 antibody communoprecipitated the 31-kDa subunit of the vacuolar H\textsuperscript{+}-ATPase. Preimmune serum did not precipitate the 31-kDa subunit of the vacuolar H\textsuperscript{+}-ATPase. These results together with the immunocytochemistry findings indicate that the two proteins are closely associated.

Functional studies. In bicarbonate-buffered Na\textsuperscript{+}- and Cl\textsuperscript{−}-free solutions, resting pH\textsubscript{i} in type A intercalated cells was 6.78 \( \pm \) 0.03 (n = 36 cells, 6 tubules). Following luminal Na\textsuperscript{+} addition, pH\textsubscript{i} increased to 6.97 \( \pm \) 0.04 with an EBF of 4.92 \( \pm \) 0.49 mM/min (Fig. 5). In similar experiments performed with EIPA (50 µM, lumen), the luminal Na\textsuperscript{+}-induced EBF was completely inhibited (0.02 \( \pm \) 0.003 mM/min; n = 18 cells, 4 tubules; P \( < \) 0.001; Fig. 5). DIDS, 1 mM (lumen), was without effect; steady-state pH\textsubscript{i} was 6.76 \( \pm \) 0.03 (18 cells, 4 tubules), and following luminal Na\textsuperscript{+} addition pH\textsubscript{i} increased to 6.95 \( \pm \) 0.02, with an EBF of 4.58 \( \pm \) 0.35 mM/min (not significant vs. control). In HEPES-buffered Na\textsuperscript{+}- and Cl\textsuperscript{−}-free solutions, the initial pH\textsubscript{i} was 6.76 \( \pm \) 0.02 (n = 33 cells, 5 tubules), and following luminal Na\textsuperscript{+} addition pH\textsubscript{i} increased to 6.95 \( \pm \) 0.02, with an EBF of 4.58 \( \pm \) 0.35 mM/min (not significant vs. control). Therefore, the EIPA-inhibitable, luminal Na\textsuperscript{+}-induced EBF was stimulated approximately twofold in the presence of bicarbonate. These results are nearly identical to those previously reported in Xenopus oocytes expressing NBC3 (34).

DISCUSSION

The results of this study demonstrate that NBC3 is localized to the apical membrane of type A intercalated cells in rabbit kidney. In both the cortical and outer medullary collecting duct, NBC3 colocalized with the vacuolar H\textsuperscript{+}-ATPase. The close association between these transporters was confirmed by their communoprecipitation. In separate experiments, type A intercalated cells in the outer medullary collecting duct (inner stripe segment) were shown to have functional EIPA-inhibitable, Cl\textsuperscript{−}-independent sodium bicarbonate co-
transport that was DIDS insensitive. These properties are nearly identical to the functional characteristics of NBC3 expressed in Xenopus oocytes (34).

High-resolution confocal images of type A intercalated cells revealed colocalization of NBC3 and the vacuolar H\(^+\)-ATPase in subapical vesicles and the apical plasma membrane. Immunelectron microscopy has confirmed that NBC3 labeling is very abundant in both the apical plasma membrane, in intracellular vesicles, and in tubulodistal profiles in the subapical domains but is absent in the basolateral plasma membrane of type A intercalated cells (unpublished results). The vesicular localization of NBC3 is significant given the results of previous studies which have demon-
Fig. 4. Western blots of rabbit kidney. A: 100 µg of crude rabbit kidney membranes was loaded onto each lane and separated by 10% SDS-PAGE. NBC3-C1 antibody (lane 1); NBC3-C1 antibody + specific peptide (10 µg/ml; lane 2). B: 100 µg of crude rabbit kidney membranes were loaded onto lane 1 and separated by 17% SDS-PAGE and probed with E11 mouse monoclonal antibody against the 31-kDa subunit of the vacuolar H^+-ATPase (indicated by arrowhead). In separate experiments, crude rabbit kidney membrane proteins were immunoprecipitated with NBC3-C1 antibody (lane 2) or with preimmune serum (lane 3) and separated by 17% SDS-PAGE and probed with the E11 antibody. IgG light chains are indicated by the arrow (IgG heavy chains not shown).

strated that vesicular trafficking is an important mechanism regulating type A intercalated cell H^+ secretion (21). For example, following NH_4Cl acid loading, the vacuolar H^+-ATPase is redistributed from the vacuolar compartment to the apical plasma membrane (7, 45). Given the colocalization of NBC3 and the vacuolar proton pump in this cell type, it will be important to determine whether NBC3 is similarly redistributed to the plasma membrane following changes in systemic acid-base balance.

We were unable, as other investigators have previously reported (39, 46), to detect basolateral plasma membrane vacuolar proton pump immunoreactivity in rabbit collecting duct type B intercalated cells on light

Fig. 5. Apical Na^+-dependent base flux in minority (type A intercalated) cells of the outer medullary collecting duct (inner stripe segment). Outer medullary collecting ducts were perfused/bathed in a Na^+- and Cl^--free solution. A: luminal Na^+ addition in bicarbonate-buffered solutions. pH_i, intracellular pH. B: luminal Na^+ addition in bicarbonate-buffered solutions in the presence of ethylisopropylamiloride (EIPA; 50 µM, lumen). C: summary of apical Na^+-dependent equivalent base flux under various conditions (see MATERIALS AND METHODS); n.s., Not significant. Starting pH_i was 6.7–6.8 in all studies.
microscopy. Similarly, the NBC3-C1 antibody failed to label the basolateral membrane of type B intercalated cells in rabbit kidney. As has been suggested, species differences most likely account for the lack of basolateral H\(^{+}\)-ATPase immunoreactivity in rabbit type B intercalated cells using identical light microscopic techniques (39, 46), since basolateral proton pump labeling is detectable in rodent kidneys (25). In rat kidney, we have recently demonstrated colabeling of the basolateral membrane of type B intercalated cells with NBC3-C1 and E11 31-kDa H\(^{+}\)-ATPase antibodies (unpublished results).

Vacular H\(^{+}\)-ATPases play an important role not only in bicarbonate transport in the collecting duct, but in addition, in the acidification of several compartments in eukaryotic cells including clathrin-coated vesicles, lysosomes, endosomes, and Golgi vesicles (21, 32, 40). The vacuolar H\(^{+}\)-ATPase is highly expressed in the apical membrane of type A intercalated cells in comparison to these intracellular organelles, permitting its detection in this cell type by immunocytochemical methods (11, 25). The widespread low level expression of vacuolar H\(^{+}\)-ATPase in intracellular organelles suggests the interesting possibility that NBC3 is also expressed at much lower levels in these organelles. Functional and immunoelectron microscopic studies of purified organelle preparations addressing this interesting question are currently in progress.

The results of this study provide the first documentation of a sodium bicarbonate cotransporter in the apical membrane of type A intercalated cells in cortical and outer medullary collecting ducts. Type A intercalated cells are thought to secrete protons (and absorb bicarbonate) via an apical vacuolar H\(^{+}\)-ATPase and H\(^{+}\)-K\(^{+}\)-ATPase (29, 48). Bicarbonate is then transported across the basolateral membrane via the basolateral AE1 anion exchanger. Type A intercalated cells are not, however, believed to mediate transepithelial Na\(^{+}\) transport. It is of interest that previous studies have demonstrated the presence of a basolateral Na\(^{+}\)/H\(^{+}\) exchanger in this cell (28, 48). The finding that net transepithelial bicarbonate transport is Na\(^{+}\) independent in the outer medullary collecting duct (inner stripe) (42) suggests rather that in type A intercalated cells, apical NBC3 and basolateral Na\(^{+}\)/H\(^{+}\) exchange may play an important role in mediating H\(^{+}\)/base transport across their respective membranes (pH\(_{i}\) regulation). It has previously been suggested that because of the Na\(^{+}\) permeability characteristics of the rabbit outer medullary collecting duct, as well as the in vivo transepithelial ion gradients, this segment mediates passive Na\(^{+}\) transport (41). Whether luminal NBC3 contributes to passive transepithelial Na\(^{+}\) transport in this segment is unknown. The potential contribution of apical NBC3 and basolateral Na\(^{+}\)/H\(^{+}\) exchange to passive transepithelial Na\(^{+}\) transport in the outer medullary collecting duct will require further study.

The distribution of NBC3 and vacuolar H\(^{+}\)-ATPase in rabbit collecting duct, as well as the com immunoprecipitation of NBC3 and the 31-kDa subunit of the vacuolar H\(^{+}\)-ATPase, suggests that the two transporters may be closely associated. There is increasing evidence that functionally unrelated ion channels and transporters may modulate each other’s activity via energetically favorable protein-protein interaction (24, 26). In cells expressing high levels of the vacuolar H\(^{+}\)-ATPase such as the type A intercalated cells, NBC3 may provide an additional means of regulating net proton secretion and bicarbonate transport. Interestingly, the rat epididymis and vas deferens, like the collecting duct, have specialized cells which express high levels of an apical vacuolar H\(^{+}\)-ATPase and are thought to play a role in mediating luminal acidification (10). We have recently found that NBC3 also colocalizes with the vacuolar H\(^{+}\)-ATPase 31-kDa subunit on the apical membrane of these cells (unpublished observations). Whether colocalization of the two transporters is a general phenomenon in all tissues expressing high levels of the vacuolar H\(^{+}\)-ATPase is currently being investigated.

Electrogenic pumping of protons generates an electric potential that can limit the pH gradient achievable by ATP hydrolysis (20, 49). In several preparations, the presence of a parallel Cl\(^{-}\) conductance results in net electroneutral transport (20, 21, 40, 49). Recent evidence suggests that the CIC-5 chloride channel, which also colocalizes with the vacuolar proton pump in type A intercalated cells, may provide the conductive pathway required for efficient vesicle acidification (22). Interestingly, previous studies of rat renal endocytotic vesicles have shown that bicarbonate, in addition to chloride, can stimulate H\(^{+}\) pump activity (38). Similar findings have been reported with the gastric H\(^{+}\)-K\(^{+}\)-ATPase and the mitochondrial H\(^{+}\)-ATPase (8, 16). The mechanism of this stimulatory effect is unknown. In vesicles derived from Dictostelium, bicarbonate stimulates vacuolar H\(^{+}\)-ATPase activity and can shunt the electrical potential generated by electrogentic proton pumping (19). In this regard, NBC3 may modulate the activity of the vacuolar H\(^{+}\)-ATPase by altering the local bicarbonate concentration.

We thank Dr. Paul Boyer for critical review of the manuscript and Dr. S. Gluck for providing the E11 antibody.

This work was supported by National Institute of Diabetes and Digestive and Kidney Diseases Grants DK-46976 and DK-50603, National Heart, Lung, and Blood Institute Grant HL-59156, The Iris and B. Gerald Cantor Foundation, the Max Factor Family Foundation, the Verna Harrah Foundation, the Richard and Linda Rosenblum Foundation, the Fredericka Taubitz Foundation, the Karen Elise Jensen Foundation, EU Commission (EU-Biotech and TMR programmes), Danish Medical Research Council, and the Danish Biotechnology Programme. N. Abuladze is supported by National Kidney Foundation of Southern California Training Grant J891002. Address for reprint requests and other correspondence: I. Kurtz, UCLA Division of Nephrology, 10833 Le Conte Ave., Room 7-155, Factor Building, Los Angeles, CA 90095-1689 (E-mail: IKurtz@mednet.ucla.edu).

Received 2 July 1999; accepted in final form 15 September 1999.

REFERENCES


