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Visualization of Tubular Interspaces in the Kidney with the Aid of Lanthanum

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Ion and water reabsorption by the kidney tubular wall is conceived as occurring mainly across the cells proper, transcellular pathway(1,2). In addition, the existence of a pathway in parallel to this transcellular pathway—a paracellular pathway—has been proposed(3–5) based mainly on the observation that in *Necturus* proximal tubule the measured transtubular resistance is, at least, 10 times smaller than the sum of the luminal cell membrane resistance plus the peritubular cell membrane resistance. Measurements pointing out the possible existence of a paracellular shunt have also been obtained in the rat and in the dog proximal tubule(4,6).

An attempt has been made to localize these paracellular pathways using lanthanum as an extracellular marker for electron microscopy in the perfused toad kidney. La $(NO_3)_3$ is soluble in water at pH lower than 7.5, while lanthanum sulfate, phosphate and bicarbonate are insoluble in water and in acetone. In the amphibian kidney, a solution perfused via the aorta filters through the glomerulus and bathes the tubular lumen. A solution perfused via the portal vein bathes mainly the peritubular spaces. Therefore, the perfusion of the kidney through the aorta with a solution containing $La(NO_3)_3$ and through the portal vein with a solution containing SO_4 (or vice versa) should result in the precipitation of insoluble $La_2(SO_4)_3$ at the sites where the two solutions meet and the electron-dense precipitate can be localized later with the electron microscope. The present paper is part of a more extensive study(7).

MATERIALS AND METHODS

Kidneys of *Bufo marinus* were perfused as previously described (8). Briefly, we cannulated anterior abdominal vein (which drains into the renal portal vein) and

aorta for inflow of perfusion fluids, and the postcaval vein for outflow of perfusion fluids. The ureters were also cannulated to collect the urine. Three perfusion fluids were used with a pH of 7.3–7.4 and an osmolality of 200–205 mOsm/kg. They all contained (in mmoles/liter) 3.5 KCl, 1 $CaCl_2$, 1 $MgCl_2$, 1 glucose. In addition, fluid A contained 86 NaCl, 16 $NaHCO_3$, 10 Na–acetate; fluid B contained 86 NaCl and 20 $La(NO_2)_3$, and fluid C contained 43 Na_2SO_4, 16 $NaHCO_3$, 10 Na–acetate, 1.6 Na_2HPO_4, 0.4 NaH_2PO_4, and 33 mannitol.

All experiments were begun by perfusing the kidneys with fluid A through both the aortic and portal circuits for 1 hr. Afterwards, fluid B was perfused via the aorta in order to bathe the tubular lumen with La^{3+} and fluid C was perfused via the portal vein and thus bathed the peritubular spaces with SO^{2-}_4 and the other lanthanum-preciptating anions.

After perfusion the kidneys were removed and immersed in buffered glutaraldehyde solution, then washed in solution C buffered with Na–cacodylate (pH 7.3–7.4). Afterwards, the tissue was dehydrated, infiltrated, and embedded in Epon 812. Pale-gold sections were double stained with uranyl acetate and lead citrate and examined with a Siemens Elmiskop 1A electron microscope.

RESULTS AND COMMENTS

Lanthanum was found to have filled about 90% of the tight junctions examined and 100% of the intercellular spaces of the proximal tubules (Fig 1a and b). The concentration of the intercellular precipitate was higher toward the tight junction than toward the basement membrane. Some lanthanum precipitate was also seen in the basement membrane. Occasionally a few vacuoles containing only small quantities of lanthanum were observed within the cell.

These observations indicate that La^{3+} and SO^{2-}_4 crossed the tight junctions and the intercellular spaces so that these ions could meet and precipitate there. It is felt that this conclusion can be safely reached because the following three alternative possibilities were ruled out. First, that La^{3+} could have precipitated in the intercellular spaces by entering backward from the peritubular capillaries (due to admixture of aortic and portal circulations) rather than by crossing the tight junctions. That the intercellular precipitate is less dense near the basement membrane argues against the possibility. Second, that $La_2(SO_4)_3$ precipitated in the intercellular spaces after La^{3+} had crossed the cell (and not the tight junction). This appears very unlikely in view of the virtual absence of La^{3+} from all cytoplasm.

Finally, the possibility was ruled out that perfusion of the kidney under the conditions used in this work opened the tight junctions allowing the abnormal movement of lanthanum through the tight junctions. Thus (a) No significant admixture of SO^{2-}_4 from the peritubular circulation into the lumen was observed as evidenced by continued transparency of the urine samples during perfusion. Turbidity would have resulted from the formation of $La_2(SO_4)_3$. (b) Urine-to-plasma sodium concentration ratios averaged 0.57 $\pm$ 0.03 (SEM, $n = 9$)

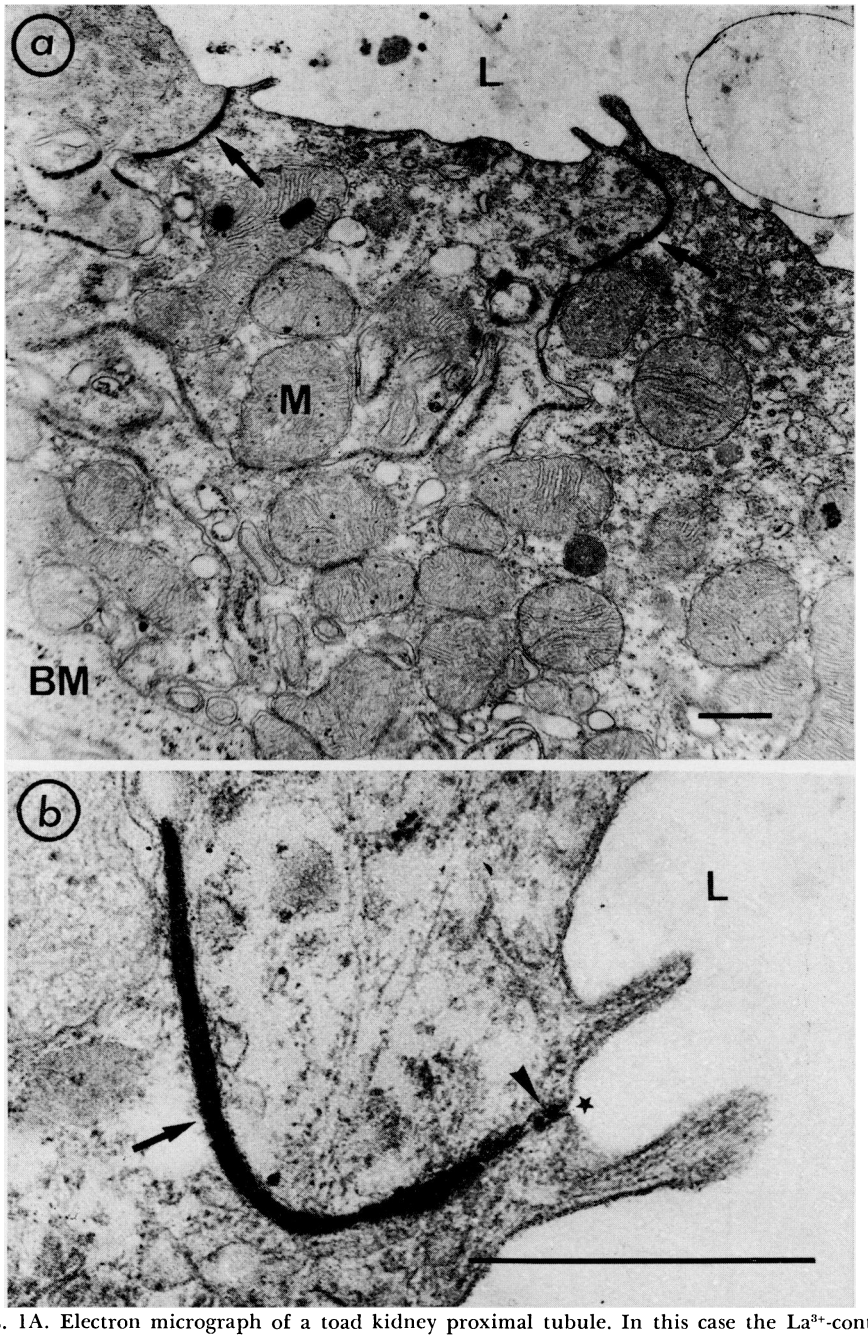

FIG. 1A. Electron micrograph of a toad kidney proximal tubule. In this case the La^{3+}-containing solution bathed the tubular lumen (L) and the SO_4^{2-}-containing solution bathed the peritubular space. Lanthanum precipitated along the intracellular space with the higher density toward the lumen (arrow). Lanthanum can also be seen precipitated in the basement membrane (BM). M, mitochondria. The bar designates 1μ. Magnification 9800×. B. Higher magnification of the luminal end of the intercellular space, showing lanthanum precipitated at the level of the tight junction (arrow head). Magnification 46,200×.

when urine-to-plasma creatinine concentration ratios averaged 1.20. This indicates that use of La^{3+} and SO^{2-}_4 did not impair tubular Na reabsorption to a greater degree than might be expected from the use of SO^{2-}_4(9). Also, lanthanum produce no deleterious effects in frog skin(10,11).

Since other investigators have used lanthanum (without sulfate) as an extracellular marker(10–12) it was important to examine whether the use of SO^{2-}_4 and the other anions (solution C) in addition to La^{3+} is advantageous over the use of La^{3+} alone. With this in mind another series of experiments was performed in which only the lanthanum-containing solution was perfused through the kidney. It was found that no lanthanum precipitated in the tight junctions and only a few intercellular spaces showed a small amount of lanthanum precipitate. Tissue lanthanum determinations indicated that, in these experiments, the lanthanum content of the tissue was 10 times lower than in the first series of experiments. These findings clearly show the advantage of precipitating La^{3+} with SO^{2-}_4 by using fluids B and C in a single experiment.

That La^{3+} permeates the tight junctions provides strong evidence that these junctions represent the sites of the high-conductance pathway measured in the proximal tubule (3–6).

The electrophysiological studies (3–6) indicate that the paracellular shunt is greater in the proximal than in the distal tubule and that its magnitude increases in the proximal tubule during saline loading(4). The tight junction permeability also seems to vary in amphibian skins. Thus, barium(13) and lanthanum(10,11) do not penetrate them under normal conditions. However, tight junctions open reversibly when hypertonic urea solutions are applied to the outside surface of the skin(10,11) or when hydrostatic pressure is applied across the epithelium in the outward direction(14). These observations indicate that the magnitude of the paracellular shunt varies from one epithelium to another and within the same epithelium according to the experimental conditions. It is tempting to speculate that the paracellular shunt may constitute a fine regulating mechanism in transepithelial transport of ions and water.

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