J. Membrane Biol. 58, 35–42 (1981)

The Journal of
Membrane Biology

Chloride Movement Across the Basolateral Membrane of Proximal Tubule Cells

T. Shindo* and K.R. Spring**

National Institutes of Health, National Heart, Lung, and Blood Institute, Bethesda, Maryland 20205

Summary. Electrophysiologic and tracer experiments have shown that Cl^- enters *Necturus* proximal tubule cells from the tubule lumen by a process coupled to the flow of Na^+, and that Cl^- entry is electrically silent. The mechanism of Cl^- exit from the cell across the basolateral membrane has not been directly studied. To evaluate the importance of the movement of Cl^- ions across the basolateral membrane, the relative conductance of Cl^- to K^+ was determined by a new method. Single-barrel ion-selective microelectrodes were used to measure intracellular Cl^- and K^+ as a function of basolateral membrane PD as it varied normally from tubule to tubule. Basolateral membrane Cl^- conductance was about 10% of K^+ conductance by this method. A second approach was to voltage clamp the basolateral PD to 20 mV above and below the spontaneous PD, while sensing intracellular Cl^- activity with the second barrel of a double-barrel microelectrode. An axial wire electrode in the tubule lumen was used to pass current across the tubular wall and thereby vary the basolateral membrane PD. Cell Cl^- activity was virtually unaffected by the PD changes. We conclude that Cl^- leaves *Necturus* proximal tubule cells by a neutral mechanism, possibly coupled to the efflux of Na^+ or K^+.

Chloride reabsorption by the cells of *Necturus* renal proximal tubules is a two-step process. Chloride enters the tubule cells from the lumen in combination with sodium. This coupled entry step is electrically silent, sodium specific, and is the sole mode of chloride movement from lumen to cell (Spring & Kimura, 1978; Kimura & Spring, 1979). The mechanism by which chloride leaves the tubule cell across the basolateral membrane is less well characterized. We had assumed that chloride exit across the basolateral membrane was a passive process – simply the diffusion of chloride down its electrochemical gradient. Intracellular chloride activity is several times greater than the equilibrium level calculated from the potential difference (PD) across the basolateral membrane. Passive chloride flow from cell to peritubular capillaries is possible only if the chloride permeability of the basolateral membrane is sufficiently large. The chloride ion transference number of the basolateral membrane of *Necturus* proximal tubule cells was estimated by Boulpaep (1967) as 0.25 to 0.41. We calculated from this value that chloride diffusion out of the tubule cell could account for about 50% of the normal rate of chloride reabsorption (Spring & Kimura, 1978). The present work demonstrates that the basolateral membrane is not significantly permeable to chloride ions and that our previous calculations were in error. The present results are consistent with the electrically silent movement of chloride across the basolateral membrane as well as across the luminal membrane of the *Necturus* proximal tubule cell.

Two independent experimental approaches were utilized to evaluate the magnitude of the basolateral membrane chloride conductance. In the first method, we estimated the chloride and potassium transference numbers of the basolateral membrane by a method free of the limitations of previous ion substitution experiments. Briefly, the experiments required the measurement of intracellular chloride and potassium activity in Ringer-perfused renal proximal tubules and the analysis of the relationship between variations in these activities and the normal distribution of basolateral membrane PDs. The second approach involved voltage clamping the basolateral membrane PD to a new value and simultaneously

* *Present address:* Department of Internal Medicine I, Nara Shijocho, Kashihara, Nara 634, Japan.
** *To whom reprint requests should be made.*

0022-2631/81/0058-0035 $01.60

monitoring intracellular chloride activity. This experiment enabled a direct assessment of the dependence of intracellular chloride activity on basolateral membrane PD. Both experiments indicated that the basolateral membrane did not exhibit significant chloride ion conductance.

Methods

Preparation of Animals

Adult speciments of *Necturus maculosus* were obtained from the Mogul-Ed Corporation (Oshkosh, Wisc.) and were stored in aquaria at 15 °C. They were anesthetized by immersion in 0.1 % tricaine methane sulfonate (Finquel, Ayerst, N.Y.) and the kidneys were double perfused as previously described (Spring & Kimura, 1978). The control Ringer solution had the following compostion: 90 mM NaCl, 2.5 mM KCl, 10 mM $NaHCO_3$, 0.5 mM NaH_2PO_4, 1.8 mM $CaCl_2$, 1.0 mM $MgCl_2$, 2.2 mM glucose, 20 g/liter dextran (average mol wt 110,000), 1,500 U/liter heparin, pH 7.60 (gassed with 99 % O_2 – 1 % CO_2), and osmolarity of 214 mOsm/kg · H_2O. Potential differences were measured with conventional 3-M KCl filled glass microelectrodes as previously described (Spring & Kimura, 1978).

Ion Sensitive Microelectrodes

Single-barrel K^+-sensitive microelectrodes were constructed as previously described (Kimura & Spring, 1978). Pyrex capillary tubing with an internal glass fiber (omega dot glass) was pulled on a vertical pipette puller (David Kopf Instruments, Tujunga, Calif.). Chlorotrimethylsilane (Aldrich Chemical Co., Milwaukee, Wisc.) in 50 % xylene solution was used for silanization. K^+-sensitive electrodes were filled with the K^+ exchanger as previously described (Kimura & Spring, 1978).

Cl^--sensitive microelectrodes were made in the same fashion as K^+ electrodes and were filled with Cl^- exchanger resin (Orion Research Inc., Mass.). After being filled, the tips of the Cl^--sensitive microelectrodes were soaked in 1 M NaCl solution and stored overnight at room temperature to enhance responsiveness. The ion-sensitive microelectrodes were connected by a chlorided silver wire to a very high impedance electrometer (input impedance $>10^{15}\,\Omega$, Model F23, WP Instruments, New Haven, Conn.); the electrometer output was displayed and digitized as previously described (Spring & Kimura, 1978). The ion-sensitive electrodes were calibrated in standard NaCl and KCl solutions (10, 50 and 100 mM in concentration), which were in Lucite wells connected by agar bridges to a calomel cell ground electrode. The regression of the electrode reading, V, on the log of the ion activity, $\log A$, was determined to calculate the standard potential and the slope for each electrode. The average slopes of the K^+ and Cl^- sensitive electrodes were 59.2 ± 0.2 and -53.2 ± 0.4 mV/10-fold change in ion activity, respectively. The correlation coefficients between V and $\log A$ were -0.9999 for K^+ and -0.999 for Cl^- electrodes. The average selectivity for K^+ electrodes over Na^+ was 51.4 ± 2.2.

Double-Barrel Ion-Sensitive Microelectrodes

Electrodes were fabricated from thick partition theta tubing (R & D Optical, Spencerville, Md.) of an outer diameter of 1.3–1.5 mm. They were pulled on a vertical pipette puller (David Kopf Instruments, Tujunga, Calif.). One barrel was silanized by exposure to dichlorodimethylsilane (Aldrich Chemical Co., Milwaukee, Wisc.) vapor for 60 sec while the other barrel was protected by wax; the pipette was then heated at 100 °C for an hour. The reference (PD) barrel was filled first with a solution of 2 M sodium formate plus 10 mM NaCl, then the Cl^- exchanger resin was put into the silanized barrel. After both barrels were filled to the electrode tip, the pipettes were polished with an abrasive spray according to the method of Odgen Citron and Pierantoni (1978). Final tip size was about 1 μm, and the reference barrel had a resistance of 30 to 70 MΩ. The tips of these electrodes were immersed in 1 M NaCl solution at room temperature overnight. The average slope of the Cl^--sensitive double-barrel microelectrodes was -47.0 ± 0.7 mV/10-fold change in ion activity, and the correlation coefficient between V and $\log A$ was -0.997.

The electrodes were connected by chlorided silver wires to a dual electrometer equipped with twin probes of very high impedance (Model FT2230 microtitrator, WP Instrument., Inc., New Haven, Conn.).

Axial Electrode

In voltage clamp experiments, an axial electrode was placed in the tubule lumen to pass current across the tubular epithelium. The design of the axial electrode and micropipette was as previously described; the indifferent electrode was a platinized coil lying under the kidney (Spring & Paganelli, 1972).

Experimental Design

The first group of experiments were designed to determine the relationship between spontaneous ionic activity in tubule cells and basolateral membrane PD. The basolateral PD was measured in several cells of a tubule and the mean value was stored in computer memory. Acceptable basolateral PD measurements exhibited a sudden decrease upon impalement of the cell, no change in input impedance, and a stable reading for at least 20 sec. Then a single-barrel Cl^-- or K^+-sensitive electrode was inserted into other cells in the same tubule. Acceptable ion-sensitive electrode punctures were assessed by the criteria previously described (Spring & Kimura, 1978; Kimura & Spring, 1979). After the calibration of ion-sensitive electrodes, the mean voltage reading of electrodes was converted into activity using the stored mean basolateral PD. Thus the mean Cl^- and K^+ activities of the tubule cells were obtained.

The second group of experiments were designed to measure the effects of changes in basolateral PD on cell Cl^- activity. Double-barrel Cl^--sensitive microelectrodes were employed and used in combination with the axial electrode. The axial electrode was inserted into the tubule lumen, then a double-barrel Cl^--sensitive microelectrode was placed in a tubule cell in the region of the axial electrode. The tubule lumen was perfused by the glomerular filtrate. By using this system, we could change the PD across the basolateral membrane of the tubule cells and observe any resultant alterations in cell Cl^- activity. After a steady-state Cl^- reading was obtained in the cell, sufficient current was passed through the axial electrode to voltage clamp the basolateral PD over a range of ±20 mV, and the changes in the Cl^- voltage as well as the basolateral PD readings were recorded continuously on an instrumentation magnetic tape recorder (Model A, A.R. Vetter Co., Rebersburg, Pa.). These records were reproduced afterwards on a chart recorder (Model 715, MFE Corporation, Salem, N.H.).

In the third group of experiments, the tubule lumen was perfused with a sodium-free solution (100 mM tetramethylammonium chloride) through the pipette which contained the axial

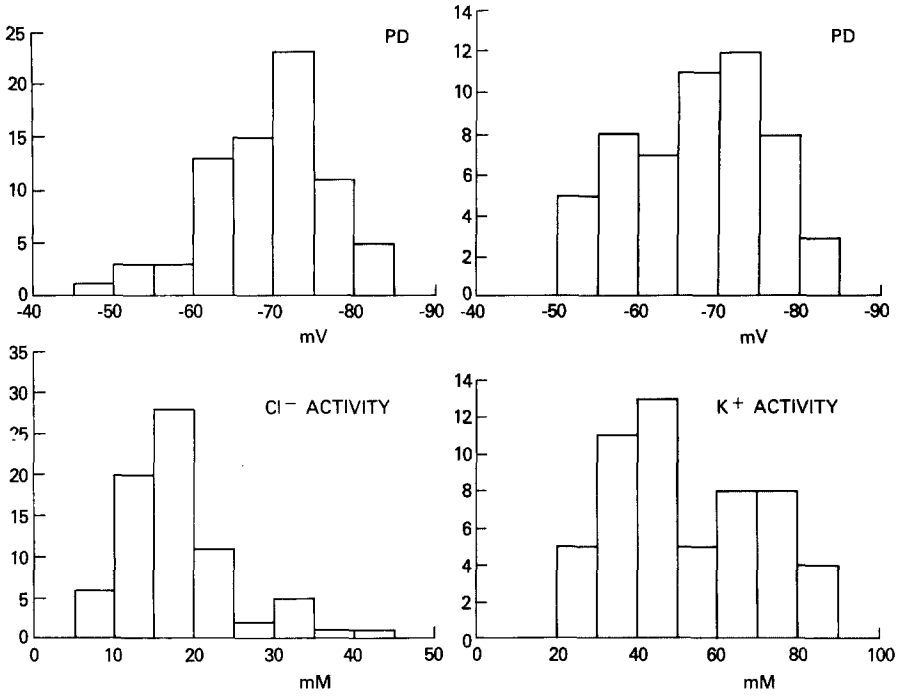

Fig. 1. Frequency histograms of basolateral membrane PD and intracellular chloride or potassium activity in *Necturus* proximal tubule cells. Number of observations on ordinate refers to the number of tubules; three to five measurements were averaged for each tubule. Basolateral PD values in upper left panel were used to correct the Cl^- readings in the lower left panel. PD values in the upper right panel were used to correct the K^+ readings

electrode. Voltage clamping of the basolateral membrane PD and simultaneous recording of Cl^- voltage was performed as described above.

Results

Spontaneous Basolateral Membrane PD and Intracellular K^+ and Cl^- Activity

In all of these experiments, the kidneys were doubly perfused with normal Ringer solution with a $[K^+]$ of 2.5 mM and a $[Cl^-]$ of 98.1 mM. Measurements were made of basolateral membrane PD from several cells in the same section of a single proximal tubule. The PD readings agreed within a few millivolts (coefficient of variation of PDs from five or more cells in the same tubule averaged 8.6%) as would be expected for such electrically coupled cells (Windhager, Boulpaep & Giebisch, 1966; Anagnostopoulos & Velu, 1974). Intracellular K^+ or Cl^- activities were measured in 3–5 cells of the same proximal tubule, and a close correlation was observed between ionic activity and spontaneous basolateral membrane PD. Although spontaneous PD is relatively constant from cell to cell in one proximal tubule, there is variation in PD from tubule to tubule. The variation of PD from tubule to tubule reflects a normal distribution of basolateral membrane potential difference. Figure 1 shows histograms of basolateral membrane PD, intracellular potassium, and chloride activities in different tubules. All are normally distributed as indicated by the Kolmogorov-Smirnov test for goodness of fit.

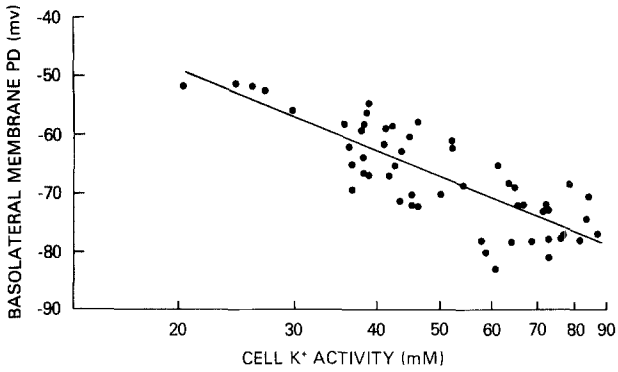

Fig. 2. Basolateral membrane PD is plotted on the ordinate against intracellular potassium activity on the abscissa. Potassium activity is on a logarithmic scale. The line is fitted to the points by the method of least squares and has the following parameters: intercept -2.63 ± 6.4 mV, slope -45.3 ± 4.5 mV/decade K^+, $r = 0.81$, slope significantly different from zero ($P < 0.001$)

Figure 2 shows the relationship between basolateral membrane PD and cell potassium activity (logarithmic scale). Each point represents the mean of at least four determinations of PD and intracellular K^+ activity in a single proximal tubule. Fifty-four tubules were studied in which the spontaneous PD varied from -52 to -83 mV. Cell K^+ activity ranged from 20 to 87 mM; low values of cell K^+ coincided with less negative cell PD and high values with more negative cell PD. Mean basolateral PD was -67.2 ± 1.1 mV

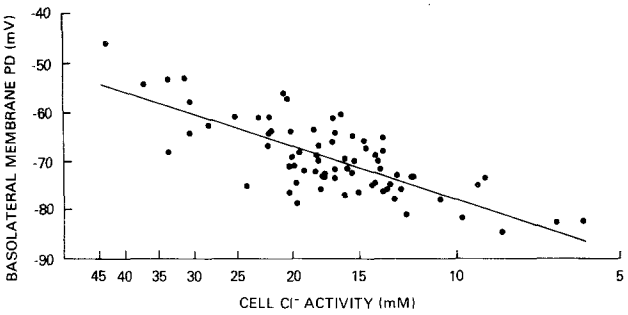

Fig. 3. Basolateral membrane PD is plotted on the ordinate against intracellular chloride activity on the abscissa. Chloride activity is on a logarithmic scale. The line is fitted to the points by the method of least squares and has the following parameters: intercept -45.6 ± 2.5 mV, slope -36.1 ± 3.7 mV/decade Cl^-, $r = 0.75$, slope significantly different from zero ($P < 0.001$)

and cell K^+ averaged 52.5 ± 2.4 mM. Both the mean and standard error of these values are comparable to previous observations (Khuri et al., 1972; Kimura & Spring, 1979). A close correlation existed between basolateral PD and cell K^+ activity. A least squares line was fitted to a plot of PD *vs.* $\log(K^+_{cell}/K^+_{cap})$, where K^+_{cell} is the intracellular K^+ activity, and K^+_{cap} is the capillary K^+ activity (i.e., 1.86 mM). The slope of the least squares line is -45.3 ± 4.5 mV/decade change in the K^+ activity ratio; the correlation coefficient is 0.81.

Figure 3 shows a similar plot of basolateral PD against the logarithm of the intracelluar chloride activity for 74 tubules. Basolateral PD varied from -46 to -84 mV, and cell chloride activity ranged from 43 mM at low PDs to 6 mM at very negative cell PDs. The mean basolateral PD was -69.5 ± 0.9 mV, and the average cell chloride activity was 16.9 ± 0.5 mM (magnitudes and errors comparable to Spring & Kimura, 1978; Khuri et al., 1975). A least squares line fitted to a plot of PD *vs.* $\log(Cl^-_{cap}/Cl^-_{cell})$ has a slope of -36.1 ± 3.7 mV/decade change in the Cl^- activity ratio. The correlation coefficient is 0.75.

Estimation of P_{Cl}/P_K

The permeability ratio (P_{Cl}/P_K) of the basolateral membrane can be caclulated from the data in Figs. 1 and 2, by using the reduced form of the Goldman equation:

$$-E = \frac{RT}{F} \ln \left(\frac{K^+_{cell} + \alpha Na^+_{cell} + \beta Cl^-_{cap}}{K^+_{cap} + \alpha Na^+_{cap} + \beta Cl^-_{cell}} \right) \quad (1)$$

where E is the electromotive force across the basolateral cell membrane, α is P_{Na}/P_K, β is P_{Cl}/P_K and A_{cell} is the ionic activity in the cell and A_{cap} is the ionic activity in the capillary. The Na^+ permeability for the basolateral membrane has been shown in previous experiments to be negligibly small (Spring & Giebisch, 1977) so that α may be neglected. β is then given by:

$$\beta = \frac{K^+_{cell} - K^+_{cap} \cdot e^{-\frac{FE}{RT}}}{Cl^-_{cell} \cdot e^{-\frac{FE}{RT}} - Cl^-_{cap}}. \quad (2)$$

Table 1.

Basolateral membrane PD (mV)	Cellular ion activity		β
	K^+ (mM)	Cl^- (mM)	
−50	24.3 (4)	43.3 (1)	0.05
−55	35.8 (3)	28.7 (5)	0.11
−60	42.5 (11)	22.3 (6)	0.14
−65	42.6 (8)	19.5 (14)	0.10
−70	58.3 (14)	17.5 (19)	0.14
−75	77.9 (6)	15.1 (21)	0.19
−80	68.1 (7)	13.1 (5)	0.10
−85	60.7 (1)	6.9 (3)	0.06
		Mean ± SE	0.11 ± 0.02

Number of tubules are in parentheses; β is the ratio P_{Cl}/P_K for the basolateral membrane

Equations (1) and (2) apply in the absence of current flow across the basolateral membrane. Circulating ionic currents would cause the basolateral membrane PD to be less than the electromotive force, E (Boulpaep, 1976*a*, *b*). Estimation of the electromotive force across the basolateral membrane was not possible from our data because we did not measure the cell membrane and shunt resistances. We chose, therefore, to use the observed potential in Eq. (2) to calculate the permeability ratio, β, with the realization that this caused a two to threefold overestimate of the true value of β. Table 1 gives the values of β calulated at different values of basolateral PD, intracellular potassium, and chloride activities. The overall mean permeability ratio was 0.11 ± 0.02. Application of Eq. (2) required two assumptions: (i) that the K^+ and Cl^- conductance ratio of the basolateral membrane of cells from different tubules were not different, (ii) the membrane and shunt resistances did not vary greatly from high to low PD tubules. Inasmuch as the values of β in Table 1 do not differ significantly from one another, the first assumption is reasonable. We cannot evaluate the second assumption because of the absence of resistance data. We therefore chose a more direct approach to the determination of the chloride conductance of the basolateral membrane by the use of a voltage-clamp technique.

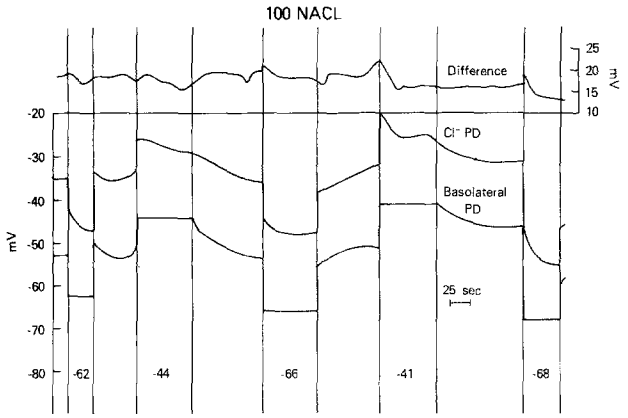

Fig. 4. Record of voltage calmp experiments in which current passage from an axial electrode in the tubule lumen is used to change basolateral membrane PD. As indicated at the top of the figure the tubule lumen contains 100 mM NaCl Ringer. The uppermost trace displays the difference between the chloride electrode PD (second trace from top) and the basolateral PD (bottom trace). The difference trace is directly proportional to cell chloride activity. The numbers at the bottom of the figure are the command voltages to which the basolateral membrane is clamped. The open-circuit PD of the basolateral membrane is −52 mV before the start of the clamp periods and approximately −47 mV at the end of the experiment. The clamp was turned on and off at times indicated by the vertical lines. (*See* text for further details)

Cell Cl^- Activity and Basolateral Membrane PD

Variations in basolateral cell membrane PD were produced by a voltage clamp with an axial electrode in the tubule lumen and a double-barrel, chloride-sensitive microelectrode in the tubule cell.

Figure 4 shows a typical tracing from a group of eight tubules whose lumen was perfused with 100 mM NaCl Ringer. The basolateral PD trace indicates changes in basolateral membrane PD in response to voltage-clamp commands. The Cl^- PD tracing represents the direct reading from the Cl^--sensitive barrel of the double-barrel microelectrode. The difference trace is a record of the chloride barrel minus the PD barrel. At the left of Fig. 4, the initial basolateral membrane PD was −52 mV. The basolateral membrane PD was then voltage clamped in the hyperpolarizing direction to −62 mV; the Cl^- PD trace showed a hyperpolarizing transient. After the clamp was released, both barrels (basolateral PD and Cl^- PD) recorded similar transients before returning to their initial values. Then a depolarizing voltage clamp was tried to −44 mV; the Cl^- PD also exhibited a small hyperpolarizing transient during the clamp period. When the clamp was released, transients were again seen in both barrels. After the reading stabilized, the basolateral membrane PD was clamped to −66 mV. A transient in the chloride reading developed as was seen in the first hyperpolarizing clamp. During another depolarizing clamp to −41 mV; the Cl^- PD exhibited an overshoot followed by a hyperpolarization similar to that seen in the first depolarizing clamp. A third hyperpolarizing clamp to −68 mV was then attempted, and similar effects were observed on the chloride-barrel readings. Hyperpolarizing transients in the Cl^- PD records occurred during each hyperpolarizing voltage clamp; they represent an increase in cell chloride during hyperpolarization of the basolateral membrane PD. Such an increase in cell chloride, after the basolateral membrane PD becomes more negative, is opposite to the expected response for simple diffusion of an anion across that membrane.

The difference trace showed only small deflections throughout the voltage clamps, which indicated that the changes in cell chloride were small (2 to 4 mM). The transients in cell chloride were most prominent during hyperpolarizing clamps and were absent or very small during depolarizing clamps (*see* Table 2). Inasmuch as the transients were always in the direction of an increase in cell chloride during a period of increased driving force for chloride ion exit from the cell, we reasoned that the observed chloride movement could be secondary to the movement of a cation. We, therefore, undertook a series of experiments in which the tubule lumen was perfused with a sodium-free solution of 100 mM tetramethylammonium chloride (TMACl).

Perfusion of the tubule lumen with 100 mM TMACl leads to a fall in cell chloride to near its equilibrium value (Spring & Kimura, 1978). Voltage-clamp experiments were done while the tubule lumen was perfused with 100 mM TMACl to investigate two related questions: (i) Would the cell chloride activity changes conform to the diffusional movement of an anion if the initial cell chloride were near its electrochemical equilibrium value? (ii) Are the hyperpolarizing chloride PD transients related to the entry of sodium from the tubule lumen into the tubule cell?

Figure 5 shows an example of successive voltage clamps of the tubular epithelium during luminal perfusion of 100 mM tetramethylammonium chloride. Only very small transients are seen in the Cl^- PD trace during the first two hyperpolarizing voltage clamps. A depolarizing voltage clamp to −60 mV made no apparent change in the Cl^- PD tracing. Another depolarizing voltage clamp to −55 mV produced an extremely small voltage change in the Cl^- PD tracing. The last depolarizing voltage clamp to −50 mV resulted in only small fluctuations in the chloride PD tracings. By the end of the experiment spontaneous cell PD was −50 mV, so that neither the basolateral not Cl^- PD trace changed when the

Table 2. Transients in Cl^- electrode reading during voltage clamp

Luminal perfusate	No. of tubules	Basolateral[a] PD (mV)	Voltage clamp direction	Transient[b] amplitude (mV)
100 mM NaCl	8	−58.1 ± 2.0 (10)	Hyperpolarizing	4.5 ± 0.8 (13)
			Depolarizing	1.5 ± 0.8 (10)
100 mM TMACl	10	−59.0 ± 2.1 (16)	Hyperpolarizing	0.6 ± 0.8 (18)
			Depolarizing	0.6 ± 0.5 (19)

[a] Basolateral PD recorded with double-barrel microelectrodes prior to voltage-clamp experiment. Values are mean ± SE with number of observations in parenthesis.

[b] Amplitude of the transients in Cl^- electrode reading during the passage of current for voltage clamping the basolateral membrane PD. Values are mean ± SE with number of observations in parenthesis.

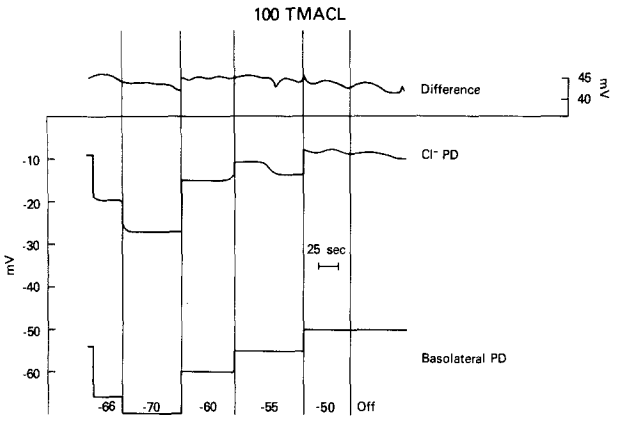

Fig. 5. Protocol as in Fig. 4 except that the tubule lumen was perfused with a solution of 100 mM TMACl. The open-circuit PD before voltage clamp was −54 mV, and the PD after the completion of the clamp periods was −50 mV. Note that the chloride PD is less negative than that in Fig. 4, indicating a low intracellular chloride activity during the luminal perfusion of a sodium-free solution

clamp was released. We conclude from these experiments that removal of mucosal sodium virtually abolished the transients in cell chloride and that chloride ions did not move passively in response to change in basolateral membrane PD, even when the cell chloride activity was near the equilibrium value (Table 2).

Discussion

Chloride apparently leaves the *Necturus* proximal tubule cell by an electrically silent process inasmuch as both of the experimental approaches in this work failed to demonstrate significant chloride conductance of the basolateral membrane of the tubule cell.

We previously reported that the replacement of peritubular capillary perfusate chloride by a wide variety of anions did not cause major alterations in basolateral membrane PD (Spring & Kimura, 1978; Kimura & Spring, 1979). These solution changes were relatively slow, and PD measurements were not made until several minutes after the ion substitution. This left open the possibility that transient PD changes occurred. However, Anagnostopoulos and Planelles (1979) recently reported a series of anion substitution experiments from which they concluded that basolateral membrane of the *Necturus* proximal tubule cell was virtually impermeable to chloride ions. Consistent with this conclusion are the recent elegant observations of Reuss (1979) on the basolateral membrane of the *Necturus* gallbladder epithelial cell. Chloride conductance of this membrane was measured, by determination of the transference number after rapid ion substitution. The transference number obtained was so small that only 3% of the transepithelial chloride flux could have been due to the diffusional movement of chloride ions across the basolateral membrane. Shunt pathway chloride ion flux was insignificant, and the total of transcellular and paracellular chloride ion diffusion was less than 10% of the observed transepithelial chloride net flux. Reuss concluded that most if not all chloride reabsorption in this tissue was electrically silent, coupled movement (Reuss, 1979; Reuss & Weinman, 1979).

Our technique for the estimation of basolateral membrane P_{Cl}/P_K did not require the determination of transference numbers and was free, therefore, of the difficulties associated with that measurement (Reuss, 1979). Our approach did require the assumptions of constancy of P_{Cl}/P_K and membrane resistance. If these assumptions were incorrect then the calculations in Table 1 would not be physiologically meaningful. The maximum chloride conductance obtained in our experiments could account for no more than 20% of the observed transepithelial chloride net flux.

The voltage-clamp experiments also suffered from limitations. The double-barrel electrodes used in the voltage-clamp experiments had a circular outer configuration and a central glass partition ("theta tub-

ing"); a geometry designed to minimize damage to the cell membrane. Most of our voltage-clamp experiments had to be rejected because of low PDs or gradual depolarization of the basolateral PD due to membrane damage during the clamp period. About 20% of the experiments were considered acceptable since they exhibited both stable voltage and chloride electrode readings throughout the clamp period and similar initial and final PD and chloride readings. The average basolateral PD recorded with the double-barrel electrodes in these experiments was approximately 10 mV lower than the single-barrel values (Table 2).

A second consideration in the voltage clamp was the magnitude of the PD changes across the luminal membrane of the tubule cell. The luminal membrane resistance is 3 to 5 times greater than the basolateral membrane (Anagnostopoulos, 1973; Boulpaep, 1967; Kimura & Spring, 1978). Transepithelial current flow caused a 3 to 5 times greater PD change across the luminal membrane than across the basolateral membrane. Thus a 20-mV hyperpolarization of the basolateral membrane PD (say from -60 to -80 mV) was accompanied by a 60 to 100 mV reduction in luminal PD. Current passage caused the transepithelial PD to change 3 to 5 times more than the basolateral PD and the electrical gradient across the luminal membrane was reduced correspondingly. Thus the driving forces for ionic movement were changed across both cell membranes in a different fashion. A reduction in electrical PD across the basolateral PD was accompanied by a large increase in luminal PD, and an increase in basolateral PD by a large decrease in luminal PD. Diffusional movement of chloride across the *Necturus* proximal tubule cell luminal membrane has been shown to be insignificant in the absence of transepithelial current passage (Spring & Kimura, 1978; Kimura & Spring, 1979). We do not know whether the large changes in luminal membrane PD induced in the voltage clamp experiments increased the chloride permeability of the luminal membrane. In view of the fact that cell chloride activity was uninfluenced by current flow, any such permeability increase must have been sufficiently small that significant changes in cell chloride were not detected.

Basolateral Membrane Chloride Conductance and Transcellular Chloride Transport

The absence of a sizeable chloride conductance of the basolateral membrane has two possible implications for transepithelial chloride transport: (i) All chloride movement bypasses the cell because of the low conductance of the basolateral membrane, or (ii) transcellular chloride movement is an electrically silent, neutral process. The first possibility, that chloride bypasses the epithelial cell during transepithelial transport, may be discounted on the basis of previous tracer and ion-sensitive microelectrode studies (Spring & Kimura, 1978; Kimura & Spring, 1979, 1980). Intracellular chloride activity falls readily when the luminal sodium or chloride concentrations are reduced, indicating that cell chloride is maintained by a steady influx across the luminal membrane (Spring & Kimura, 1978). Tracer chloride experiments also clearly indicate a large transcellular chloride flux (Kimura & Spring, 1978, 1980).

Evidence for a significant neutral chloride flux across the basolateral membrane of *Necturus* proximal tubule has not been previously reported. Neutral chloride movement across the basolateral membrane could be in form of coupled NaCl or KCl extrusion from the cell or anion exchange across that boundary. There is evidence against a chloride-bicarbonate exchange at the basolateral membrane. Sodium chloride reabsorption was not significantly affected by the removal of bicarbonate from the peritubular perfusate, and its replacement by chloride (Whittembury et al., 1975). Since other exchangeable anions are not present in sufficient concentration in *Necturus* Ringer there seems to be little likelihood of an anion exchange mechanism across the basolateral membrane. Coupled movement of chloride and hydrogen or hydroxyl ion also seems unlikely in view of the fact that *Necturus* proximal tubule does not acidify the luminal fluid (Giebisch, 1961; Whittembury et al., 1975). The most probable mechanism for the extrusion of chloride involves a linkage to the active transport of sodium. It is also possible that a portion of the Cl^- crosses the basolateral membrane together with K^+ in a neutral complex and that K^+ is recycled by the Na–K pump; the net result would be NaCl transport. Inasmuch as we do not have direct evidence which can further delineate the nature of basolateral chloride transfer, further discussion of this question is unwarranted.

The observed relationship between cell potassium and chloride activities and basolateral membrane PD is of considerable interest. It is obvious from Fig. 2 and 3 that both K^+ and Cl^- were oppositely dependent on the basolateral PD. At low PDs cell K^+ was low and Cl^- was high, while at high PDs K^+ was high and Cl^- low. Although we did not measure intracellular sodium activities, we would expect Na^+ and Cl^- to show a similar PD dependence. The apparent dependence of Cl^- on basolateral membrane PD could simply be a reflection of changes in cell Na^+ activity and in the transcellular NaCl transport rate.

In summary, the low basolateral membrane chloride conductance observed in our experiments is consistent with the neutral transport of chloride across the basolateral membrane possibly linked to the exit of sodium and potassium.

The authors wish to acknowledge the helpful technical assistance of Lynne Des Jardins and the theoretical guidance provided by Dr. C. Patlak.

References

Anagnostopoulos, T. 1973. Biionic potentials in the proximal tubule of *Necturus* kidney. *J. Physiol. (London)* **233(2)**:375

Anagnostopoulos, T., Planelles, G. 1979. Organic anion permeation at the proximal tubule of *Necturus*. *Pfluegers Arch.* **381**:231

Anagnostopoulos, T., Velu, E. 1974. Electrical resistance of cell membranes in *Necturus* kidney. *Pfluegers Arch.* **346**:327

Boulpaep, E.L. 1967. Ion permeability of the peritubular and luminal membrane of the renal tubular cell. *In:* Transport und Funktion Intracellulärer Electrolyte. F. Krück, editor, pp. 98–107. Urban & Schwarzenberg, Munich

Boulpaep, E.L. 1976*a*. Electrical phenomena in the nephron. *Kidney Int.* **9**:88

Boulpaep, E.L. 1976*b*. Recent advances in electrophysiology of the nephron. *Annu. Rev. Physiol.* **38**:20

Giebisch, G. 1961. Measurements of electrical potential differences on single nephrons of the perfused *Necturus* kidney. *J. Gen. Physiol.* **44**:659

Khuri, R.N., Agulian, S., Bogharian, K., Aklanjian, D. 1975. Electrochemical potentials of chloride in proximal renal tubule of *Necturus* maculosus. *Comp. Biochem. Physiol.* **50A**:695

Khuri, R.N., Hajjar, J.J., Agulian, S., Bogharian, K., Kalloghlian, A., Bizri, H. 1972. Intracellular potassium in cells of the proximal tubule of *Necturus* maculosis. *Pfluegers Arch.* **338**:73

Kimura, G., Spring, K.R. 1978. Transcellular and paracellular trace chloride fluxes in *Necturus* proximal tubule. *Am. J. Physiol.* **235(6)**:*F*617 or *Am. J. Physiol.:* Renal Fluid Electrolyte Physiol. **4(6)**:*F*617

Kimura, G., Spring, K.R. 1979. Luminal Na^+ entry into *Necturus* proximal tubule cells. *Am. J. Physiol.* **236(3)**:*F*295 or *Am. J. Physiol:* Renal Fluid Electrolyte Physiol. **5(3)**:*F*295

Kimura, G., Spring, K.R. 1980. Ionic conductance of the cell membranes and shunts of *Necturus* proximal tubule. *Curr. Topics Membr. Transp.* **13**:265

Ogden, T.E., Citron, M.C., Pierantoni, R. 1978. The jetstream microbeveller: An inexpensive way to bevel ultrafine glass micropipettes. *Science.* **201**:469

Reuss, L. 1979. Electrical properties of the cellular transepithelial pathway in *Necturus* gallbladder. III. Ionic permeability of the basolateral cell membrane. *J. Membrane Biol.* **47**:239

Reuss, L., Weinman, S.A. 1979. Intracellular ionic activities and transmembrane electrochemical potential differences in gallbladder epithelium. *J. Membrane Biol* **49**:345

Spring, K.R., Giebisch, G. 1977. Tracer Na fluxes in *Necturus* proximal tubule. *Am. J. Physiol.* **232**:*F*461 or *Am. J. Physiol.:* Renal Fluid. *Electrolyte Physiol.* **1**:*F*461

Spring, K.R., Kimura, G. 1978. Chloride reabsorption by renal proximal tubules of *Necturus*. *J. Membrane Biol.* **38**:233

Spring, K.R., Paganelli, C.V. 1972. Sodium flux in *Necturus* proximal tubule under voltage clamp. *J. Gen. Physiol.* **60**:181

Whittembury, G., Diezi, F., Diezi, J., Spring, K., Giebisch, G. 1975. Some aspects of proximal tubular sodium chloride reabsorption in *Necturus* kidney. *Kidney Int.* **7**:293

Windhager, E.E., Boulpaep, E.L., Giebisch, G. 1966. Electrophysiological studies on single nephrons. *Proc. Int. Congr. Nephrol.* (3rd, Washington, D.C.) **1**:35

Received 7 April 1980; revised 28 July 1980