TRANSEPITHELIAL VOLTAGES AND RESISTANCES ACROSS ISOLATED PERFUSED RENAL TUBULES OF THE WINTER FLOUNDER

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INTRODUCTION

With the technique of in vitro microperfusion of renal tubuels (Burg et al., Am. J. Physiol. 210: 1293, 1966) it is now possible to examine structure-function correlations directly in specific segments of isolated renal tubules. In the present study we perfused what we thought were proximal tubules from the winter flounder and measured transepithelial voltages and resistances.

MATERIALS AND METHODS

Isolation of renal tubules. Winter flounder (Pseudopleuronectes americanus), weighing 500 g on the average, were collected by trawl and kept without food in running sea water for no longer than one week. A male or female flounder was decapitated, its kidney was removed from the body cavity and placed in ice-cold Ringers. Slices measuring 1x2x2 mm were cut from the caudal kidney and transferred to Ringers containing 0.25 mM/l chlorphenol red.

To our knowledge the anatomy of the renal tubule in the winter flounder has not been described. It is presumed to be similar to that of the English sole (Trump and Bulger, Lab. Invest. 16: 453, 1967). Identification of the initial proximal tubules is usually easy because of its attachment to the glomerulus which is easily recognized. However, in the kidney of the winter flounder the number of glomeruli is small, 8,000 to 12,000 (Nash, Am. J. Anat. 47: 425, 1931), and in the present study glomeruli could not be seen in the dorsal caudal slices through a stereomicroscope under 200x magnification. Therefore, we incubated the kidney slices in chlorphenol red at room temperature for 15 minutes since flounder proximal tubules are known to sequester chlorphenol red in their tubule lumen (Kinter, Am. J. Physiol. 211: 1152, 1966). Chlorphenol red containing tubules, 1 to 2 mm long, were then dissected free with needles and forceps.

In vitro perfusion of isolated tubules. The flounder renal tubule is a fragile tissue compared to the proximal and distal tubules of the garter snake (Beyenbach and Dantzler, Am. J. Physiol. 234: 238, 1978). To maintain the functional and electrical integrity of these renal tubules for the duration of an experiment (~1 hour) we perfused the tubular lumen with pipet pressures less than 20 cm H_2O. As the tubule lumen was perfused at rates less than 8 nl/min, the bath was perfused with a flow of 4 ml/min. Perfusion temperature was $12 \pm 2^{\circ}C$. The basic solution used for dissection, incubation, perfusion of the lumen, and superfusion of the bath contained in mmoles/l: NaCl 145, $NaHCO_3$ 20, KCl 5, $MgSO_4$ 1, $CaCl_2$ 1, Na_2HPO_4 1.65, NaH_2PO_4 0.3. No transepithelial ionic and osmotic gradients existed during in vitro microperfusion except for those introduced by the addition of glucose (10 mM/l) and acetate (3 mM/l) to the bath.

Measurement of transepithelial voltage and resistance. The transepithelial voltage was measured at the proximal and distal end of the perfused segment with respect to ground in the bath (Ag:AgCl electrodes in 4% agar-Ringer bridges). The transepithelial resistance was measured by cable analysis (Helman, Yale J. Biol. Med. 45: 339, 1972), injecting constant current pulses of 100 nA for 600 msec duration through the perfusion pipet into the tubule lumen. Tubules shorter than 4 length constants ($\lambda < 100\ \mu m$) were perfused in this series of experiments. Inner and outer diameters of perfused segments were respectively 21.1 ± 1.0 and $49.6 \pm 1.3\ \mu m$ (n=17).

RESULTS

Pre-perfusion incubation in chlorphenol red. In the chlorphenol red incubations we made the following observations: 1. In some fish the bluish-red color was not observed at all in spite of prolonged incubations in chlorphenol red. 2. The tubular lumen of freshly caught winter flounder appeared more intensely bluish-red than those from flounder kept in the holding facilities without food. 3. In 1x1x2 mm slices of the caudal kidney only tubules at the periphery of the slice showed the bluish-red color in their lumen. The lumen of tubules below the peripheral layer appeared colorless. 4. The intensity of the bluish-red color varied in different tubules at the periphery of the slice; it also varied with length along a single tubule; and in a single tubule a clear, colorless segment could be found interposed between two lumen-red segments. 5. The lumen of joining tubules and those of large tubules joining with several smaller tubules at right angles also appeared bluish-red, some of them very intensely. Trump and Bulger (1967) made these same observations in the English sole Parophrys vetulus.

The muscular layer of winter flounder tubules. Trump and Bulger (1967) have reported that a smooth muscular layer more or less completely surrounds the renal tubule of the English sole. In the winter flounder this muscular layer is thought to allow movement of the tubule (Townsley and Scott J. Fisheries Board, Canada, 20: 243, 1963) and to cause broken ends of tubules to close off and seal (Kinter, 1966; Maack and Kinter, Am. J. Physiol. 216: 1034, 1969; Renfro, Am. J. Physiol. 234: F522, 1978). The latter reports concerned us since we would be unable to perfuse these renal tubules if this constriction and sealing also occurred in isolated perfused tubules. However, muscular constriction interferred only rarely in our perfusion, 2 times in 95 perfusions. It is possible that the muscular layer is stripped from the basement membrane during dissection. It is also possible that our method of dissecting a tubule frays the ends to such an extent that closure and sealing does not occur. Still, we occasionally observed movement by isolated tubules in the dissecting dish and radial constrictions of perfused tubules. In the tubule photographed in Fig. 1, such a radial constriction is seen to invaginate the basement membrane from one side. The invagination began after 32 minutes of perfusion and then continued to relax and contract averaging 3 cycles per minute. The electrophysiological response to this constriction is discussed below (Fig. 3).

Transepithelial voltages and resistances of chlorphenol red containing tubules. Table 1 lists the mean values of the transepithelial voltage (V_T^{oc}) of all tubules successfully perfused. This mean value derives from 93 lumen-negative potentials. Two tubules with lumen-positive potentials (1.5 and 3.1 mV) are not included. Only one value per tubule is used. This value is the supposed steady state V_T^{oc} taken usually 15 to 30 minutes after the onset of perfusion. The highest transepithelial potentials were observed within 2 minutes after perfusion was started. Thereafter potentials approached stable values usually 10 to 15 minutes into the experiment.

Table 1 also lists the mean value of the transepithelial resistance for 20 lumen-negative V_T^{oc} tubules in which it was measured. One value per tubule was taken at the steady state value of the V_T^{oc}. Values of R_T for lumen-positive tubules are not included in Table 1. It is of interest that the two lumen-positive tubules encountered in 95 perfusions also had high transepithelial resistances 7,721 and 13,811 Ωcm).

The short-circuit current (I_{sc}) is calculated as the ratio V_T^{oc}/R_T. This ratio is not a measure of the rate of transepithelial net ion movement under open-circuit conditions, but it is an estimate of the ionic current that might flow if it were possible to short-circuit this epithelium.

Frequency distribution of the transepithelial voltage and resistance. The frequency distributions of the data summarized in Table 1 are illustrated in Fig. 2 along with the values from the lumen-positive tubules. Transepithelial voltages varied from +3.1 mV to -10.1 mV. The distribution is skewed towards the lumen-negative values close to zero mV.

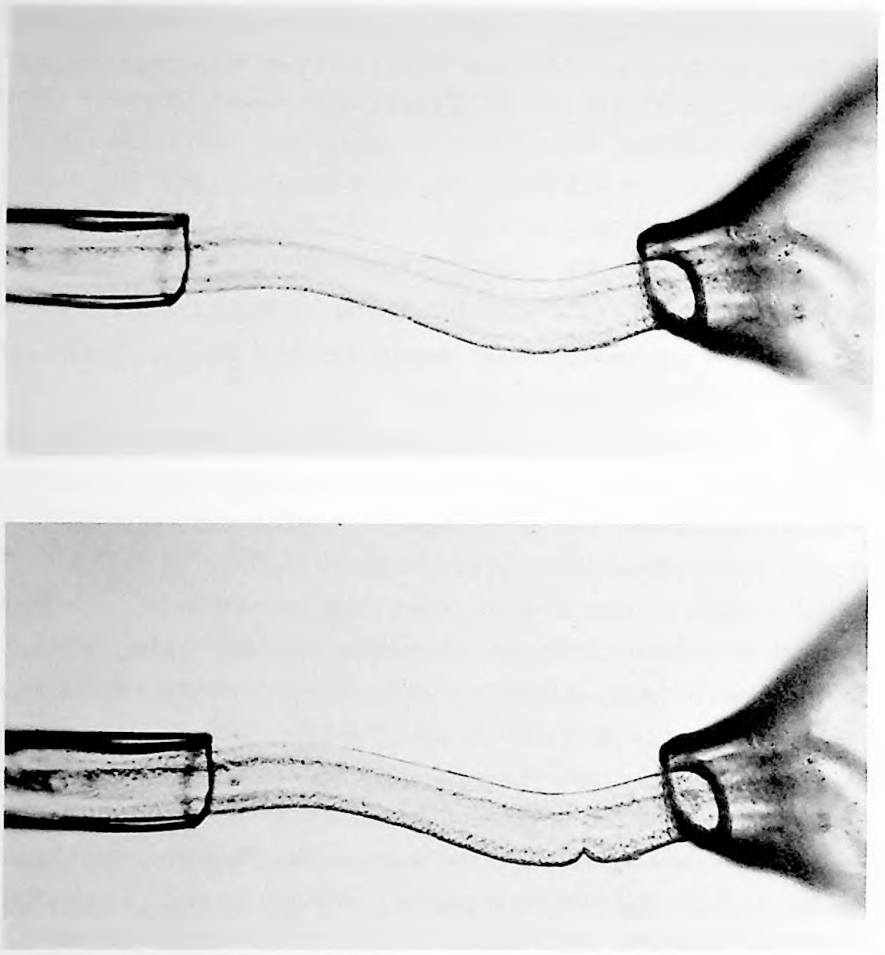

Figure 1.--Radial constriction of flounder renal tubule. The segment is perfused from left to right. The transepithelial electrical responses to this constriction are shown in Figure 3.

Table 1.--Electrical variables of isolated perfused renal tubules with lumen-negative transepithelial potentials.

	V_T^{oc} (mV)	R_T (Ω cm)	I_{sc} (μA/cm)	D_e (μm)	D_o (μm)
$\bar{X}$	-2.24	3,373	0.78×10^{-6}	21.3	20.8
S.E.	0.20	268	0.15×10^{-6}	1.7	1.1
n	93	20	20	20	20

V_T^{oc} open-circuit voltage; R_T transepithelial resistance; I_{sc} short-circuit current calculated as V_T^{oc}/R_T D_e lumen diameter calculated from the electrical measurement; D_o lumen diameter determined from the optical measurement.

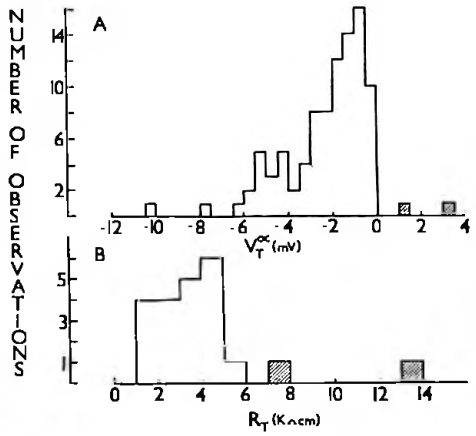

Figure 2.--Distribution of the transepithelial voltage. (V_T^{oc}) and resistance (R_T) measured in respectively 95 and 22 perfused renal tubules. V_T^{oc} is measured with respect to ground in bath. Open bars: data from lumen-negative tubules; striped bars: data from lumen-positive tubules. All tubules had chlorphenol red staining lumen.

Transepithelial resistance varied from 1,599 to 5,600 Ωcm for the lumen-negative V_T^{oc} tubules but was 7,721 and 13,811 Ωcm for the two lumen-positive V_T^{oc} tubules found among the tubules having the bluish-red colored lumen.

Effect of tubule constriction on transepithelial electrical variables. Figure 3 depicts the time course of the

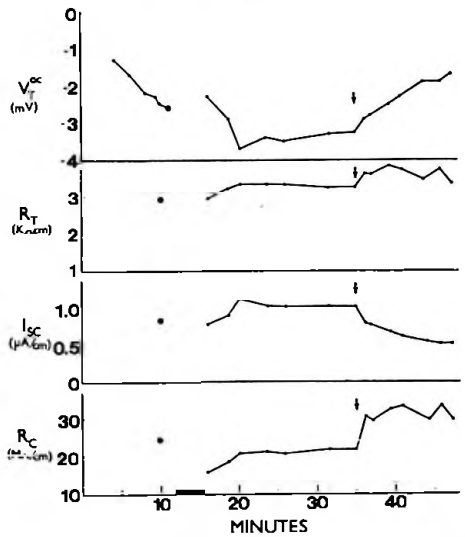

Figure 3.--Time course of transepithelial electrical variables for the tubule shown in Figure 1. The single heavy dots indicate a single measurement of the transepithelial voltage (V_T^{oc}), resistance (R_T), short-circuit current (I_{sc}), and core resistance (R_c). The solid bar on the ordinate indicates the period of pipet adjustments to improve electrical seals. The arrow marks the onset of the rhythmical constrictions near the distal end of the perfused segment.

electrical variables of the tubule shown in Figure 1. Data from this single tubule are shown to illustrate that changes in V_T^{oc}, R_T, I_{sc}, and R_c may be brought about by the spontaneous constriction of the tubule lumen.

At time 0 min, the perfusion was begun. The transepithelial voltage became increasingly more lumen-negative, and when the optical diameter did not agree with the electrical diameter (t=10 min), the perfusion pipet (sensing electrode) was advanced further into the tubule lumen. At the same time the tubule was drawn further into the

proximal holding pipet. This maneuver improved the electrical seal as is indicated by the increase of R_T and V_T^{oc}, and the agreement between D_e and D_o, respectively the lumen diameter measured electrically (cable analysis) and optically (ocular micrometer). At 35 minutes the tubule began its rhythmical constrictions near the collection (distal holding) pipet (Fig. 1). From this moment on, V_T^{oc} drifted towards zero mV, R_T and R_c increased, and I_{sc} decreased (Fig. 3).

DISCUSSION

In the present study we incubated kidney slices of the winter flounder in chlorphenol red in order to identify proximal tubules which are known to accumulate this dye in their lumen. However, chlorphenol red appeared in the lumen of tubules with lumen-negative and lumen-positive voltages, with low and high transepithelial resistances, and tubules of the collecting system. These observations reveal that chlorphenol red cannot be used as a selective marker for proximal tubules.

The vast majority of perfused tubules had lumen-negative transepithelial voltages when flounder Ringers was present on both sides of the epithelium. The ion dependence of this potential is unknown. However, the generation and the maintenance of the transepithelial potential appears to be dependent on perfusion flow through the tubule lumen. When the perfusion pressure is lowered to halt perfusion, the transepithelial voltage soon declines towards zero mV. The transepithelial voltage also declines when the tubules spontaneously constrict. This constriction can be expected to narrow the tubule lumen thereby 1) increasing the core resistance R_c (Fig. 3), and 2) increasing the hydraulic resistance to axial flow, and 3) reducing perfusion flow. Reduction of perfusion flow would lower transepithelial voltages and short-circuit currents, and increase transepithelial resistances if the luminal concentration of a transported species now become the rate-limiting factor of transepithelial transport (Figs. 1, 3).

The transepithelial resistance of the lumen-negative tubules is low, 3,400 Ωcm tubule length. Converted to resistance per cm^2 luminal surface (tubule inner diameter, 21 μm) the resistance is 22.3 Ωcm^2; on the basis of the peritubular surface area (tubule outer diameter, 50 μm) R_T is 53.0 Ωcm^2. These values are not as low as those measured in rat proximal tubules, 5 Ωcm^2 (Hegel et al., Pflügers Arch. 294: 274, 1967). In contrast the two tubules with lumen-positive potentials had significantly higher transepithelial resistances than those with lumen-negative potentials. They measured 71.0 Ωcm^2 and 169.8 Ωcm^2 on the basis of luminal and peritubular surface areas respectively.

The function of the muscular layer partially or completely covering the renal tubule of the flounder is unknown. The present study showed that the tubule wall is capable of spontaneous invaginations. Presumably these derive from the thin muscular layer that is thought to exist in winter flounder renal tubules (Kinter, 1966; Maack and Kinter, Am. J. Physiol. 216: 1034, 1969; Renfro, Am. J. Physiol. 234: F522, 1978). In Fig. 1 the single constriction was sufficient to reduce the average electrical diameter of the lumen from 38 μm to 30 μm and to decrease luminal perfusion flow. In other tubules as many as 10 such radial constrictions could be observed in a segment less than 1 mm long. In vivo, where the hydrostatic pressure for fluid flow through the tubule lumen might be low, these radial constrictions may serve to advance tubular fluid along the length of the nephron. Supported by a grant from the Whitehall Foundation to A. K., Cornell University start-up funds to K.W.B., and NIH 2 SO7 RR 05764-06 to MDIBL.

THE VOLTAGE-CLAMPED DOGFISH GASTRIC MUCOSA: DEPRESSION OF NET H^+ SECRETION, CONDUCTANCE CHANGE AND ANOMALOUS "BASE" SECRETION

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It is asserted that proton transport across the apical border of the oxyntic cell is electrogenic because hydrogen