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Insertion of an Axial Electrode into Renal Proximal Tubule*

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INTRODUCTION

The renal proximal convoluted tubule of the amphibian, *Necturus maculosus,* carries out isosmotic reabsorption of sodium chloride and water, and concomitantly maintains a transepithelial electrical potential difference (PD), with tubular lumen some 20 mV negative to the peritubular extracellular fluid(6). Understanding how this transepithelial PD influences the reabsorption of salt and water is fundamental to our knowledge of renal tubular function in particular, and of epithelial transport processes in general. A method is here described which permits systematic alteration (voltage-clamping) of transepithelial PD in long, straight segments of *Necturus* proximal tubule, and simultaneous measurement of salt and water reabsorption in the same segments. The method involves placing an axial wire electrode, through which current can be passed from an external source, in the lumen of the tubule (average i.d. 110 μm). Neurophysiologists have long made use of such axial wire electrodes to voltage clamp large areas of squid axonal membrane, but voltage-clamp analysis of cylindrical structures less than several hundred microns in diameter has been impeded by difficulties in the placement of an axial electrode.

Guard electrodes, which are necessary in experiments on squid axon to assure uniform current density near the ends of the voltage-clamped region, are not needed in renal tubular voltage clamps because of the presence of insulating columns of oil which delimit a droplet of Ringer's solution within the tubular lumen, as shown in Fig. 1. Current flow is thus restricted to the area containing

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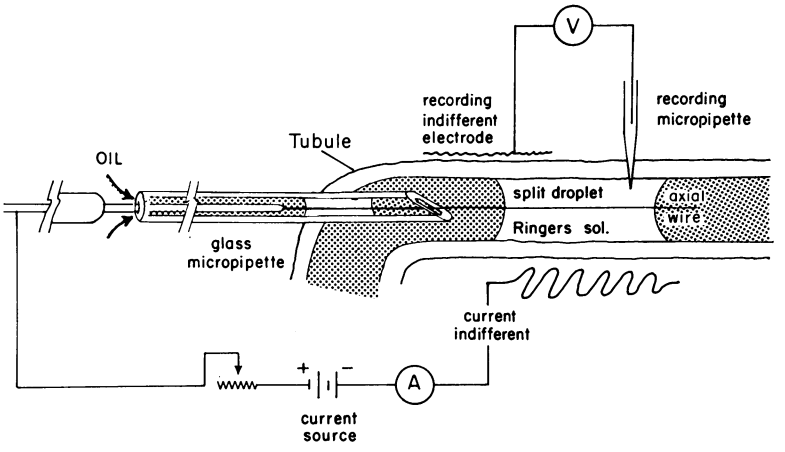

FIG. 1. Electrode arrangement for voltage clamping of an injected droplet. The variable resistor in the circuit enables control of the current so that the potential difference is held at the desired value. The current indifferent electrode is a large platinum wire lying under the kidney; the current record is displayed on an ammeter (A). The axial wire is moved hydraulically across the droplet, and additional droplets may be injected from the micropipet after reabsorption occurs. The voltage-recording micropipet is a 3 *M* KCl-filled glass microelectrode; the recording indifferent is a calomel wick-electrode. V represents an electrometer–voltmeter–oscilloscope combination.

Ringer's solution, and complex electrical fields at the ends of the axial wire are avoided. The indifferent electrode for the current circuit, a platinized silver chloride wire lying under the kidney, is made as large as possible to aid in the development of a uniform electrical field. The axial wire is advanced down the axis of the tubule through the barrel of a sharpened micropipet which has previously penetrated the tubular wall (Fig. 1). The transepithelial PD may now be clamped to any desired value by passage of an appropriate current through the wire, while salt and water reabsorption can be measured simultaneously by direct microscopic observation of the change in length of the droplet of Ringer's solution contained between the oil columns [the "split droplet method" of Gertz(3)].

The apparatus described below is used both to inject oil and Ringer's solution from a micropipet into the tubular lumen, and to advance the axial wire electrode. The apparatus meets the following criteria:

(1) The axial wire must move smoothly, without disturbing the pipet or interrupting perfusate injection, and must not change position upon injection of perfusate.

(2) The axial wire should be nonpolarizable at the expected current densities, rigid enough to support its own weight at unfavorable length to diameter ratios, and easily replaced if damaged.

(3) Electrical connections to the wire must be easily made.

(4) Passage of current through the axial wire must not cause appreciable changes in pH or composition of the tubular contents. The presence of the axial wire in the tubular lumen should not affect the spontaneous rate of fluid transport in the absence of current passage.

METHODS

The apparatus (Fig. 2) consists of two separable units: an anterior chamber, used to inject solutions through the micropipet, and a posterior chamber, a modified syringe, which is used to move the axial wire hydraulically. The anterior chamber, with a female luer connector at one end and a pipet chuck at the other, is filled with 300 centipoise (cP) mineral oil and connected through a side arm to a 20 cc syringe. Pressure applied to the syringe results in injection of oil from the pipet tip or injection of a droplet of Ringer's solution that has been drawn into the pipet prior to its insertion into the tubular lumen. The posterior chamber is a precision-fit 5 cc syringe with the following modifications: A side arm, soldered to the base of the syringe tip, is connected with polyethylene tubing to a 2 cc syringe, and the entire system is filled with 300 CP mineral oil. The 5-cc syringe plunger is drilled out to receive a 14-cm length of 0.72-mm diameter stainless-steel tubing (outer tube), which is cemented to the plunger with 6 cm extending beyond the forward end of the plunger. An "O" ring fitting in the syringe tip allows the outer tube to move in and out freely with the plunger, but prevents the escape of mineral oil from the 5-cc syringe. The "O" ring also prevents the movement of oil from anterior to posterior chamber when the apparatus is assembled. The outer tube acts as a guide for the holder of the axial wire. It has a slightly tapered tip which fits into the lumen of the shaft of the glass micropipet. Electrical connections are made at the rear of the outer tube.

A 22-cm length of 0.315-mm diameter tubing (inner tube) with a 0.13-mm diameter stainless-steel pin soldered into the anterior end and a short length of polyethylene tubing on the posterior end is slid inside the outer tube. The pin fitting enables easy replacement of wires; the platinized wire, soldered into a short length of tubing, slips snugly onto the pin. The polyethylene tube seals the posterior end of the outer tube and prevents reflux of oil from the anterior chamber through the outer tube.

The axial electrode is made from a 2-cm length of 5-μm diameter tungsten

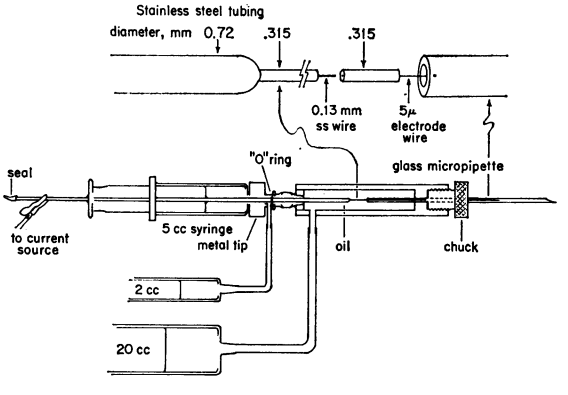

FIG. 2. Apparatus used for electrode insertion. See text for detailed discussion.

wire[1] soldered into a 1-cm piece of 0.315-mm diameter stainless-steel tubing and platinized by a modification of the method of Cole and Kishimoto(2). The wire is washed in distilled water, 70% ethanol, concentrated nitric acid, once again in distilled water, and finally placed in a Kohlrausch salt solution.[2] It is connected to the cathode of a Grass SD5 stimulator (Grass Instrument Co., MA), and a clean platinum wire is used as the anode. Monophasic pulses of 10-msec duration and 4.0-V amplitude are passed at 30 per second through the electrodes for 60–120 sec. Plating current density is 20–30 mA/cm^2 and the total charge deposited ranges from 0.6–1.0 C/cm^2. The initial resistance of the electrode is usually 125 Ω/cm or less. The tungsten wire then displays a dull, gray coating of platinum black with a slight increase in diameter (8–10 μm total diameter).

Large micropipets (o.d. 20–30 μm) are used to puncture the tubule since there must be sufficient clearance in the pipet tip to allow the wire to pass freely. The tubular micropipet is carefully aligned with a long, straight segment of oil-filled proximal tubule, and inserted so that its long axis is parallel to the center of the tubular lumen. The voltage recording electrode, a 3 *M* KCl-filled glass micropipet, is inserted into the tubular lumen and Ringer's solution is injected from the tubular micropipet. The droplet thus formed is moved distal to the puncture site by the injection of oil from the glomerular micropipet. The axial wire is advanced hydraulically across the droplet until it enters the distal oil column. The spontaneous droplet shrinkage rate is recorded prior to the passage of current. A small amount of oil is aspirated into the micropipet tip to effect both a mechanical and electrical seal.

RESULTS

Fluid flux in *Necturus* proximal tubules as a function of transtubular PD has been studied using the equipment described above (manuscript in preparation). The presence of the axial wire and the voltage-recording micropipet in the split droplet did not alter the rate of fluid transport, as indicated by the half-time for reabsorption of an injected droplet of Ringer's solution (Table 1). Spontaneous fluid reabsorption rate and transepithelial PD after passage of current were similar to those values observed in a control period prior to clamping.

The steady-state resistance of the axial wire was unchanged during prolonged (up to 10 min) clamping experiments, in which the current carried by the wire was as high as 1 mA/cm^2. The source voltage necessary to pass currents of up to 2×10^{-7} A was generally 1 V or less. Occasionally currents were observed that were exceptionally high for a given change in transepithelial PD. Such high currents could usually be reduced by a slight repositioning of the wire. These high currents were attributed to shorting caused by contact between the axial electrode and the microvilli of the tubular cells, especially in the immediate vicinity of the puncture site.

[1] General Electric Co., Tungsten Process, Cleveland Wire Plant, Cleveland, OH.

[2] 3% Platinic chloride, 0.025% lead acetate, 0.025 *N* hydrochloric acid.

DISCUSSION

The physical and electrical properties of the axial wire electrode are of primary concern in the application of voltage-clamp techniques to small tubules. Tungsten is the material of choice because of its rigidity. Neither pure silver nor platinum wire has sufficient rigidity, at the requisite length to diameter ratios, to act as an axial electrode in this system. The tungsten wire must be plated to eliminate electrode polarization. Practical nonpolarizable surfaces are platinum black and chlorided silver. However, a chlorided silver surface may be toxic to the tissue under study and exhibits high electrical impedance(4). The platinized electrode, prized for its superior electrical characteristics, may cause pH shifts and the generation of gas (hydrogen, oxygen or chlorine)(5).

The platinized electrode has performed well in voltage-clamp studies of the proximal tubule. The platinum black layer adheres reasonably well to the tungsten wire and does not flake off as the wire passes through the micropipet tip. Gas generation, which must be prevented since it not only damages the tubule cells but also precludes accurate determination of split droplet volume, could be avoided by preventing contact of the wire with the tubular epithelium and by keeping the source voltage below 2 V. The slow generation of acid (HCl or hypochlorite) at the anode and base (NaOH) at the cathode of the platinized electrodes does not seem to interfere with tubular function since flux rate and PD returned to control values after each clamping period. The buffering capacity of the tubular and extracellular fluids, together with the relative rapidity of ionic movements in this system should combine to minimize pH changes.

The application of voltage-clamp techniques to smaller cylindrical structures than ever before seems possible. The tubules must be straight enough to permit the insertion of the wire, and the tubular wall rigid enough to be punctured by a sharpened micropipet. Thus, the split-drop voltage-clamp method may prove suitable for mammalian proximal tubules, mammalian salivary ducts, and large axons. The versatility and power of the voltage-clamp approach can now be utilized in the analysis of ionic fluxes and of membrane properties of these and other tubular structures.

TABLE 1

PROXIMAL TUBULAR FLUID REABSORPTION MEASURED BY SPLIT-DROPLET METHOD

	Number of observations	Half-time (min)	
Control (no axial wire)	8	29.5 ± 7.2[a]	
			$P>0.6$[b]
Axial wire and microelectrode in place	20	31.4 ± 10.1	
Transepithelial PD clamped to Zero	15	13.7 ± 2.4	

[a] Mean ± SD.

[b] t test for differences between sample means.

In summary, a new technique is described which enables voltage clamping of long, straight segments of *Necturus* proximal tubule, *in vivo*, and simultaneously determining volume flux by the split-droplet method. Current is passed from an axial electrode, consisting of a platinized, 5-μm diameter, tungsten wire, which is advanced hydraulically through the barrel of the tubular pipet across the split droplet. The wire is in contact with oil throughout its length except in the area of the droplet, restricting current flow to this area and eliminating the need for guard electrodes. Procedures for the fabrication of axial electrodes and details of the construction of the apparatus used to advance the axial wire are given. The presence of the axial wire in the tubular lumen does not significantly alter the spontaneous rate of fluid transport, and prolonged current passage apparently does not damage the tubular epithelium. It is suggested that this technique may be utilized in voltage-clamp analysis of many tubules previously considered too small for such experiments.

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REFERENCES

1. Cole, K. S., *Membranes, Ions and Impulses,* pp. 242–244. Univ. of California Press. Berkeley, 1968.
2. Cole, K. S., and Kishimoto, U., Platinized silver chloride electrode. *Science* **136,** 381–382 (1962).
3. Gertz, K. H. Transtubuläre Natriumchloridflüsse und Permeabilität für Nichtelektrolyte im proximalen und distalen Konvolut der Rattenniere. *Pflügers Archiv.* **276,** 336–356 (1963).
4. Moore, J. W., and Cole, K. S., Voltage clamp techniques. *in Physical Techniques in Biological Research, Vol. 6* (W. L. Nastuk, ed.), Academic Press, New York, 1963.
5. Watlington, C. O., Smith, T. C., and Huf, E. G., Direct electrical currents in metabolizing epithelial membranes. *in Experiments in Physiology and Biochemistry* (G. A. Kerkut, ed.), Vol. 3, pp. 49–159. Academic Press, New York, 1970.
6. Windhager, E. E., and Giebisch, G., Electrophysiology of the nephron. *Physiol. Rev.* **45,** 214–244, 1965.