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Effects of Antidiuretic Hormone on Cellular Conductive Pathways in Mouse Medullary Thick Ascending Limbs of Henle: II. Determinants of the ADH-Mediated Increases in Transepithelial Voltage and in Net Cl^- Absorption

Steven C. Hebert* and Thomas E. Andreoli
Division of Nephrology, Department of Internal Medicine, University of Texas Medical School, Houston, Texas

Summary. Cellular impalements were used in combination with standard transepithelial electrical measurements to evaluate some of the determinants of the spontaneous lumen-positive voltage, V_e, which attends net Cl^- absorption, J_{Cl}^{net}, and to assess how ADH might augment both J_{Cl}^{net} and V_e in the mouse medullary thick ascending limb of Henle microperfused *in vitro*. Substituting luminal 5 mM Ba^{++} for 5 mM K^+ resulted in a tenfold increase in the apical-to-basal membrane resistance ratio, R_a/R_{bl}, and increasing luminal K^+ from 5 to 50 mM in the presence of luminal 10^{-4} M furosemide resulted in a 53-mV depolarization of apical membrane voltage, V_a. Thus K^+ accounted for at least 85% of apical membrane conductance. Either with or without ADH, 10^{-4} M luminal furosemide reduced V_e and J_{Cl}^{net} to near zero values and hyperpolarized both V_a and V_{bl}, the voltage across basolateral membranes; however, the depolarization of V_{bl} was greater in the presence than in the absence of hormone while the hormone had no significant effect on the depolarization of V_a. Thus ADH-dependent increases in V_e were referable to greater depolarizations of V_{bl} in the presence of ADH than in the absence of ADH. 68% of the furosemide-induced hyperpolarization of V_a was referable to a decrease in the K^+ current across apical membranes, but, at a minimum, only 19% of the hyperpolarization of V_{bl} could be accounted for by a furosemide-induced reduction in basolateral membrane Cl^- current. Thus an increase in intracellular Cl^- activity may have contributed to the depolarization of V_{bl} during net Cl^- absorption, and the intracellular Cl^- activity was likely greater with ADH than without hormone. Since ADH increases apical K^+ conductance and since the chemical driving force for electroneutral Na^+, K^+, $2Cl^-$ cotransport from lumen to cell may have been less in the presence of ADH than in the absence of hormone, the cardinal effects of ADH may have been to increase the functional number of both Ba^{++}-sensitive conductance K^+ channels and electroneutral Na^+, K^+, $2Cl^-$ cotransport units in apical plasma membranes.

Key Words rental tubule transport · medullary thick ascending limb · intracellular voltage recording · ADH · K^+ conductance

* Established Investigator of the American Heart Association.

Introduction

The purpose of the experiments reported in the present paper was to analyze the factors responsible for the lumen-positive spontaneous transepithelial voltage (V_e, mV) which attends net Cl^- absorption (J_{Cl}^{net}) in single medullary thick ascending limbs of Henle isolated from mouse kidney; to provide a model which explains, at least in part, how ADH, operating via cAMP, augments simultaneously V_e and J_{Cl}^{net}; and to inquire whether a homology might exist between the ADH-dependent mechanisms leading to increased rates of Cl^- absorption and V_e in the isolated mouse mTALH, and those ADH-mediated events resulting in stimulation of net Na^+ absorption and osmotic water permeability by ADH in amphibian epithelia, and the enhancement of osmotic water permeability by ADH in isolated mammalian collecting tubules.

We argued, in the preceding paper (Hebert, Friedman & Andreoli, 1984), that salt absorption in the isolated mouse mTALH involved an electroneutral Na^+, K^+, $2Cl^-$ apical membrane entry step and conductive net Cl^- efflux across basolateral membranes; the majority of K^+ entering cells in this cotransport step was accounted for by K^+ recycling to luminal fluids across apical membranes. Net Na^+ absorption was rationalized in terms of two processes, each of which contributed approximately 50% to the total Na^+ flux: net transcellular Na^+ flux mediated by basolateral membrane (Na^+ + K^+)-ATPase; and dissipative paracellular Na^+ flux driven by the lumen-positive voltage. The cardinal effects of ADH on this system included: simultaneous increases in J_{Cl}^{net} and in V_e (Hebert, Culpepper & Andreoli, 1981*a*; Hebert et al., 1984); an increase in transcellular conductance which occurred even when net rates of Cl^- absorption and V_e were virtu-

ally abolished (Hebert et al., 1984); and an increase in the Cl^- conductance of basolateral membranes which occurred only when J_{Cl}^{net} was also increased by the hormone (Hebert et al., 1984).

The present experiments utilized cellular impalement techniques in these isolated mTALH tubules to address a number of issues relevant to these arguments. More specifically, the major issues for analysis were: whether K^+ conductance accounted for the majority of apical membrane conductance; whether the magnitude of basolateral membrane depolarization during net Cl^- absorption was sufficiently greater than the magnitude of apical membrane depolarization to account for a lumen-positive V_e; how hormone-dependent increases in J_{Cl}^{net} resulted in an increased V_e; and the extent to which furosemide-induced hyperpolarization of apical and basolateral membranes could be accounted for by furosemide-induced reductions in apical K^+ currents and basolateral Cl^- currents. These results were integrated into a model which postulates that, in the mouse mTALH: the primary effect of ADH responsible for hormone-mediated increases in J_{Cl}^{net} was the consequence of an ADH-dependent increase in the functional number of electroneutral Na^+, K^+, $2Cl^-$ cotransport units and of conductive K^+ units in apical membranes; the lumen-positive V_e occurred because, during net Cl^- absorption, basolateral membranes were depolarized to a greater extent than apical membranes; and ADH increased V_e by increasing the degree of basolateral membrane depolarization.

Materials and Methods

The methods used in these experiments were identical, with the exceptions noted below, to those used in the companion paper (Hebert et al., 1984). In brief, white male Swiss mice weighing between 25–30 g were sacrificed with no prior treatment, and 0.15–0.30 mm segments of mTALH were dissected from quartered kidneys immersed in cold HEPES (N-2 hydroxyethyl-piperazine-N'-2 ethanosulfonic acid)-buffered bathing solution. The mTALH tubule segments were transferred to a perfusion chamber fitted to the stage of an inverted microscope and were mounted between double lumen theta glass perfusion pipettes and standard collection pipettes as described previously (Hebert et al., 1984). In all experiments, the perfusion rate was maintained between 10–20 nl/min to minimize flow-dependent changes in the transepithelial voltage (V_e, mV) and in the axial composition of luminal fluids (Hebert, Culpepper & Andreoli, 1981*b*). During perfusion, the temperature was maintained at 37 ± 0.5°C.

Several modifications of the flow systems for the perfusion and bathing solutions were required to reduce tubule vibrations and/or to avoid tubule movement during solution changes so that stable cell impalements could be obtained. First, the perfusate flow system was open-ended (*see* Fig. 1 in Hebert et al., 1984), allowing perfusion solution to flow continuously through one-half of the theta glass perfusion pipette, via an exchange pipette, at a rate of 5–10 ml/min. This design permitted rapid (<15 sec) perfusate solution changes without flow-related pressure transients. Second, the perfusion chamber used in the present experiments, which was similar in design to those described by others (Biagi, Kubota, Sohtell & Giebisch, 1981; Koeppen, Biagi & Giebisch, 1983), permitted bathing solution to flow continuously through the perfusion chamber at rates of 5–10 ml/min, after having been equilibrated with 100% O_2 and warmed to 37°C. In this manner, the tubule could be supplied continuously with fresh oxygenated bathing solution while producing no detectable tubule vibrations. Finally, the entire perfusion system was placed on an air suspension table (MICRO-g, Bacher-Coring Corp., Peabody, MA) to isolate the tubule from external sources of vibration.

Composition of Solutions

The composition of routine solutions was identical to that described in detail in the preceding paper (Hebert et al., 1984) and contained (mM): 140 NaCl, 5.0 KCl, 1.0 $CaCl_2$, 1.2 $MgCl_2$, and 3.0 HEPES. In addition, bathing solutions contained 5.5 mM glucose and 0.4 g/100 ml of exhaustively dialyzed bovine serum albumin (Fraction V, Armour Pharmaceuticals, Tarrytown, NY). All solutions were adjusted to an osmolality of 300 mOsm/kg H_2O with urea, and to a pH of 7.40 after equilibration with 100% O_2. The ionic composition of the bath was not changed when $BaCl_2$ was added to, and KCl removed from, the perfusate, since these differences in perfusate and bath composition produced negligible dilution voltages. Perfusate KCl concentrations were increased to 50 mM by replacing NaCl isosmotically with KCl.

Where indicated, ADH (synthetic arginine vasopressin, Grade V, Sigma Chemical Co., St. Louis, MO) was added to the bath at a concentration of 10 μU/ml, which produces maximal increases in V_e and net rates of Cl^- absorption in the mouse mTALH (Hebert et al., 1981*a,b*). Furosemide was kindly provided by Hoechst Pharmaceuticals, Somerville, NJ.

Microelectrode Impalement of Single mTALH Cells

The general methods used to impale cells of the isolated perfused mouse mTALH were similar to those described previously by others (Biagi et al., 1981; Greger, Frömter & Schlatter, 1981; Greger & Schlatter, 1983; Koeppen et al., 1983). Cells were impaled across basolateral membranes using microelectrodes made from 1.2 or 1.5 mm OD thick-walled capillary glass containing an inner filament (Kwick-Fil, W-P Instruments, Inc., New Haven, CT). A Brown-Fleming puller (Model P-77, Sutter Instrument Co., San Francisco, CA) was used to fabricate microelectrode tips having shanks 12-18 mm long and resistances between 120–200 MΩ when filled with 0.5 M KCl. This relatively low concentration of KCl was used to fill the microelectrodes since Fromm and Schultz (1981) and Blatt and Slayman (1983) have reported recently that higher concentrations of KCl can result in high rates of K^+ and Cl^- leakage from microelectrodes, which could lead to unstable transmembrane voltages and large increases in cell K^+ and Cl^- activities. The microelectrodes pulled and filled with 0.5 M KCl produced no visible cell swelling when cells were punctured.

Microelectrodes were positioned in the bath using a hydraulic-drive, remote-control micromanipulator (MO-102R, Narishige Scientific Instrument Lab, Tokyo). Cell impalements were made by positioning the microelectrode tip on the tubule basement membrane at an angle of 20–30°, advancing the tip to produce slight dimpling of the basement membrane, and either lightly tapping on the micromanipulator base or using an piezoelectric device built into the electrometer holding the microelectrode (W.P. Instruments electrometer Model 57071A). All cell impalements were made within 100 μm of the tip of the perfusion pipette.

Electrical Measurements

The electrical circuit used for the measurement of V_e and the transepithelial resistance (R_e, Ω cm^2) or conductance (G_e, mS cm^{-2}) was identical to that described in the first paper of this series (Fig. 1 in Hebert et al., 1984). The equivalent short circuit (J_{sc}, A cm^{-2}), or equivalent ionic flux (J_e, pEq sec^{-1} cm^{-2}), was calculated from Ohm's law, using the spontaneous open-circuit transepithelial voltage and the measured value of G_e, as (Schultz, Frizzell & Nellans, 1977; Hebert et al., 1984)

$$J_e = I_{sc}/F = \frac{V_e G_e}{F}. \tag{1}$$

As indicated in the preceding paper, J_e values calculated in this fashion were virtually identical in magnitude to measured rates of net Cl^- absorption in the mouse mTALH (Hebert et al., 1984).

For intracellular recordings, the voltage across the basolateral cell membrane (V_{bl}, mV) was measured between the microelectrode impaling the cell and the bath electrode (e_2 in Fig. 1 of Hebert et al., 1984), using a high impedence differential amplifier (W.P.-Instruments, Model 7071A, New Haven, CT). The voltage across the apical cell membrane (V_a, mV) was calculated as ($V_e - V_{bl}$). All voltages were measured with reference to bath ground and recorded on a strip chart recorder (Model 2200S, Gould, Inc., Cleveland, OH).

Transepithelial electrical conductances were calculated as described in the preceding paper (Hebert et al., 1984). The apical membrane to basolateral membrane resistance ratio (R_a/R_{bl}) was estimated from voltage divider ratio measurements as

$$R_a/R_{bl} = \frac{\Delta V_x}{\Delta V_{bl}} - 1 \tag{2}$$

where R_a and R_{bl} are the apical and basolateral membrane resistances, respectively, and ΔV_x and ΔV_{bl} are the voltage deflections produced by positive direct current pulses across the tubule at the point of cell impalement and across the basolateral membrane, respectively. ΔV_x as calculated from cable equations as (Sackin & Boulpaep, 1981; and Koeppen et al., 1983):

$$\Delta V_x = \Delta V_0 \frac{\cosh(x/\lambda - L/\lambda)}{\cosh(L/\lambda)} \tag{3}$$

where L is the length of the perfused segment of tubule, x is the distance from the tip of the perfusion pipette to the tip of the microelectrode, and λ is the length constant.

We wish to stress in this regard that there are a number of theoretical uncertainties associated with the determination of R_a/R_{bl} values in electrically leaky epithelia. First, in an electrically leaky tubular epithelium such as *Necturus* proximal tubule, multiple cables may exist [e.g., cell-to-cell electrical communication of "cross-talk" (Anagnostopoulos, Teulon & Edelman, 1980; Boulpaep & Sackin, 1980)] such that the R_a/R_{bl} ratio will vary along the length of the tubule. While the possibility of significant cell-to-cell coupling could not be tested directly in the mTALH (because the simultaneous insertion of two microelectrodes in the same tubule was not technically feasible), such cell-to-cell electrical coupling may produce little error in the estimate of R_a/R_{bl}, judging by the analysis of Guggino, Stanon and Giebisch (1982*b*). Second, voltage drops across junctional complexes can be considerable in leaky epithelia and may add to the measured ΔV_{bl} value, thus resulting in an underestimate of the R_a/R_{bl} ratio (Boulpaep & Sackin, 1980). The significance of this latter problem is unknown in the mouse mTALH.

Because of these uncertainties, we accept the R_a/R_{bl} ratios reported in this paper as, at best, approximate estimates of the actual R_a/R_{bl} ratios in these mTALH segments. Consequently, all interpretations of the effects of various experimental maneuvers on the R_a/R_{bl} ratio were derived exclusively from paired analyses. All voltage deflections, except ΔV_x, were read from the strip chart recorder at 100–150 mS after the onset of the current pulse, since pipette and tubule capacitance changes were complete at this time (Hebert et al., 1984).

It is also pertinent to state clearly that, in our experience, the technical constraints of the system were such that a significant fraction of the cellular impalement experiments did not yield stable, acceptable measurements of V_{bl}. In more than half of the cellular impalements with microelectrodes, V_{bl} began to decay towards zero almost immediately after the initial impalement voltage spike, and reached near zero values in less than 1–3 min. Since the voltage divider ratio ($\Delta V_{bl}/\Delta V_x$) decreased as V_{bl} depolarized toward zero, these impalements were considered unacceptable. In other words, approximately 50% of all experiments in which a tubule had been mounted for routine perfusion were not successful in terms of cellular impalement.

The origin of such voltage decays in V_{bl} cannot be defined explicitly, but we have assumed that they occurred because of large basolateral cell membrane shunts produced by insertion of the microelectrode. Acceptable microelectrode impalements were characterized by steady-state values of V_{bl} that were equal to or greater than that of the initial impalement spike. Most of these latter impalements could be maintained for at least 15 min, but rarely for more than 30 min. Additional criteria for acceptable impalements included: return of the electrode to within 5 mV of baseline after spontaneous or intentional withdrawal of the microelectrode from the cell; and microelectrode tip potentials of less than 5–6 mV.

Statistical Analyses

Three to five current injections were performed for each experimental condition in a given tubule, and G_e and R_a/R_{bl} values were calculated for each injection. The average of these values provided the mean value for the tubule for that condition. These average values from a number of tubules were used to compute a mean ± SEM for the indicated number of tubules (n). Statistical significance for mean paired differences were evaluated by the Student's t test and probabilities (P) were computed from the t distribution with significance being <0.05. A Hewlett-Packard 9845B computer was used to perform all calculations.

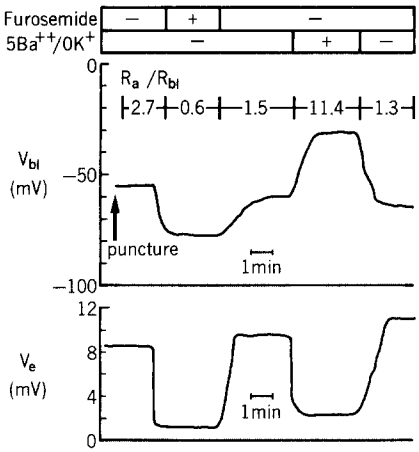

Fig. 1. Representative cell impalement of an *in vitro* microperfused mouse mTALH segment. ADH was uniformly present in the bath at a concentration of 10 μU/ml. The arrow in the upper panel indicates the point of cell impalement across the basolateral membrane. The solid line in the upper panel represents V_{bl}, the voltage across the basolateral membrane, under control conditions and when either 10^{-4} luminal furosemide or luminal 5 mM Ba^{++}, zero K^+ was present. The apical-to-basolateral membrane resistance ratio, R_a/R_{bl}, for each of these conditions is shown at the top of this panel. The lower panel shows the spontaneous transepithelial voltage, V_e, corresponding to each of the steady-state V_{bl} values shown in the upper level

Results

Experimental Design

Figure 1 presents the results of a representative cell impalement experiment that was considered acceptable. The mTALH tubule segment was perfused at 37°C, with ADH uniformly present in the bath. After V_e and G_e reached steady-state values considered to represent the ADH-dependent case (Hebert et al., 1981*a*; and Fig. 4 in Hebert et al., 1984), a cell was impaled across the basolateral membrane, indicated by the arrow in the upper panel of Fig. 1. The results in Fig. 1 illustrate that either 10^{-4} M luminal furosemide or luminal 5 mM Ba, zero K^+ produced falls in V_e to near-zero values. However, these two maneuvers had different effects on the values of V_{bl} and R_a/R_{bl}. 10^{-4} M luminal furosemide hyperpolarized V_{bl} and reduced R_a/R_{bl} while luminal 5 mM Ba^{++}, zero K^+ depolarized V_{bl} and dramatically increased R_a/R_{bl}. Finally, Fig. 1 shows that both the furosemide effect and the luminal 5 mM Ba^{++}, zero K^+ effect were reversible when control conditions were reinstituted. This latter reversal to control values when control luminal and bathing solutions replaced experimental solutions was also used in our experiments as a required test of acceptability for cellular impalement studies.

Table 1. Effect of luminal zero K^+, 5 mM Ba^{++} on the ADH-dependent R_a/R_{bl}

Lumen K^+	Lumen Ba^{++}	R_a/R_{bl}
(mM)	(mM)	
5	0	1.9 ± 0.6
0	5	12.9 ± 2.7
	Mean paired difference (n = 3)	10.8 ± 3.3 ($P < 0.02$)

Paired measurements of the R_a/R_{bl} ratio were made in the indicated number of tubules using the protocol illustrated in Fig. 1. The results are expressed as mean values ± SEM.

It will be noted from Fig. 1 that the required experimental sequence control period : experimental period : control period, using either luminal zero K^+, 5 mM Ba^{++} or 10^{-4} M luminal furosemide during the experimental period, could be completed easily during the time course of a successful impalement, that is, approximately 15 min (*cf.* Materials and Methods). Thus in the present studies, it was technically feasible to carry out paired observations on the effects of either luminal zero K^+, 5 mM Ba^{++} (Table 1) or luminal 10^{-4} M furosemide (Figs 2 and 3) on intracellular electrical recordings.

However, as noted previously for these mTALH segments (Hebert et al., 1981*a*, 1984), ADH-mediated increases in V_e, G_e and J_{Cl}^{net} generally reach steady-state values approximately 15–20 min after exposure of a tubule to hormone. Since the present successful cellular impalements ordinarily lasted only 15 min (*cf.* Materials and Methods), it was not possible to carry out paired observations on the effects of ADH on intracellular electrical recordings. Rather, all the experiments reported in this paper were carried out either for the ADH-dependent case (Figs. 1 and 3; Table 1) or for the ADH-independent (Fig. 2) case.

Effect of Luminal 5 mM Ba^{++}, Zero K^+ on R_a/R_{bl}

In order to test directly the contribution of K^+ to apical membrane conductive pathways, we evaluated the effect of luminal zero K^+, 5 mM Ba^{++} on the R_a/R_{bl} ratio. The results of a series of studies using the protocol illustrated in Fig. 1 are presented in Table 1. The data indicate clearly that replacing control luminal solutions with those containing luminal zero K^+, 5 mM Ba^{++} produced more than a tenfold increase in the R_a/R_{bl} ratio. This finding is in close accord with the observations of Greger and

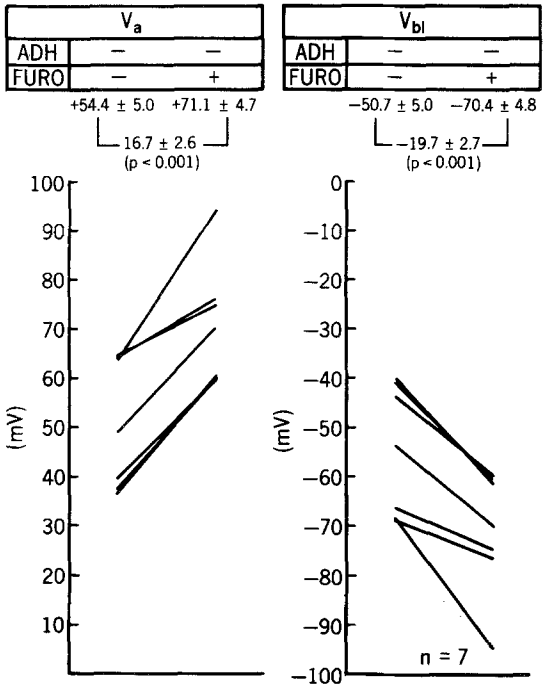

Fig. 2. The effect of 10^{-4} M luminal furosemide on the voltage across the apical membrane, V_a, and basolateral membrane, V_{bl}, the mouse mTALH, in the absence of ADH. The mTALH cells were impaled when V_e and G_e reached the steady-state nadir described in the text. Each line connects V_a or V_{bl} values for individual tubules

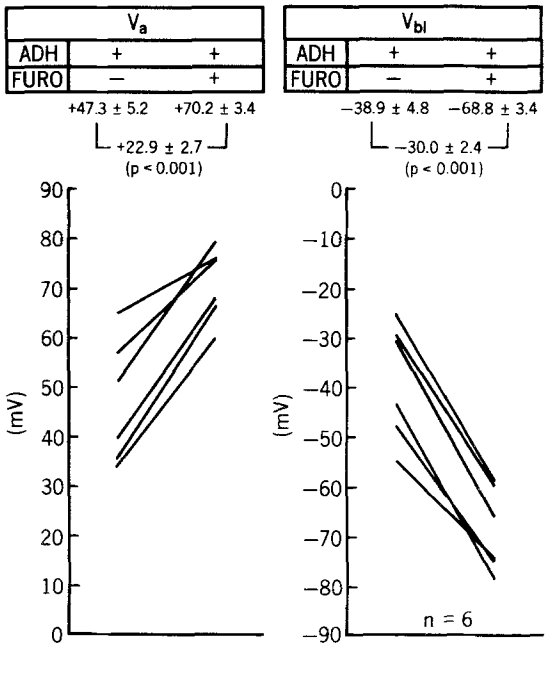

Fig. 3. The effect of 10^{-4} M luminal furosemide on the voltage across the apical membrane, V_a, and basolateral membrane, V_{bl}, in the mTALH in the presence of ADH (10 μU/ml). The mTALH cells were impaled when V_e and G_e reached the steady-state maximum values described in the text. Each line connects V_a or V_{bl} values for individual tubules

Schlatter (1983), in the rabbit cTALH, and of Guggino et al. (1982*a*) and Oberleithner et al. (1983*b*), in the early distal tubule of *Amphiuma* kidney. In these latter two diluting segments, both sets of investigators found that the combination of luminal K^+ omission and Ba^{++} addition increased the R_a/R_{bl} ratio from threefold to more than tenfold and deduced, from these observations, that apical membranes in those diluting segments contained significant K^+ conductances. Thus the results presented in Table 1 are consistent with the arguments presented in the preceding paper (Hebert et al., 1984) and with the conclusions of Greger and Schlatter (1983), Guggino, Stanton & Giebisch (1982*a*) and Oberleithner et al. (1983*b*) for other renal diluting segments, namely, that K^+ was the primary conductive species traversing apical membranes.

Alternatively, it could be argued that, since lumina zero K^+, 5 mM Ba^{++} also reduced dramatically the rate of net Cl^- absorption in these segments (Table 7 in Hebert et al., 1984), the increase in the R_a/R_{bl} ratio shown in Table 1 might have been due to a reduction in R_{bl} attendant on a fall in the rate of net Cl^- absorption. However, this latter possibility seems improbable since, as shown in Fig. 1 (and subsequently in Tables 2 and 3), inhibition of net Cl^- absorption by 10^{-4} M luminal furosemide resulted in a reduction in the R_a/R_{bl} ratio. These results with furosemide thus imply that a reduction in the rate of net Cl^- absorption may have raised, rather than lowered R_{bl}, a conclusion supported directly by the observation that 10^{-4} M luminal furosemide blocked the ADH-dependent increase in basolateral Cl^- conductance in these tubules (Tables 2 and 3 in Hebert et al., 1984).

Thus it is plausible to consider that the increase in R_a/R_{bl} produced by luminal zero K^+, 5 mM Ba^{++} was primarily the consequence of an increase in R_a, and that, for such circumstances, R_{bl} may have increased rather than decreased. While the latter argument has not been verified explicitly, the results in Table 1 therefore suggest that K^+ accounted for at least 85% of apical membrane conductance, a result in accord with the arguments set forth in the preceding paper (Hebert et al., 1984).

Another way to test the contribution of K^+ to apical membrane conductance was to evaluate the change in apical membrane voltage (V_e) produced by raising luminal K^+ concentrations from 5 to 50 mM in the presence of 10^{-4} M luminal furosemide,

Table 2. Effect of 10^{-4} M luminal furosemide on the ADH-independent V_e and R_a/R_{bl}

Luminal furosemide	V_e	R_a/R_{bl}
(M)	(mV)	
0	3.7 ± 0.3	1.2 ± 0.4
10^{-4}	0.7 ± 0.2	0.5 ± 0.1
Mean paired difference	3.0 ± 0.3 ($P < 0.001$; $n = 7$)	0.7 ± 0.3 ($P < 0.1$; $n = 3$)

The values of V_e are for all the paired experiments shown in Fig. 2. The values of R_a/R_{bl} are for three of the tubules shown in Fig. 2.

Table 3. Effect of 10^{-4} luminal furosemide on ADH-dependent V_e and R_a/R_{bl}

Luminal furosemide	V_e	R_a/R_{bl}
(M)	(mV)	
0	+8.4 ± 0.7	2.2 ± 0.6
10^{-4}	+1.3 ± 0.3	0.6 ± 0.1
Mean paired difference	7.1 ± 0.7 ($P < 0.001$; $n = 6$)	1.6 ± 0.4 ($P < 0.02$; $n = 6$)

The values of both V_e and R_a/R_{bl} are for all the paired experiments shown in Fig. 3.

that is, under conditions where apical and basolateral membranes were clamped to virtually identical values with symmetrical external solutions, and V_e was zero (Figs. 1–3). For this tenfold change in luminal K^+ concentration under these conditions, the mean change in V_e was 53.1 mV, which represents 87% of the 61.4 mV change predicted for ideal Nerstian behavior and a K^+ transference number of unity for apical membranes. These results therefore show that K^+ was the major conductive species traversing apical membranes.

Effect of Luminal Furosemide on V_a, V_{bl} and R_a/R_{bl} in the Presence and Absence of ADH

A general observation in a variety of furosemide-sensitive Cl^--transporting epithelia is that furosemide hyperpolarizes apical and basolateral membranes and abolishes the spontaneous transepithelial voltage. In the early distal tubule of *Amphiuma* kidney, Oberleithner *et al.* (Oberleithner, Giebisch, Lang & Wang, 1982*a*; Oberleithner, Guggino & Giebisch, 1982*b*, 1983*a*) found that the reduction of the spontaneous lumen-positive V_e produced by luminal furosemide could be accounted for entirely by hyperpolarization of the basolateral cell membrane; these workers did not, however, describe the effects of furosemide on the R_a/R_{bl} ratio. In perfused diluting segments of salamander kidney, the preliminary results of Sackin Morgunov and Boulpaep (1982) indicate that 10^{-5} M luminal furosemide hyperpolarized apical and basolateral membranes concomitantly with abolishing V_e. Furthermore, entirely comparable results were reported by Greger (1981) for the rabbit cTALH.

The effects of 10^{-4} M luminal furosemide on simultaneously measured values of V_e, V_a, V_{bl} and R_a/R_{bl} in the isolated mouse mTALH, using the protocol illustrated in Fig. 1, are presented in Figs. 2 and 3 and in Tables 2 and 3. We note in this regard that, when isolated mouse mTALH segments are first mounted, V_e, G_e and the net rate of Cl^- absorption are relatively high and decline, generally over 20–40 min, to steady-state nadir values which are taken to represent the ADH-independent case. The subsequent rises in V_e, G_e and the net rate of Cl^- absorption when ADH is added to the bath are taken to represent the ADH-dependent case (Hebert et al., 1981*a*; and Fig. 4 in Hebert et al., 1984). However, the measurement of both the ADH-independent and the ADH-dependent values of V_e, G_e and J_e in a given tubule generally requires at least 30 min (Fig. 4 in Hebert et al., 1984), while, as indicated in Materials and Methods, our cellular impalements could generally be maintained for 15 min, but rarely for more than 30 min.

Accordingly, in the present experiments, intracellular impalements were carried out in two separate sets of tubules. In one group of tubules, cellular impalements were performed when V_e and G_e had declined to steady-state nadir values (Fig. 4 in Hebert et al., 1984); these data, presented in Fig. 2 and Table 2, were taken to represent ADH-independent values. In the second group of tubules, 10 μU/ml ADH was added to bathing solutions after V_e and G_e had declined to steady-state nadir values, and cellular impalements were carried out after V_e and G_e had increased to steady-state maximal values (Fig. 4 in Hebert et al., 1984); these data, presented in Fig. 3 and Table 3, were taken to represent ADH-dependent values.

The data presented in Fig. 2 and Table 2 indicate that, in paired observations for the ADH-independent case, the application of 10^{-4} M luminal furosemide hyperpolarized significantly both V_a and V_{bl} to virtually identical values ($P < 0.001$ in both cases; Fig. 2), while V_e fell to a value indistinguishable from zero. The R_a/R_{bl} ratio was reduced (Table 2), but the mean paired difference between R_a/R_{bl} values with and without furosemide listed in Table 2 did not achieve statistical significance; however,

Table 4. Effects of ADH and furosemide on apical and basolateral membrane voltages

Source	ADH	$(V_a - V_a^f)$	$(V_{bl} - V_{bl}^f)$	$(V_e - V_e^f)$	
		(mean paired differences, mV)			
Fig. 3; Table 2	−	−16.7 ± 2.6	19.7 ± 2.7	3.0 ± 0.3	($P < 0.001$)
Fig. 4; Table 3	+	−22.9 ± 2.7	30.0 ± 2.4	7.1 ± 0.7	($P < 0.001$)
Mean unpaired differences		6.2 ± 3.8 (NS)	10.3 ± 3.7 ($P < 0.02$)	4.1 ± 0.7 ($P < 0.001$)	

The first horizontal row provides the mean paired differences for the indicated data without ADH from Fig. 2 and Table 2. The second horizontal row provides the mean paired differences for the data with ADH from Fig. 3 and Table 3. The vertical columns represent mean unpaired differences between each of the indicated values, without and with hormone.

these R_a/R_{bl} values were measured in only three of the seven tubules listed in Fig. 2.

The data presented in Fig. 3 and Table 3 indicate that entirely comparable results obtained in paired observations for the ADH-dependent case. Thus the results shown in Fig. 3 indicate that 10^{-4} M luminal furosemide hyperpolarized significantly both V_a and V_{bl} ($P < 0.001$ in both cases), and that, with furosemide, V_a and V_{bl} were virtually identical. The values listed in Table 3 indicate that, simultaneously, V_e fell significantly ($P < 0.001$) to a value near zero, while the R_a/R_{bl} ratio was reduced by approximately 67%. In the latter circumstance, the R_a/R_{bl} ratios were measured in all of the tubules shown in Fig. 3, and the mean paired difference between R_a/R_{bl} values with and without furosemide was clearly significant ($P < 0.02$).

Analysis of the ADH Effect

The results in Figs. 2 and 3 and Tables 2 and 3 indicate that, in the presence of 10^{-4} M luminal furosemide, the V_e values with or without ADH were virtually zero (in close accord with previous results; Hebert et al., 1981*a*, 1984), and that the values of V_{bl}, either with or without ADH, were nearly identical; thus the values of V_a (calculated as $[V_e - V_{bl}]$) in the presence of furosemide were also identical, with or without ADH. Now since the net rate of Cl^- absorption in these tubules, either measured chemically (Hebert et al., 1981*a*, 1984) or estimated electrically from the equivalent short-circuit current (Hebert et al., 1984) is also virtually zero with 10^{-4} M luminal furosemide, we may use the results with 10^{-4} M luminal furosemide as a basal, or ground, set of data for assessing the factors responsible for the change in V_e that accompanies net rates of Cl^- absorption, and the effect of ADH on these processes.

By definition, V_e may be expressed as

$$V_e = V_a + V_{bl}. \tag{4}$$

Since the luminal and peritubular solutions were symmetrical, it may be inferred that transport-related events across apical and basolateral membranes were the primary determinants of the lumen-positive V_e, although obviously the magnitude of the latter was also dependent on the magnitude of the ratio between cellular and paracellular conductances. According to this view, a spontaneous lumen-positive voltage accompanying net Cl^- absorption requires that, during net Cl^- absorption (i.e., in the absence of furosemide), V_{bl} depolarized to a greater degree than V_a. Similarly, since ADH increases V_e in this nephron segment (Tables 2 and 3; Hebert et al., 1981*a*, 1984), it may be argued that, during net Cl^- absorption, V_{bl} may have depolarized to a greater degree when ADH was present rather than absent.

A phenomenological analysis of these factors using the data from Figs. 2 and 3 and Tables 2 and 3 is presented in Table 4. Each horizontal row represents data from paired observations (Fig. 2 and Table 2, without ADH; Fig. 3 and Table 3, with ADH), while the vertical columns provide unpaired group comparisons between the ADH-dependent and ADH-independent cases. The term $(V_a - V_a^f)$ is the mean paired difference between apical membrane voltages without and with furosemide, respectively (Fig. 2, without ADH; Fig. 3, with ADH); the term $(V_{bl} - V_{bl}^f)$ is the mean paired difference between basolateral membrane voltages without and with furosemide, respectively (Fig. 2, without ADH; Fig. 3, with ADH); and the term $(V_e - V_e^f)$ is the mean paired difference between transepithelial voltages without and with furosemide, respectively (Table 2, without ADH; Table 3, with ADH).

Inspection of the paired comparisons in Table 4 (the horizontal rows) indicates that, either with or without hormone, basolateral membranes depolarized to a significantly greater degree than apical

membranes in the presence of net Cl^- absorption (i.e., without furosemide). Thus the furosemide effect on V_e (Tables 2 and 3 could be accounted for entirely by the simultaneous hyperpolarization of apical and basolateral cell membranes to identical values, with the degree of furosemide-induced basolateral membranes hyperpolarization clearly exceeding the furosemide-induced hyperpolarization of apical membranes. These observations coincide closely with the earlier conclusions of Oberleithner et al. (1982*a*,*b*, 1983*a*) in early distal tubules of *Amphiuma* kidney, and Greger (1981), in the rabbit cTALH, about the furosemide-induced abolition of a lumen-positive V_e in renal diluting segments.

An inspection of the unpaired data in Table 4 (i.e., without and with ADH) indicates that the unpaired difference between the ADH-independent and ADH-dependent values of $(V_a - V_a^f)$ was 6.1 ± 3.8 mV; this difference did not achieve statistical significance. Thus this unpaired comparison does not permit unambiguously the conclusion that, during net Cl^- absorption, there was a greater depolarization of apical membranes with ADH than without ADH; but the results are at least consistent with such a possibility.

However, the results in Table 4 also show that the unpaired comparison of $(V_{bl} - V_{bl}^f)$ value without and with ADH was 10.3 ± 3.7 mV and that this difference was clearly significant. In other words, these comparisons are consistent with the view that the ADH-mediated increase in V_e (Table 4; 4.1 mV) observed in these tubules during net Cl^- absorption occurred because the ADH-mediated change in apical membrane depolarization (Table 4; 6.1 ± 3.8; NS) was less than the ADH-mediated increase in basolateral membrane depolarization (Table 4; 10.3 ± 3.7; $P < 0.02$). It is relevant in this regard to stress the fact that ADH increases both J_{Cl}^{net} and the net electrical flux (J_e, pEq sec^{-1} cm^{-2}) in these tubules. Thus the observation that V_{bl} was depolarized to a greater degree with ADH than without hormone (Table 4) is consistent with the view (Hebert et al., 1983) that the majority of net Cl^- efflux across basolateral membranes was conductive.

It is pertinent to compare the present results with previously reported cellular impalement data on renal tubular diluting segments. In other isolated renal diluting segments, either mammalian (Greger, 1981; Murer & Greger, 1982) or amphibian (Oberleithner et al., 1982*a*,*b*, 1983*a*), the hyperpolarized value of V_{bl} in the presence of luminal furosemide has been noted to be in the range of 77–84 mV, with one added report of 91 mV in *Amphiuma* diluting segments (Guggino et al., 1982*b*). Thus the mean values of V_{bl} with luminal furosemide reported in the present experiments, either with (Fig. 3) or without (Fig. 2) ADH, are within 10% of the values for the furosemide-dependent V_{bl} in other renal diluting segments.

In contrast, the ADH-dependent value of V_{bl} without furosemide, that is, during net Cl^{-1} absorption (Fig. 3, −38.9 ± 4.8 mV), is considerably less than −65 to −75 mV, which represents the range of values for V_{bl} during net Cl^- absorption reported for the rabbit cTALH (Greger, 1981; Murer & Greger, 1982) and for amphibian diluting segments (Oberleithner et al., 1982*a*,*b*, 1983*a*). However, in these latter nephron segments, the rate of net Cl^- absorption, measured either from net electrical flux studies or from net Cl^- fluxes, is appreciably smaller than in the mouse mTALH: in the mouse mTALH, the ADH-dependent value of J_{Cl}^{net}, for a transepithelial V_e of 10 mV, is approximately 10,000 pEq sec^{-1} cm^{-2} (Table 5; Hebert et al., 1981*a*, 1984); in the rabbit cTALH, J_e is in the range of 2,000 pEq sec^{-1} cm^{-2} at a V_e of approximately 6–10 mV (Greger, 1981; Murer & Greger, 1982); and in isolated amphibian renal diluting segments, J_{Cl}^{net} is approximately 450–500 pEq sec^{-1} cm^{-2} at a V_e of approximately 9 mV. Thus the observation that, during net Cl^- absorption, V_{bl} is smaller in the mouse mTALH than in other renal tubular diluting segments correlates closely with the fact that the rate of net Cl^- absorption in the mouse mTALH exposed to ADH is considerably greater than in other renal tubular diluting segments.

Partial Analysis of the Determinants of V_e

The results in the preceding section indicate that the onset of the net Cl^- absorption, using cellular voltage data with 10^{-4} M luminal furosemide as the ground state, resulted in simultaneous depolarization of V_a and V_{bl}, with V_e being lumen-positive because, during net Cl^- absorption, V_{bl} depolarized to a greater degree than V_a (Table 4). Accordingly, it was relevant to examine the potential contributions of an apical K^+ current and a basolateral Cl^- current to the depolarization of V_a and V_{bl}, respectively, during net Cl^- transport; or conversely, to the contribution of reduced apical K^+ currents and basolateral Cl^- currents to the furosemide-induced hyperpolarization of V_a and V_{bl} (Figs. 2, 3).

The frame of reference for these experiments derives from the following considerations. From Ohm's law, V_a may be defined as:

$$V_a = E_a^K - i_a^K/g_a^K \tag{5}$$

where E_a^K is the K^+ equilibrium voltage across apical membranes during net Cl^- absorption, i_a^K is the

K^+ current across apical membranes during net Cl^- absorption, and g_a^K is the K^+ conductance of apical membranes under these conditions.

To calculate i_a^K, we follow the same line of argument developed in the preceding paper (Fig. 9 and Table 6 of Hebert et al., 1984). Thus G_c^{min}, the minimum conductance of the transcellular pathway, may be taken to be

$$G_c^{min} = G_e - G_s^{max} \tag{6}$$

where G_e is the control transepithelial conductance measured using positive luminal current injections with luminal 5 K^+, zero Ba^{++}; G_s^{max} is the transepithelial conductance measured in the same tubule, also with positive current injections, with luminal zero K^+, 5 mM Ba^{++}; and G_c^{min} is the mean paired difference between G_e and G_s^{max}. Since K^+ is the predominant conductive species across apical membranes (Table 1; and Fig. 9 and Table 6 in Hebert et al., 1984), we have, for the parallel array of apical membranes and the paracellular pathway [Eqs. (8)–(11) in Hebert et al., 1984]

$$i_a \cong i_a^K \leqq G_s^{max} V_e. \tag{7}$$

For R_a/R_{bl} ratios measured in tubules where G_e and G_s^{max} are simultaneously measured, we have

$$R_a + R_{bl} \leqq \frac{1}{G_c^{min}} \tag{8a}$$

$$R_a/R_{bl} = \alpha \tag{8b}$$

and therefore:

$$R_a \leqq \frac{1}{G_c^{min}(1 + 1/\alpha)}. \tag{8c}$$

Thus for K^+ as the major conductive species of apical membranes (Table 1; Hebert et al., 1984), we obtain

$$g_a^K \cong 1/R_a. \tag{8d}$$

Accordingly, Eq. (5) may be rewritten, using Eqs. (6)–(8d), as

$$V_a \cong E_a^K - [(G_s^{max} V_e) R_a]. \tag{9}$$

In the case of Cl^- transport across basolateral membranes, Eq. (5) becomes

$$V_{bl} = E_{bl}^{Cl} - i_{bl}^{Cl}/g_{bl}^{Cl} \tag{10}$$

where E_{bl}^{Cl} is the Cl^- equilibrium voltage across apical plasma membranes during net Cl^- absorption, i_{bl}^{Cl} is the Cl^- current across basolateral membranes during net Cl^- absorption (given by $J_e F$, as indicated in Figs. 7 and 8 of Hebert et al., 1984), and g_{bl}^{Cl} is the Cl^- conductance of basolateral membranes for that circumstance. Similarly, from Eqs. (8a) and (8b), we have

$$R_{bl} \leqq \frac{1}{G_c^{min}(1 + \alpha)}. \tag{11}$$

Clearly, g_{bl}^{Cl} will be less than $1/R_{bl}$: from the analysis presented in the Introduction and in the prior paper (Table 10 in Hebert et al., 1984), ions other than Cl^- also carry significant current across basolateral membranes. Hence by substituting R_{bl} from Eq. (11) for the g_{bl}^{Cl} term in Eq. (10), we obtain

$$V_{bl} = E_{bl}^{Cl} - [(J_e F)(>R_{bl})] \tag{12}$$

where the term $[(J_e F)(>R_{bl})]$ provides a minimal estimate of the depolarizing effect of Cl^- current on the difference between V_{bl} and V_{bl}^f.

The experimental protocol involved paired measurements of V_e, V_a, V_{bl}, $(V_a - V_a^f)$ and $(V_{bl} - V_{bl}^f)$ with and without furosemide using the same protocol shown in Fig. 3 and Table 3; and in the same tubules, control measurements of G_e and J_e, and assessment of G_c^{min} as $(G_e - G_s^{max})$ according to Eq. (6), using the same protocol illustrated in the preceding paper (Fig. 9 and Table 6 in Hebert et al., 1984). The results of these experiments are presented, for the ADH-dependent case, in Table 5.

A comparison of the V_a, $(V_a - V_a^f)$, V_{bl} and $(V_{bl} - V_{bl}^f)$ values shown in Table 5 with those presented in Fig. 3 and Table 4 indicates a remarkably close concordance of experimental values between the

Table 5. Simultaneous analysis of the determinants of the ADH-dependent V_e

A. Voltage Values

V_e	V_a	$(V_a - V_a^f)$	V_{bl}	$(V_{bl} - V_{bl}^f)$
		(mV)		
8.0 ± 0.9	46.7 ± 7.4	−22.5 ± 4.0	−38.5 ± 6.7	29.4 ± 3.3

B. Conductance, R_a/R_{bl} and J_e values

G_e	G_c^{min}	G_s^{max}	R_a/R_{bl}	J_e
	(mS cm^{-2})			(pEq sec^{-1} cm^{-2})
153.9 ± 32.4	42.1 ± 5.1	111.9 ± 27.7 (n = 4)	2.5 ± 0.8	11,060 ± 1,900

In these four tubules, V_e, V_a, V_{bl}, J_e, R_a/R_{bl} and G_e were measured simultaneously with control luminal solutions containing 5 mM K^+, zero Ba^{++}. $(V_a - V_a^f)$ and $(V_{bl} - V_{bl}^f)$ were measured inpaired observations as shown in Fig. 3 and Table 4. G_c^{min} and G_s^{max} were computed as described in the text according to Eq. (6), also in the same tubules. The data are expressed as mean values ± SEM.

Table 6. Analysis of the data in Table 5 in terms of the theoretical frame of reference [Eqs. (5)–(12)]

A. Apical depolarization by K^+ currents		
$(V_a - V_a^f)$	$[(G_s^{max}V_e)R_a]$	$[(G_s^{max}V_e)R_a]/(V_a - V_a^f)$
(mV)	(mV)	(%)
−22.5 ± 4.0	15.2	67.6
B. Minimal estimate of basolateral depolarization by Cl^- currents		
$(V_{bl} - V_{bl}^f)$	$[(J_eF)(>R_{bl})]$	$[(J_eF)(>R_{bl})]/(V_{bl} - V_{bl}^f)$
(mV)	(mV)	(%)
−38.5 ± 6.7	7.2	18.7

The values of $(V_a - V_a^f)$ and $(V_{bl} - V_{bl}^f)$ are from Table 5. $[(G_s^{max}V_e)R_a]$ and $[(J_eF)(>R_{bl})]$ were computed from Eqs. (9) and (12), respectively, using the data in Table 5.

two different sets of experiments. Likewise, the ADH-dependent V_e value of 8.0 ± 0.9 mV and the R_a/R_{bl} value of 2.5 ± 0.8 listed in Table 5 were virtually the same as those presented in Table 3 for a different group of experiments under identical conditions. Finally, a comparison of the G_e, G_c^{min} and G_s^{max} values listed in Table 5 with those presented in the preceding paper (Fig. 9 and Table 6 in Hebert et al., 1984) indicates a close concordance between these experimental values. In other words, the data in Table 5, all obtained in paired observations on the same set of tubules, constitute a reasonably representative set of values for these parameters in the ADH-dependent case.

The data in the upper half of Table 6 compare the measured values of $(V_a - V_a^f)$ with the values of $[(G_s^{max}V_e)R_a]$ calculated from Eq. (9). These results show that 68% of the apical membrane depolarization occurring during net Cl^- absorption (or alternatively, that 68% of the furosemide-induced hyperpolarization of apical membranes) could be accounted for by a depolarizing K^+ current across apical membranes which occurred during net Cl^- absorption (i.e., when furosemide was absent). The discrepancy listed in Table 6 between the measured value of $(V_a - V_a^f)$ and the $[(G_s^{max}V_e)R_a]$ term in Eq. (9) remains unexplained. In principle, such a discrepancy might occur because intracellular K^+ activities fell during net Cl^- absorption or because other ions had finite conductances across apical membranes. These possibilities, and other alternate explanations, require additional evaluation with technologies not yet routinely applicable for mTALH cells having small heights, i.e., 4 μM.

The results in the lower half of Table 6 show that the $[(J_eF)(>R_{bl})]$ term in Eq. (12) accounted for 19% of the measured $(V_{bl} - V_{bl}^f)$ term from Table 5. It should be emphasized again that the value of $[(J_eF)(>R_{bl})]$ in Eq. (12) is a minimal estimate of the extent to which basolateral conductive Cl^- flux contributed to basolateral membrane depolarization during net Cl^- absorption. In other words, Cl^- currents across basolateral membranes probably contributed significantly more than 19% of the measured difference between $(V_{bl} - V_{bl}^f)$.

Discussion

The studies reported in this paper were designed to assess, using cellular impalement techniques in combination with standard transepithelial electrical measurements, certain aspects of the model for net Cl^- absorption in the isolated mouse mTALH (Hebert et al., 1984), some of the determinants of the spontaneous lumen-positive V_e that attends net Cl^- absorption, and how ADH might augment both J_{Cl}^{net} and V_e in these tubules. The data reported in this paper permit the following general conclusions.

First, the fact that substituting luminal 5 mM Ba^{++}, zero K^+ for luminal 5 mM K^+ resulted in a tenfold increment in the R_a/R_{bl} ratio (Table 1) is consistent with the argument (Hebert et al., 1984) that K^+ was the primary conductive species traversing apical plasma membranes. Further evidence for the view that K^+ was the major conductive species crossing apical membranes obtains from the observation that tenfold increments in luminal K^+ concentrations produced a mean change of 53.1 mV in V_a.

Second, we note again that, with 10^{-4} M furosemide: V_a and V_{bl} had virtually the same values (Figs. 2 and 3), so that V_e was near zero (Tables 2 and 3; Hebert et al., 1981*a*, 1984); and J_{Cl}^{net} was also indistinguishable from zero (Hebert et al., 1981*a*, 1984). Thus it is permissible to consider experimental results with 10^{-4} M luminal furosemide as the ground, or basal, state for assessing the relations between V_e and J_{Cl}^{net} and the effects of ADH in these processes.

The results in Figs. 2 and 3 and Table 4, which were derived from paired observations with and without furosemide, indicate that 10^{-4} M luminal furosemide hyperpolarized V_{bl} to a greater degree than V_a. Stated differently, lumen-positive values of V_e (Table 2 and 3) occurred because, during net Cl^- absorption, V_{bl} depolarized to a greater degree than V_a. Moreover, the unpaired comparisons among tubules with and without ADH (the bottom row in Table 4), indicate that the ADH-dependent increase in V_e was referable, at least in part, to the fact that, during net Cl^- absorption, V_{bl} depolarized to a

greater degree in the presence of ADH than in the absence of ADH.

Third, a comparison of the ADH-dependent value of $(V_a - V_a^f)$ in Tables 5 and 6 with the value of i_a^K/g_a^K [Eq. (5)] estimated from the $[(G_s^{max}V_e)R_a]$ term in Eq. (9) indicates that 68% of the furosemide-induced hyperpolarization of apical membranes was referable to a reduction in the $[G_s^{max}V_e)R_a]$ term in Eq. (9). Since V_e with furosemide was virtually zero (Tables 2 and 3), the shunt current i_s also approached zero. Thus for K^+ as the major conductive species traversing apical plasma membranes, it may be argued that the 15.2 mV reduction in the $[(G_s^{max}V_e)R_a]$ term listed in Table 6 was due primarily to the fact that the i_a^K term in Eq. (5) was reduced strikingly.

Finally, the results in Tables 5 and 6 indicate that the calculated value of $[(J_eF)(>R_{bl})]$ was 7.2 mV, which represents 19% of the $(V_{bl} - V_{bl}^f)$ values measured simultaneously in paired experiments (Tables 5, 6). However, as indicated in Eq. (12), the $[(J_eF)(>R_{bl})]$ term in Table 6 represents a minimal estimate of the i_{bl}^{Cl}/g_{bl}^{Cl} term in Eq. (10). Thus these data indicate that at least 19% of the hyperpolarization of V_{bl} produced by furosemide was referable to a reduction in Cl^- current across basolateral membranes.

The present experiments do not permit a direct estimate of the $(i_{bl}^{Cl}/g_{bl}^{Cl})$ term in Eq. (10), so it is not possible to determine the extent to which the $[(J_eF)(>R_{bl})]$ term in Eq. (12) underestimated the contribution of a depolarizing Cl^- current to the difference $(V_{bl} - V_{bl}^f)$. However, it is particularly relevant to note that, in other renal diluting epithelia sensitive to furosemide, the latter agent consistently reduces intracellular Cl^- activities (Obeleithner et al., 1982*b*; Greger et al., 1983); and that precisely the same results have obtained in Cl^--transporting renal tubular diluting segments in which the net Cl^- absorption is abolished with luminal furosemide (Murer & Greger, 1982; Oberleithner et al., 1982*b*).

It is also reasonable to consider that an increase in intracellular Cl^- activity may have contributed in part to the depolarization of V_{bl} during net Cl^- transport (Figs. 2 and 3; Table 5), and that this increase was greater with hormone than without hormone. The line of argument is as follows. For conductive Cl^- efflux from cells to bath, the electrochemical gradient for net Cl^- flux across basolateral membranes was greater with ADH than without hormone, since, from Tables 1–3 of the preceding paper (Hebert et al., 1984) the ADH-mediated increase in the Cl^- conductance of basolateral membranes was secondary to hormone-induced increases in net Cl^- absorption. But the results in Table 4 indicate that, during net Cl^- absorption, the depolarization of basolateral membranes was greater with ADH than without ADH. Thus we argue that the ADH-mediated increase in J_{Cl}^{net} (Hebert et al., 1981*a*, 1984) was the consequence of a hormone-mediated increase in intracellular Cl^- activities.

Speculations on the ADH Effect

The results in the preceding paper (Hebert et al., 1984) led to the hypothesis that ADH increased the K^+ conductance of apical membranes in the isolated mouse mTALH. Furthermore, since ADH increases J_{Cl}^{net} in this nephron segment (Hebert et al., 1981*a*, 1984), it is obvious that the rate of net Cl^- flux across apical membranes must be greater with ADH than without hormone. But from the argument presented in connection with Tables 5 and 6 (*cf.* above), it is likely that the intracellular Cl^- activity in these tubules was greater with hormone than without hormone; and, for such a circumstance, the chemical driving force for electroneutral Na^+, K^+, $2Cl^-$ cotransport from lumen to cell may have been less in the presence of ADH than in the absence of hormone. These latter considerations, namely, the possibility of the simultaneous occurrence of ADH-mediated increases in J_{Cl}^{net} and in intracellular Cl^- activity, indicate that ADH may also have increased the functional number of apical membrane Na^+, K^+, $2Cl^-$ cotransport units.

An insight into how an ADH-mediated increase in the functional number of apical membrane K^+ conductance units and apical membrane Na^+, K^+, $2Cl^-$ cotransport units might contribute to hormone-mediated increases in net NaCl absorption derives from the following considerations. Specifically, we (Hebert et al., 1984) have argued that, in the mouse mTALH, approximately 50% of net Na^+ absorption traversed the paracellular route. Thus for apical membranes in parallel with an electrically leaky paracellular pathway, an ADH-mediated increase in conductive K^+ flux from cells to lumen, with the majority of this conductive K^+ flux recycled electroneutrally back into cells (Hebert et al., 1984), permitted an increase in net Na^+ absorption through the paracellular pathway. Likewise, a hormone-mediated increase in apical membrane Na^+, K^+, $2Cl^-$ cotransport units may have resulted in: an increased rate of K^+ recycling into cells; an increased driving force for net Cl^- efflux across basolateral membranes, presumably because of a rise in intracellular Cl^- activity (*see* above); and an increased net paracellular Na^+ absorption because of the rise in V_e produced by hormone-dependent in-

creases in basolateral membrane depolarization (Table 4).

Finally, it should be noted that, in apical membranes of amphibian epithelia, ADH increases both the functional number of small channels for water transport (Hays & Franki, 1970; Hays, Bourguet & Chevalier 1981; Levine et al., 1983) and the functional number of Na^+-conductive channels (Li, Palmer, Edelman & Lindemann, 1982). Likewise, in apical membranes of rabbit cortical collecting tubules, ADH increases the functional number of narrow water channels for transepithelial osmosis (Al-Zahid, Schafer, Troutman & Andreoli, 1977; Hebert & Andreoli, 1980). And with respect to the mouse mTALH, we have argued that ADH increased the functional number of both Ba^{++}-sensitive K^+-conductive channels and electroneutral Na^+, K^+, Cl^- cotransport units. Thus there may exist a degree of homology with respect to the action of ADH in hormone-sensitive epithelia, namely, to increase the functional number of transport units in apical membranes for those molecular species whose flux is augmented by ADH.

In amphibian epithelia (Leaf, 1965; Li et al., 1982), in the rabbit cortical collecting tubule (Grantham & Burg, 1966; Schafer & Andreoli, 1972), and in the mouse mTALH (Hebert et al., 1981*a*, 1984), ADH-mediated increases in transepithelial transport rates occur within minutes of hormone application to basolateral solutions. In other words, that ADH-mediated alterations in apical membrane transport process occur at rates sufficiently rapid to require activation or translocation of existing transport units, that is, by a recruitment process rather than by *de novo* synthesis.

In this regard, the fusion of sub-apical vacuoles into intramembranous aggregates in apical membranes (Hays et al., 1981; Wade, Stetson & Lewis, 1981) correlates reasonably well with the ADH-mediated hydroosmotic response (Chevalier, Bourguet & Hugon, 1974; Chevalier, Parisi & Bourguet, 1979; Bourquet, Chevalier & Hugon, 1976; Kachadorian, Wade, Uiterwyk & DiScala, 1977) in amphibian epithelia; however, these results have not yet established the explicit relation between apical membrane aggregates and ADH-dependent water channels in apical membranes. Alternatively, Li et al. (1982) have provided convincing evidence that the natriferic response of toad urinary bladder involves the conversion, or recruitment, or electrically silent Na^+ channels in apical membranes to amiloride-sensitive conductive Na^+ channels in these membranes. It is plausible that similar recruitment mechanisms may underlie ADH-dependent increases in apical membrane transport rates for hormone-targeted molecular species in different ADH-sensitive epithelia.

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