Immunocytochemical Localization of Sodium-Calcium Exchanger in Canine Nephron

James E. Bourdeau, A. N. Taylor, A. M. Iacopino

J.E. Bourdeau, Section of Nephrology, Department of Medicine, Department of Veterans Affairs Medical Center and Department of Physiology and Biophysics, University of Oklahoma Health Sciences Center, Oklahoma City, OK
A.N. Taylor, A.M. Iacopino, Department of Biomedical Sciences, Baylor College of Dentistry, Dallas, TX

ABSTRACT
The sodium-calcium exchanger was localized in tissue sections of canine kidneys with two different polyclonal antisera raised against purified sodium-calcium exchanger protein derived from dog cardiac sarcolemma. The sodium-calcium exchanger was prominent in the basolateral plasmalemmae of the majority of cells in all connecting tubules. In contrast, it was observed rarely and inconspicuously over the basolateral aspects of proximal tubular cells. The sodium-calcium exchanger was undetectable in all other portions of the nephron.

Key Words: Antipporter, basolateral plasma/emma, calcium ion transport, connecting tubule, cytosolic Ca²⁺ concentration

Sodium-calcium exchange activity is present in the basolateral plasmalemmae of renal epithelial cells. Isolated basolateral membrane vesicles derived from kidney tubular cells in the cortices of rats (1–3), dogs (4,5), and cattle (6) demonstrate such activity. It also is expressed in Xenopus laevis oocytes injected with poly(A)⁺ RNA isolated from the renal cortices of either rabbits or rats (7). A cDNA encoding a sodium-calcium exchanger from rabbit kidney cortex, which is homologous to the canine sarcolemmal sodium-calcium exchanger, has been cloned (8,9). Additionally, a 452-nucleotide sequence homologous to a portion of the canine kidney sodium-calcium exchanger cDNA has been cloned from rat kidney cortex (10). The preponderance of proximal tubules in the cortex (11) and technical factors related to the preparation of the canine vesicles (4,5) suggest that the exchanger is located in proximal tubular cells. Functional studies (12–14) and Western and Northern blot analyses (15) in isolated rat proximal tubules provide direct evidence for sodium-calcium exchanger activity, protein, and mRNA, respectively, in these cells. However, because cortical nephrons are composed of heterogeneous epithelia (16), none of the above studies exclude the presence of sodium-calcium exchange activity in distal nephron segments. In fact, the existence of sodium-calcium antiporters in the basolateral plasmalemmae of cells within the distal nephron is supported by several lines of evidence. First, purified basolateral cell membranes from rabbit distal tubules demonstrate sodium-calcium exchange activity (17). Second, physiologic studies in isolated perfused rabbit connecting tubules show sodium-calcium exchange activity exclusively in the basolateral plasmalemmae (18). Finally, individually microdissected distal tubules from rat kidneys demonstrate mRNA for the sodium-calcium exchanger (10).

The dual distribution of the sodium-calcium exchanger in the proximal and distal segments of mammalian nephrons is controversial. On the basis of membrane vesicle transport studies, Ramachandran and Brunette (17) reported that the antiporter was located exclusively in the distal tubules of rabbit kidneys. Using reverse transcription-polymerase chain reaction, Yu et al. (10) observed that mRNA for the exchanger was present in five of eight distal convoluted tubules microdissected from rat kidneys but only one of eight proximal tubules, supporting a selective presence in the distal nephron. In contrast, Dominguez et al. (12–15) have provided strong evidence for the expression of the sodium-calcium exchanger in rat proximal tubules, as discussed above. To further investigate the spatial distribution of the sodium-calcium exchanger along the nephron, we reacted sections of dog kidneys fixed by freeze-sub-
stitution with two different polyclonal antisera specific for the canine cardiac sarcolemmal sodium-calcium exchanger.

METHODS

Animals

This work was conducted in accord with the NIH Guide for the Care and Use of Laboratory Animals. Three female and three male mongrel dogs (16 to 23 kg) were maintained on a standard canine feed (Field and Farm; Purina, St. Louis, MO). They were anesthetized with sodium pentobarbital, 33 mg/kg, and an abdominal incision was made that exposed both renal arteries. One renal artery was cannulated and perfused with Lactated Ringer's Injection USP (McGaw, Irvine, CA) at room temperature until the kidney blanched (200 to 300 mL; 3 to 5 min). This kidney was removed rapidly, and 1- to 2-mm-thick slices perpendicular to the long axis were cut and frozen quickly (vide infra). The second kidney was handled similarly.

Tissue Processing

The tissue slices were frozen rapidly in isopentane maintained at its melting point (−160°C) in liquid nitrogen. They were transferred without thawing to absolute ethanol (−75°C) and fixed by freeze-substitution for 2 wk (19). Thereafter, the slices were brought to room temperature and immersed successively in absolute ethanol (1 h), acetone containing 8 g of Drierite (W.A. Hammond Drierite Company, Xenia, OH)/L (two changes, 0.5 h each), paraffin (Paraplast Plus; Stat Labs Medical Products, Dallas, TX) at 60°C for 1 h, and fresh paraffin for 2 h. After paraffin embedding, microscopic sections (6 µm) of the tissue were cut, floated briefly on water, attached to microscope slides, and dried. Before immunocytochemistry, the paraffin was removed from the sections by successively immersing the slides in toluene (two changes, 10 min each), 50–50 (vol/vol) toluene–absolute ethanol (5 min), and ethanol-water solutions of decreasing ethanol concentration: 100, 95, 75, and 50% (two changes, 2 min each). Finally, the sections were rinsed with phosphate-buffered saline (10 min) and were treated with 3% H2O2 in phosphate-buffered saline (10 min) to reduce endogenous peroxidase activity.

Immunohistochemistry

Two different polyclonal antisera raised by immunizing rabbits with purified canine cardiac sodium-calcium exchanger were obtained: one from Dr. Kenneth D. Phillipson et al. (20) and the other from the laboratory of Dr. George E. Lindenmayer (21). The exchanger was immunostained with each antiserum by a modification of a previously published procedure (22). Sections were incubated at room temperature for 30 min in Tris-buffered saline (TBS) containing 1% normal sheep serum and 2% BSA (pH 7.6; SA-TBS). The primary antiserum, rabbit anti-dog cardiac sarcolemmal sodium-calcium exchanger, was diluted 1:1,000 in TBS (pH 7.6) and placed over the tissue sections in a humid chamber at 4°C overnight. The sections were brought to room temperature for successive incubations in SA-TBS (30 min), biotinylated donkey–anti-rabbit γ-globulin (1:70 in TBS; 1 h), SA-TBS (30 min), streptavidin conjugated to horseradish peroxidase (1:70 in TBS), TBS (2 h), and 0.02% 3,3′-diaminobenzidine tetrahydrochloride in TBS (5 min). After being rinsed with TBS, the sections were counterstained with either periodic acid–Schiff reagent/hematoxylin or hematoxylin and eosin (23).

RESULTS

Immunolocalization of the sodium-calcium exchanger in canine kidney with the two different antisera to dog heart sarcolemmal exchanger was identical. Connecting tubule cells containing the sodium-calcium exchanger stained brown by the avidin-biotin procedure, as shown in Figure 1. The brown staining was specific for the sodium-calcium exchanger protein because it was absent from the same cells of adjacent immunocontrol sections (Figure 2). The

Figure 1. Immunostaining (brown) of dog kidney cortex (capsule at top). In addition to prominent labeling of cells in the centrally located connecting tubule, several surrounding connecting tubules also stain positively (arrowheads). Immunostaining was absent in the cells of proximal convoluted tubules (red brush borders). Periodic acid–Schiff/hematoxylin counterstain. Scale bar, 100 µm.
majority of connecting tubule cells stained brown, and the staining was localized over the basal poles of these cells (Figure 3). Staining for the sodium exchanger was observed primarily over connecting tubules. However, faint immunostaining was present infrequently over the basal aspects of proximal convoluted tubule cells (Figure 3A). Distal convoluted tubules (arising a short distance beyond the macula densa) did not stain for the sodium-calcium exchanger nor did cortical collecting ducts (Figure 4). Staining for the sodium-calcium exchanger was absent from cells in the renal corpuscle, Henle’s loop, and medullary collecting ducts.

DISCUSSION

Reilly and Shugrue (9) used the polymerase chain reaction and library screening to clone a cDNA for a rabbit kidney sodium-calcium exchanger on the basis of homology with the canine cardiac sarcoldemal sodium-calcium exchanger cDNA sequence (8). The mRNA for the exchanger was found in the cortex but not in the medulla of the rabbit kidney. Subsequently, Reilly et al. (24) reported that a monoclonal antibody raised against the purified canine cardiac sodium-calcium exchanger selectively labeled the basolateral surface of rabbit connecting tubule (but not intercalated) cells. Yu et al. (10) localized mRNA for the sodium-calcium exchanger to the distal convoluted tubule of rat kidney (vide supra). Because the transition between the distal convoluted tubule per se and the connecting tubule in the rat is gradual (25) and because the latter portion of the distal tubule in the rat corresponds to the connecting tubule, it is reasonable to speculate that the three tubule segments in which mRNA for the sodium-calcium exchanger was not found correspond to the distal convoluted tubule proper and that the five in which it was present correspond to the connecting tubule.

In this study, the sodium-calcium exchanger was localized in canine kidneys by immunostaining with rabbit polyclonal antibodies directed against purified dog cardiac sarcoldemal sodium-calcium exchanger. In agreement with the observations of Reilly et al. (24), the exchanger was located primarily in the basal poles of connecting tubule cells. These results confirm physiologic studies demonstrating the presence of sodium-calcium exchange activity in the basolateral cell membranes of connecting tubules (18) and are consonant with the findings of Ramachandran and Brunette (17) and Yu et al. (10) that the renal sodium-calcium exchanger is located predominantly in the distal tubule.

Because there is strong evidence for the presence
Figure 3. Basal poles of most connecting tubule cells stain brown for sodium-calcium exchanger. Unstained cells are indicated by arrows. Brush borders of proximal convoluted tubules stain reddish purple (*), distal convoluted tubule. (A) Tail of lower arrow overlies a cortical radial artery. Faint immunostaining may be present over the basal aspects of two proximal convoluted tubules (arrowheads). (B) Cortical radial artery is located in the right lower corner. Periodic acid–Schiff/hematoxylin counterstain. Scale bars, 50 μm.

Figure 4. Two examples (A and B) of transitions from distal convoluted (d) to connecting tubules (c). p, proximal convoluted tubule; d, cortical collecting duct; g, glomerulus; t, cortical thick ascending limb of Henle’s loop. Hematoxylin and eosin counterstain. Scale bars, 50 μm.
of a sodium-calcium exchanger in the proximal tubule (12–15) and reasonable evidence for the presence of sodium-calcium exchange activity in the thick ascending limb of Henle’s loop (26), the distal convoluted tubule (27), and the cortical collecting duct (28,29), it is important to question why we failed to detect this antiporter in these nephron segments. Lack of staining could result for several reasons. Two major possibilities are the existence of different isoforms of the exchanger in different tubular segments or differences in the numbers of antiporters in each cell type. Because the cDNA for the sodium-calcium exchanger that were isolated from rat proximal (15) and distal (10) tubules were homologous to the cDNA for the rat and dog cardiac sarcolemmal sodium-calcium exchanger, respectively, it seems unlikely that different isoforms of the exchanger that are unrecognized by our antisera are present. Accordingly, we speculate that the number of sodium-calcium antiporters in the cells of these other nephron segments is smaller than that in connecting tubule cells, which is supported by the faint immunostaining observed over the basal poles of some proximal convoluted tubule cells. A definitive answer to this question will require further experimental investigation.

In conclusion, this study demonstrates that a sodium-calcium exchanger related antigenically to the cardiac sarcolemmal sodium-exchanger exists in the basal poles of connecting tubule cells in dog kidney.

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REFERENCES

22. Iacopino AM, Christakos S, Modl P, Altar CA:


I was in Richard’s office one summer afternoon in 1941. The phone rang. A brief conversation ended, “Yes, I will see you.” For a long time he sat in silence looking down at his desk. Then he raised his head and said, “I must be a fraud. I deceive people. Why else does he think I can do the job.” Vannevar Bush had called to ask him to serve as chairman of the Committee on Medical Research (C.M.R.) in the Office of Scientific Research and Development (O.S.R.D.). Richards did, of course. His stern sense of duty forced him.”

“In 1941, Howard Florey, later Lord Florey, president of the Royal Society, came to Richards to discuss the possibility of making penicillin synthetically. Florey had worked with Richards in Philadelphia many years before, and they now began a British-American cooperative venture which, under Richard’s insistence, promoted the production of penicillin by natural fermentation with cultures of Fleming’s original mold. Consequently, by 1944, there was enough penicillin from American and British sources to treat the casualties of the Normandy Invasion.”