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THE TRANSEPITHELIAL SHUNT PATHWAY IN THE RENAL PROXIMAL TUBULE OF NEWT KIDNEY

TAKEO MARUYAMA

Department of Applied Physiology, Tohoku University School of Medicine, Sendai 980 (Japan)

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Summary

The transepithelial shunt pathway of newt proximal tubule was examined with glass micro-electrode and electron microscopic methods. The input resistance of the peritubular (basal) membrane and tubular wall were found to be $4.2 \pm 0.1 \cdot 10^6$ (mean ± S.E., $n = 16$) and $11.4 \pm 0.2 \cdot 10^4$ ($n = 11$), respectively. The input resistance of the peritubular membrane was approximately 40-times larger than that of the tubular wall. When the kidneys were perfused in a lanthanum solution, the lanthanum ions were then observed in the junctional complexes and in the intercellular spaces on both the basal and apical sides. The results indicate that the electrical shunt pathway corresponds to the apical junctional complexes and the intercellular spaces, and that the tight junctions are not truly 'tight' for the transepithelial movement of small ions in the proximal tubule of the newt kidney.

The renal proximal tubule transports electrolytes, non-electrolytes and water from lumen to peritubular spaces. Whether this transport occurs through the cell body or through an intercellular route such as a paracellular shunt pathway remains a problem. The electrical paracellular shunt pathway in the renal proximal tubule was suggested to be the likely pathway by Hoshi and Sakai [1] in the newt and by Windhager et al. [2] in the Necturus based on the finding that the conductance of the proximal tubular wall was higher than that of the peritubular membrane alone. The same conclusion was reached by Bloupaep and Seely [3] in dog kidney from comparison of the overall epithelial membrane resistance with the peritubular membrane resistance. Further electrical evidence has indicated the existence of a paracellular

shunt pathway in Necturus kidney [4], rabbit gall-bladder [5] and frog choroid plexus [6]. Although electron microscopic observations indicate that the zonula occludens (or 'tight junction') in the renal proximal tubule is impermeable to large molecules like hemoglobin [7], albumin [8] and ferritin [9], the junction complex could still be 'leaky' to small molecules such as water, electrolytes and non-electrolytes [10—12]. The lanthanum ions may also penetrate the tight junction in the renal proximal tubule [13]. We report, here, electrical and morphological studies which were designed to detect the existence of a passive shunt pathway in the renal proximal tubule of newt kidney and to determine its location in the tubular wall.

23 adult male japanese newts (*Triturus pyrrhogaster*) were used in all experiments. The methods of experimentation have been described previously [14]. Micro-injection and electrical measurement of the proximal tubular wall (16 animals) were carried out in the following sequence. First, mineral oil saturated with Sudan black II was injected through a single-barreled glass pipette into one of the Bowman's capsules under a widefield stereomicroscope (Leitz) and illuminated with a glass fiber optic with a tungsten halide light source (Olympus, Japan). The outer diameter of the sharpened tip of the pipette was less than 20 μm. The purpose of this procedure was to block the fluid flow and the electrical current leakage out of the glomerulus. Thereafter, the proximal tubule was punctured by a single-barreled pipette which was filled with Ringer's solution. The outer diameter of the pipette was less than 15 μm. The Ringer's solution used in the present study had the following composition: 105 mM NaCl, 2.65 mM KCl, 1.8 mM $CaCl_2$, 2.25 mM Na_2HPO_4; 0.75 mM NaH_2PO_4, 11 mM D-mannitol, pH 7.2. The osmolality of the solution was approx. 230 mosM per kg H_2O. The input resistance and the potential difference of the proximal tubular wall were measured by the bridge balance method with a single glass micro-electrode: the same glass micro-electrode was used simultaneously for both the intercellular pulse current and potential recording (Fig. 1). Electrodes having electrical resistances of 15—20 · 10^6 Ω and tip potentials less than 5 mV were selected for use. Morphological experiments were made as follows. Kidneys of seven animals were perfused with a test solution (105 mM NaCl, 5 mM $La(NO_3)_3$, 10 mM Tris-HCl buffer, pH 7.2; the osmolality of the solution was approx. 230 mosM per kg H_2O) using the methods of Whittembury and Fishman [15]. The inflows of perfusion pressure of the abdominal artery and the portal vein were approx. 30 cm H_2O and 15 cm H_2O, respectively. After a 5-min perfusion with the fixative, the kidneys were excised, cut into about 1 mm

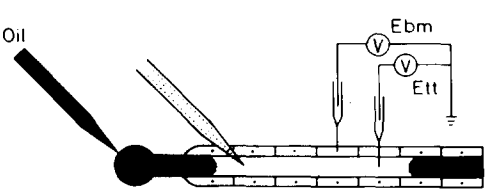

Fig. 1. A schematic presentation of the method for applying a perfusion fluid and recording potential differences. First, colored mineral oil was injected into a Bowman's capsule through a single-barreled micropipette, then the Ringer's solution was injected with constant pressure into the oil column to split it. During the perfusion, both the transmembrane potential across the peritubular membrane and the tubular wall potential were recorded by the bridge balance method with a single glass micro-electrode.

blocks and immersed for 2 h in ice-cold 1% OsO_4 (in 0.1 M cacodylate buffer, pH 7.2). After fixation, the blocks were embedded in Epon 812. Ultra-thin sections were prepared and double stained with 1% uranyl acetate and examined with a Hitachi HS-7S or a JEOL-1000 electron microscope.

The peritubular (i.e., basal) membrane potential and the tubular wall potential during perfusion with the Ringer's solution were -71.8 ± 3.4 mV (mean ± S.E., $n = 16$) and -4.5 ± 0.4 mV ($n = 11$), respectively.

Fig. 2a. shows the potential changes in the peritubular membrane produced by the application of square-wave pulses through an intracellular micro-electrode. There was no significant difference between the depolarizing

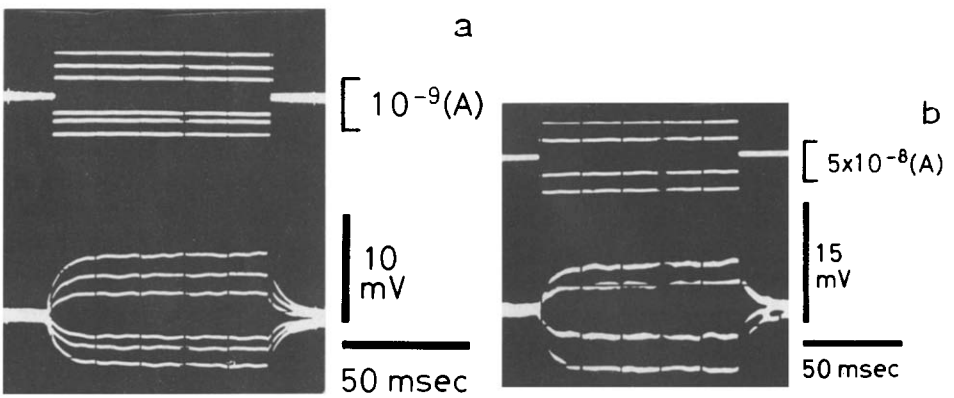

Fig. 2(a). The change in the peritubular membrane potential of the proximal tubular cell of the newt kidney caused by the intracellular application of current pulses. The upper tracing show the injected current and the lower tracings show the evoked membrane potential changes. (b) The changes in the tubular wall potential of the proximal tubule in the newt kidney caused by the tubular wall application of current pulses. The upper tracings show the injected current and the lower tracings show the evoked membrane potential changes.

and the hyperpolarizing responses. From the linearity of the current-voltage relationship, the input resistance of the peritubular membrane was calculated to be $4.2 \pm 0.1 \cdot 10^6\ \Omega$ ($n = 16$). From similar experiments (Fig. 2b), the input resistance of the tubular wall was calculated to be $11.4 \pm 0.4 \cdot 10^4\ \Omega$ ($n = 11$). The input resistance of the peritubular membrane was approximately 40-times larger than that of the tubular wall. These results suggest that a low resistance transepithelial shunt pathway exists in the renal proximal tubule of newt kidney.

In order to determine whether the transepithelial shunt pathway of the proximal tubular wall passes through the cells or through the extracellular spaces, the kidneys were perfused with the lanthanum solution. The lanthanum ions were then observed in the junctional complexes and in the intercellular spaces on the basal and apical sides of the complexes (Fig. 3a, b). However, since there is the possibility that the lanthanum ions may find their way to the cytoplasm and be retained specifically within the intracellular pinocytotic vesicles, a high-voltage electron microscope (JEOL JEM-1000) was employed. The lanthanum was then observed in the center of the entire junctional complex and in the intercellular space, whereas the lanthanum could not be detected within the cytoplasm or the intracellular vacuoles (Fig. 4). The fact

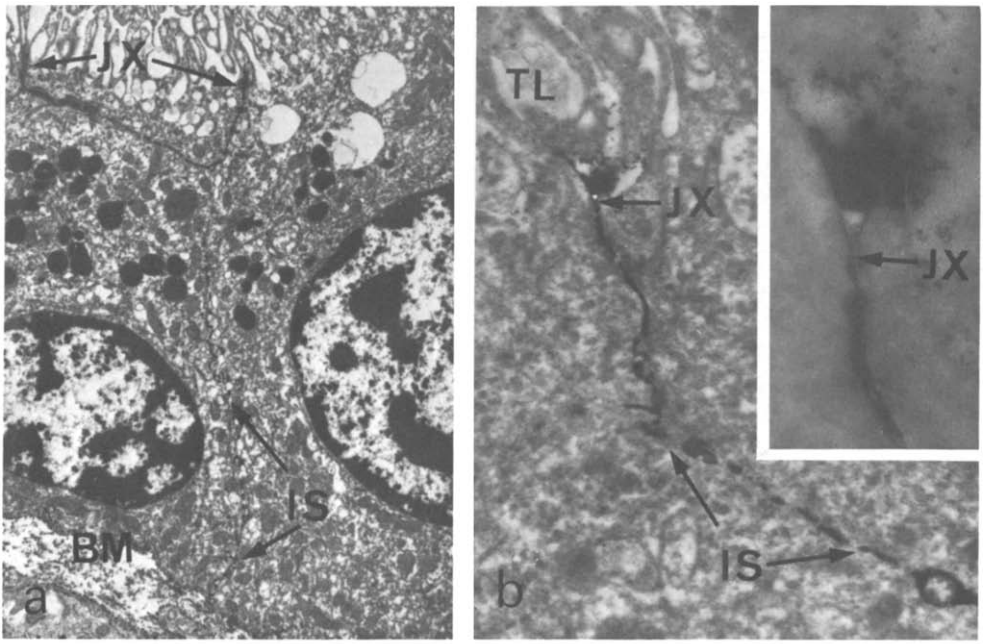

Fig. 3(a). This micrograph illustrates part of the tubular wall in the tissue treated with the lanthanum solution. Lanthanum is observed in the junctional complex (JX) closely associated with the cell membrane, in the intercellular space (IS) and within the basement membrane (BM) (magnification, X 2000). The section was stained with uranyl acetate and lead citrate. (b) This micrograph demonstrates the apical part of two cells in the tissue treated with lanthanum solution. Lanthanum is seen in the intercellular spaces (IS), in the junctional complex (JX) and in the tubule lumen (TL) (magnification, X 16 500; right insert, X 98 000. The section was stained with uranyl acetate and lead citrate.

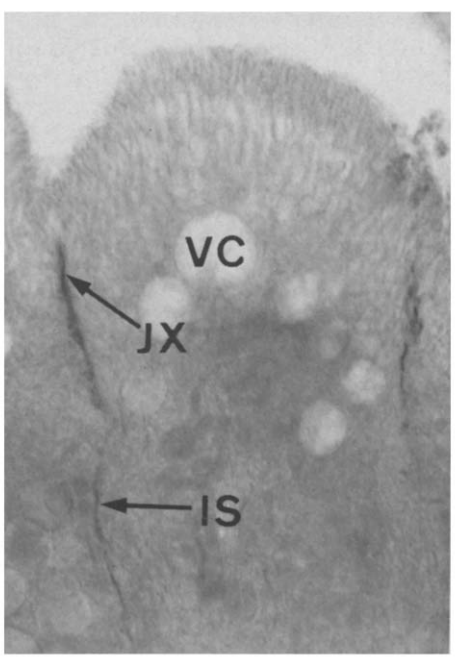

Fig. 4. This micrograph shows parts of the wall in the tubule in tissue treated with the lanthanum solution. Lanthanum is present in the junctional complex (JX) and in the intercellular spaces (IS), but is not observed in the cytoplasm or vacuoles (VC). Unstained section, 2 μm thick (magnification, X 3000).

that the lanthanum ions permeate the junctional complexes provides strong evidence that the junctional complexes and the intercellular spaces represent the sites of the low electrical transepithelial shunt pathway in the renal proximal tubule of newt kidney.

In transporting epithelia, e.g., the proximal tubular epithelium and gallbladder, the transport of fluid and solutes takes place mainly by the active transport of sodium into the intercellular spaces, where a local high concentration of sodium results in the movement of water into the intercellular spaces [16]. The flow of fluid is directed towards the peritubular spaces, since the tight junction restricts passage of fluid into tubule lumen [16]. Electron microscopic observations suggest that a low transepithelial electrical conductance in tissues is associated with well-developed tight junctions and a high transepithelial conductance with poorly-developed junctions [11]. In the proximal tubule of newt kidney, the high transepithelial conductance can be ascribed to the existence of a low-resistance intercellular shunt pathway allowing the ionic current to bypass the luminal, lateral and basal cell membranes [18].

In these experiments, we have demonstrated that the conductance of the tubular wall is 40-times higher than that of the peritubular membrane and that the lanthanum ions are able to penetrate the junctional complexes. The results strongly suggest that the electrical shunt pathway may correspond to the apical junction complexes and the intercellular spaces and that the 'tight' junctions are not truly tight for the transepithelial movement of small ions in the proximal tubule of the newt kidney. The difference in the electrical resistance of the transepithelial shunt pathway in various epithelial tissues may reflect the difference of permeability in the zonula occludens.

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