ELECTROPHYSIOLOGICAL INVESTIGATION OF THE RAT NEPHRON

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Electrophysiological investigations of the nephron of the rat kidney showed that its proximal portion has a low transtubular potential (−5.6 mV) and a low effective electrical resistance of the tubule wall (235 kΩ) by contrast with the distal tubule, with a tubular potential of −24.5 mV and a resistance of 840 kΩ. The results are evidence of higher ionic permeability of the proximal portion of the nephron. Active sodium transport was investigated in that segment by the short-circuit current method; its value was $8.0 \cdot 10^{-9}$ A. Marking the portion of the nephron investigated with Evans' blue enabled the position of the microelectrode to be located with an accuracy of several microns.

One of the most important functions of the mammalian kidney is to regulate water and salt metabolism. The transport of the principal minerals of the internal milieu of the organism by the cell systems of the renal epithelium and the phenomena of ionic permeability of biological membranes can be studied by electrophysiological microelectrode research methods. From investigations carried out by others and from results obtained by the present writers it is clear that this method can be used successfully for the further study of kidney function at the cellular level of organization.

This paper describes a study of the electrophysiological characteristics of the renal epithelium of a single nephron of the rat kidney.

EXPERIMENTAL METHOD

Albino rats weighing 200-400 g were anesthetized with thiopental (50 mg/kg). Tracheotomy was carried out and a catheter introduced into the external jugular vein for intravenous infusion. Through a midline laparotomy incision the left kidney was placed in a dish without stretching its vessels and immersed in 2% agar in 0.9% sodium chloride solution, while a polyethylene catheter was inserted into the left ureter to obtain a free flow of urine.

To measure the electrical characteristics of the renal tubules glass microelectrodes with a tip about 1-2 μ in diameter, filled with 3 M KCl solution, were used. In some experiments electrodes filled with a 5% aqueous solution of Evans' blue were used, for the dye is precipitated in potassium chloride solution. Potentials were recorded by a V2-11 high-ohmic, high-sensitivity dc amplifier and recorded graphically on the automatic writer of a type EPP-09 dc electronic potentiometer. The effective resistance of the wall of the renal tubule and the short-circuit current were measured by the method of Eigler and Krager [4-6] using a single intratubular microelectrode.

To determine the location of the electrode, Evans' blue was injected iontophoretically at the end of the experiment, so that the location of the microelectrode could be determined visually. The kidney was embedded in paraffin wax, sections were cut to a thickness of 5 μ, and they were studied under the microscope (400-600×) to locate the marked portion of the nephron. Micropuncture of the superficial renal

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TABLE 1. Electrophysiological Characteristics of Proximal and Distal Portions of Renal Tubules of Rats (M ± m)

Index studied	Proximal portion	Distal portion
Transtubular potential (in mV)	—5,6±0,3	—24,5±1,5
Transepithelial resistance (in MΩ)	0,235±0,020	0,840±0,070
Short-circuit current ($\times 10^{-9}$ A)	8,0±0,7	—

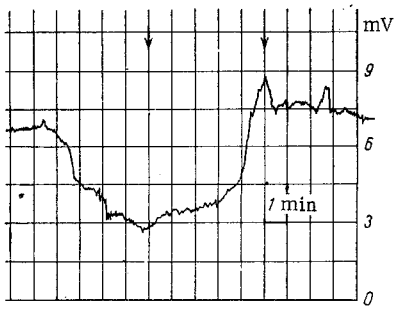

Fig. 1. Fall of transtubular potential of renal tubule on clamping renal artery for 5 min (reading from right to left).

tubules was carried out under the control of the MBS-2 stereoscopic microscope under a magnification of 90-120×.

EXPERIMENTAL RESULTS

The surface of the kidney consists of a mass of convoluted tubules. The proximal tubules were differentiated from the distal by intravenous injection of indigocarmine 20 min before the beginning of the experiment [3] – the distal tubules thereby acquire a deep blue color while the proximal portions remain colorless. By intravenous injection of 0.05 ml of 5% Lissamine green solution [9] the proximal portion of the nephron could be quickly identified, for this dye appeared in the lumen of the tubule on account of ultrafiltration. The dye was injected only once at the end of the experiment because of its ability to inhibit proximal reabsorption of fluid and electrolytes [8].

Introduction of the microelectrode into the lumen of the tubule was accompanied by recording of a transtubular potential (the reference electrode was applied to the kidney surface). It was extremely difficult to record a stable transtubular potential in the proximal tubule; the stable potential recorded for 1-2 min was taken as an adequate measurement. Sometimes a potential could be recorded in the distal portion of the nephron for up to 30 min. Disturbance of the renal blood flow was accompanied by a marked decrease in the transtubular potential, which returned to normal when the circulation was restored (Fig. 1).

Investigation of the electrophysiological characteristics of the nephron showed that the proximal tubule differs from the distal in having a lower transtubular potential and transepithelial resistance (Table 1). These results indicate the higher ionic permeability of the wall of the proximal portion of the nephron, where most of ultrafiltrate is reabsorbed.

Active transport of sodium can be studied in the proximal tubule by the short-circuit current (SCC) method. In this portion of the nephron iso-osmotic reabsorption of fluid takes place and concentration gra-

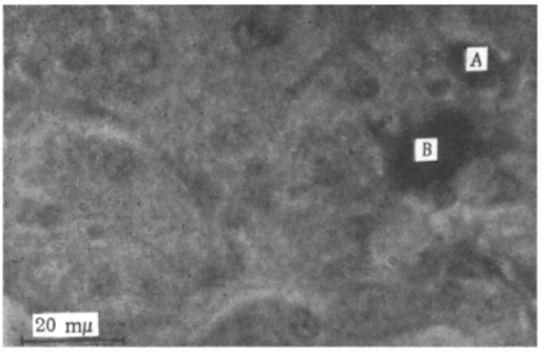

Fig. 2. Unstained section through rat kidney, 5 μ in thickness, 400×. Explanation in text.

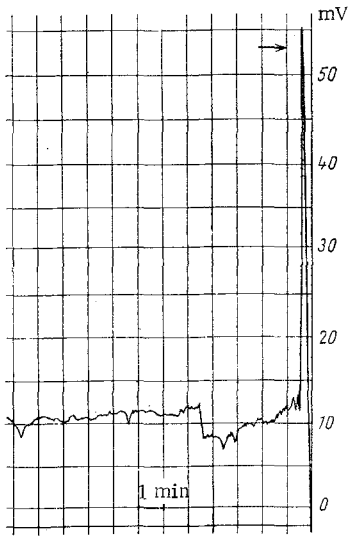

Fig. 3. Peritubular (marked by arrow) and transtubular potentials of renal epithelium (reading from right to left).

dients for most ions through the tubule wall are absent. With these facts in mind, and also the disappearance of the electrical gradient at the time of the short circuit of the tubular epithelium, it can be taken that factors (electrical and concentration gradients) determining the passive ion transport are absent. Under these conditions active transport of the sodium ion only takes place and the SCC is a criterion of the active transport of the ion through the tubule wall [4]. In experiments to study active sodium transport in the proximal tubules of the rat kidney it was found that the results obtained by the SCC method are similar to those obtained by other methods (calculation, the use of the radioactive sodium isotope [7]). The SCC was studied only in the proximal tubule, for in the distal portion of the nephron there are considerable concentration gradients for sodium and other ions, preventing active sodium transport to be investigated adequately by this method. In this portion of the nephron the electrical gradient in the present experiments varied from −10 to −65 mV, as a result of changes in the magnitude of the transtubular potential along the length of the nephron [11]. An increase in the potential was found from the beginning to the end of the distal tubule of the rat kidney.

An important aspect of the investigation was the determination of the position of the microelectrode tip relative to the lumen of the tubule in order to avoid recording cell (membrane) potentials. During penetration of the tubule wall a high-amplitude potential of between −50 and −70 mV often was recorded, together with high electrical resistance – about 2-3 mΩ. These values characterize electrical phenomena through the peritubular membrane, which separates the contents of the cell from the interstitial space. The small size of the cell (5-10 μ) prevents prolonged recording of the transmembrane phenomena [10]. The method of iontophoretic injection of Evans' blue was used to facilitate visual identification of the position of the electrode and subsequent histological confirmation. Escape of the dye into the tubule was accompanied by filling of the lumen of the nephron and movement of the tubular fluid with the current along the length of the nephron, while if the microelectrode was intracellular in position only a local contrasting spot could be seen around the tip of the electrode. A characteristic feature of the intracellular localization was a high-amplitude potential of −55 mV (Fig. 3); a spot could be seen in the section in the wall of the tubule (Fig. 2A). If the microelectrode lay in the lumen of the tubule a stable tubular potential of −12 mV was recorded (Fig. 3); filling of the lumen of the tubule with the dye can be seen in the section (Fig. 2B). By this method the position of the electrode in that portion of the nephron studied can be identified with an accuracy of several microns.

The use of microelectrodes with Evans' blue is suitable for recording bioelectrical potentials. Passage of a current through the electrode during iontophoretic injection of the dye did not significantly change the diffusion potential of the microelectrode and the transtubular potential of the renal tubule. Recording of the potential was regarded as adequate if, after injection of the dye, the tubular potential and diffusion potential of the electrode tip, measured in 0.9% sodium chloride solution surrounding the kidney, did not change by more than 1 mV. Microelectrodes with the dye have a higher internal resistance which may vary during the passage of an electric current, thus complicating the measurement of the SCC and the effective electrical resistance of the tubule wall. To investigate a combination of electrophysiological characteristics of the nephron electrodes filled with 3 M sodium chloride solution were used.

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