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Volume Regulation in the Early Proximal Tubule of the *Necturus* Kidney

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Summary. The ability of early proximal tubule cells of the *Necturus* kidney to regulate volume was evaluated using light microscopy, video analysis and conventional microelectrodes. *Necturus* proximal tubule cells regulate volume in both hyper- and hyposmotic solutions. Volume regulation in hyperosmotic fluids is HCO_3^- dependent and is associated with a decrease in the relative K^+ conductance of the basolateral cell membrane and a decrease in the resistance ratio, R_a/R_{bl}. Volume regulation in hyposmotic solutions is also dependent upon the presence of HCO_3^- but is also inhibited by 2 mM Ba^{2+} in the basolateral solution. Hyposmotic regulation is accompanied by an increase in the relative K^+ conductance of the basolateral cell membrane and an increase in R_a/R_{bl}. Neither hypo- nor hyposmotic regulation have any affect on the depolarization of the basolateral cell membrane potential induced by HCO_3^- removal. We conclude that volume regulation in the early proximal tubule of the kidney involves both HCO_3^--dependent transport systems and the basolateral K^+ conductance.

Key Words cell volume · bicarbonate · potassium · hypertonicity · hypotonicity

Introduction

In order to maintain flow through the kidney, it is very important that renal cells maintain their cell volume both in response to changes in plasma osmolality and to variations in the rate of solute and water reabsorption. To regulate volume, a renal cell may utilize transporters which are entirely separate from those involved in the net transport of solutes. This will in essence separate the transport function from volume regulation, ensuring that net transport is maintained during volume regulation. For example, it has been shown that the mouse medullary thick ascending limb (Hebert, 1986*a*,*b*) regulates cell volume in hyperosmotic solutions by means of parallel Na^+/H^+ and Cl^-/HCO_3^- exchangers. These two exchangers function in volume regulation and are not involved in the reabsorption of NaCl.

In contrast, in some epithelia changes in extracellular fluid osmolality have profound effect on net solute transport. For example, Ussing (1965) showed several years ago that there is a relationship between the frog skin cell's ability to control its volume and the rate of net transport such that increased osmolality decreases short-circuit current and net Na^+ reabsorption and an enhanced short-circuit current is associated with hyposmotic solutions. Likewise, in the *Necturus* proximal tubule Whittembury and Hill (1982) observed an inverse relationship between fluid reabsorption and extracellular fluid osmolality. It is obvious that if some of the same transporters are used for volume regulation and for the net transport of ions, net ion transport must be disrupted, particularly during osmotic volume regulation, since to maintain volume in different osmolalities the cells must alter their intracellular solute content by modifying the rate of one or more of its transport pathways.

The aim of the present study is to determine if *Necturus* early proximal tubule cells regulate their cell volume in anisotonic solutions and, if so, to determine the role of K^+ and HCO_3^- transport pathways in the regulation.

Materials and Methods

Kidney Preparation

Adult male and female *Necturus maculosus* obtained from Carolina Biological Supply (Burlington, NC) were kept in aquaria at 5°C until use. Kidneys were removed from decapitated animals, cut into thin cross sections and placed into ice-cold Ringer's solutions. Early proximal tubules were perfused according to the techniques first described by Burg et al. (1966). Briefly, tubules were placed in a perfusion chamber and mounted on a concentric perfusion pipette system. On the perfusion side were an outer

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Table 1. Composition in mM of the perfusion solutions

Ion	1	2	3	4	5	6	7	8	9	10	11	12
Na^+	100	100	100	100	82.5	82.5	75	75	75	75	57.5	57.5
K^+	2.5	2.5	2.5	2.5	20	20	2.5	2.5	2.5	2.5	20.0	20.0
Cl^-	98	98	98	98	98	98	73	73	73	73	73	73
HCO_3^-	10	10	—	—	10	10	10	10	—	—	10	—
HEPES	—	—	10	10	—	—	—	—	10	10	—	—
Raffinose	—	50	—	50	—	50	50	—	50	—	50	—

In all the solutions the concentrations in mM of Ca^{2+} (1.8), Mg^{2+} (1.0), and $H_2PO_4^-$ (0.5) remain unchanged. All solutions except those HCO_3^- free were bubbled with 2% CO_2 balance O_2 and contained 2.2 mM glucose and 0.25 mM glycine. HCO_3^--free solutions were bubbled with O_2. The solution pH was 7.5. All ion concentrations are in mM. Ba^{2+} was added to either solution 2 or 7. The control osmolality for all experiments was 202 mOsmol. The hyperosmotic solutions were 249 mOsmol and the hyposmotic solutions were 145 mOsmol.

holding pipette and an inner perfusion pipette. The collection side had a holding pipette with Sylgard 184 (Dow Corning, Midland, MI) for electrical isolation. The tubule chamber consisted of a trough cut in a Plexiglas® plate. The chamber volume was approximately 0.5 ml, and the perfusion rate was typically 10 ml/min. The $t_{1/2}$ of lumen and bath exchange was 1 to 2 sec (measured for the luminal exchange from the depolarization induced by glucose addition to substrate depleted tubules and for the bath from the depolarization induced by increasing basolateral K^+ to 20 mM). The composition of the perfusion solution is given in Table 1.

In experiments involving osmotic shrinking, Raffinose was added to control solutions (solutions 1, 3, and 5 are at control osmolality while 2, 4 and 6 are hyperosmotic, Table 1). In all experiments involving osmotic swelling, the cells were first bathed on both apical and basolateral sides with a solution in which the NaCl concentration was reduced and replaced with enough raffinose to match the osmolality of the control solution (solutions 7, 9 and 11, Table 1). The osmolality was reduced by perfusing a solution with the identical ionic composition but without raffinose (solutions 8, 10 and 12, Table 1). In HCO_3^--free solutions (solutions 3, 4, 9 and 10, Table 1), pH was maintained with HEPES. Thus all changes in osmolality were performed at constant ionic composition. Solution osmolality was measured with a Wescor model 5100C (Logan, UT) vapor pressure osmometer.

Electrical Measurements

The transepithelial potential difference (V_{te}) was measured at the tip of the perfusion pipette by means of a Ringer-agar bridge—Ag/AgCl electrode located on the outlet port of the perfusion system and referenced to another Ringer-agar bridge—Ag/AgCl electrode located in the outflow of the perfusion chamber. V_{te} was measured by an electrometer (Model 7000, W-P instruments, New Haven, CT) and recorded on a stripchart recorder (model 2400, Gould, Cleveland, OH). Measurements of the basolateral cell membrane potential were made with microelectrodes manufactured on a Narishige (model PD-5, Narishige Scientific Instruments, Tokyo, Japan) from 1.2 mm-o.d., 0.5 mm-i.d. Omega-dot capillaries and filled with 1 mM KCl. Electrometer-microelectrode connections were made by Ag/AgCl wires. Manipulation of microelectrodes was performed with a hydraulic micromanipulator (model MOR, Narishige Scientific Instruments). Cells were impaled by means of a sharp tap on the back of the manipulator with a plastic lunchroom knife.

The resistance ratios, R_a/R_{bl}, and the apparent K^+ transference number, T_K, were determined as described previously (Guggino, 1986). T_K is an apparent transference number since it was determined from the change in V_{bl} following a change in K^+ concentration (*see* Guggino, 1986).

Video-Optical Techniques

The optical and video hardware used in these experiments was the same as described previously (Guggino, Oberleithner & Giebisch, 1985). To evaluate cell volume, the level of focus was adjusted at approximately the center of the tubule lumen to give a side view of the cells along a portion of the tubule similar to previous experiments in the *Amphiuma* diluting segment (Guggino et al., 1985). Images of cells were recorded during experiments and analyzed later by tracing the outline of a portion of the tubule, typically 56 μm, onto plastic sheets without considering any surface amplifications such as basal foldings or brush border surface. The thickness of the cell layer (H) including the thickness of the brush border was measured at several points along a portion of the tubule and averaged. The cross-sectional area (A_s) of each cell layer was calculated as follows:

$$A_s = L \cdot H \tag{1}$$

and the volume V of an annulus of length L was determined according to the equation:

$$V = \pi L((R_o)^2 - (R_o - H)^2) \tag{2}$$

where R_o is the outer tubule radius. This equation differs from experiments on *Amphiuma* diluting segments (Guggino et al., 1985). Equation (2) was selected because the change in cell height associated with volume changes were more uniform in *Necturus* proximal tubule cells than in the *Amphiuma* early distal tubule. In the proximal tubule cells the whole cell layer increases in height, whereas in the *Amphiuma* early distal tubule increases in cell height are more pronounced in the center of the cell than at the edges. However, in order to make sure that Eq. (1) was an

Table 2. Volume measurements

	$H(\mu m)$	$V(\mu m^3/\mu m)$	$A_b(\mu m^2)$
Control	19 ± 1	6718 ± 392	575 ± 102
Hyposmotic	23 ± 1	7850 ± 423	588 ± 77
n	17	17	8
P	<0.001	<0.001	NS
Control	20 ± 1	7414 ± 611	—
Hyperosmotic	17 ± 1	6637 ± 511	—
n	15	15	—
P	<0.001	<0.001	—

The average outer tubular diameter for tubules used for hyperosmotic experiments was 132 ± 3 μm and for hyposmotic experiments, 138 ± 4 μm. A_b is the area of the base of the cell facing the cover slip. V is in μm^3 per μm of tubule length.

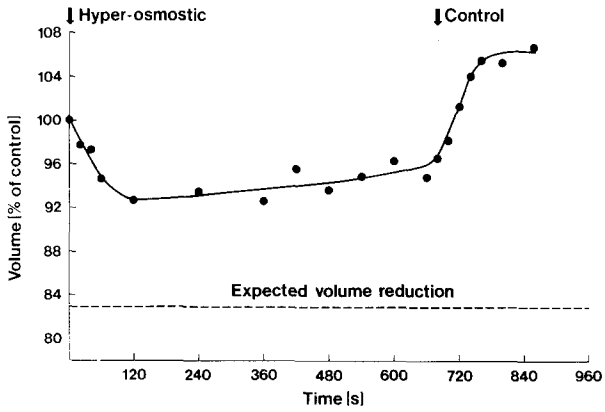

Fig. 1. Cell volume relative to control following an increase in osmolality of the perfusion solutions. The perfusion solution osmolality was switched from control at time zero

accurate representation of the cell outline, we measured the cross-sectional area of the cell outlines with a planimeter and compared it to values calculated from Eq. (1).

Statistics

All data are given as means ± SE. Difference between means was determined from a two-tailed Student's *t* test. Paired or unpaired *t* tests were used as necessary; *n* is the number of tubules.

Results

Volume Measurements

Our technique for estimating volume involves focusing the microscope on the center of the lumen and using a side view of the cells to determine the average cell height along a length of tubule, typically 56 μm long. The cell volume was then determined according to Eq. (2). The average height of the cell layer was monitored continuously, while the outer tubule diameter, o.d., was determined only at the beginning of the experiment. For this technique to be accurate, changes in volume must occur primarily by either increases or decreases in cell height without significant variation in length or width. Although we could not detect any significant changes in o.d. or length in swelling or shrinking experiments, we tested this assumption by estimating the cross sectional area of the base of cells, following a reduction in perfusion osmolality. This was accomplished by altering the level of focus of the microscope to the base of the cell nearest to the cover slip, tracing the outline of the base and determining the area with a planimeter. The results are given in Table 2. It is clear that, although cell height and volume increase significantly in hyposmotic solutions, the cross sectional area of the base of the cells remains unchanged. The observation that the cross sectional area of the base of the cells remains constant despite alterations in cell height strongly suggests that both o.d. and L also remain constant during our experiments. Moreover, it also indicates that osmotically-induced alterations in cell volume occur primarily by changes in cell height. Similar observations were also made in experiments on *Amphiuma* diluting segments (Guggino et al., 1985). As an additional check of our techniques, we compared A_s, the area of the side view of the cells, from Eq. (2) and by tracing the cell outline with a planimeter. The former yielded an A_s of 982 ± 80 μm^2 and the latter an A_s of 951 ± 73 μm^2 (n = 10 cells).

It should be mentioned that since our techniques consider the cell as having a single smooth surface without accounting for structures that amplify the membrane area, we cannot determine the exact cell volume; therefore, we report all the data as a percent of control.

Volume Regulation in Hyperosmotic Solutions

Figure 1 shows the effect on cell volume of increasing the perfusion fluid osmolality. This maneuver causes this early proximal tubule cell to shrink followed by a gradual return toward the original volume. In addition, upon return to normal osmolality the cells swell beyond their original, pre-experiment volume. This "overshoot" has been observed in cells that volume regulate in hyperosmotic solution by increasing intracellular solute content (*see* He-

Table 3. Influence of hyperosmotic solutions

	% Change in volume	*n*	*P*
Hyperosmolality	−11 ± 2	15	—
Hyperosmolality minus HCO_3^-	−23 ± 3	6	<0.001
Hyperosmolality plus 2 mM Ba^{2+}	−10 ± 3	5	NS

Both apical and basolateral solutions were made hyperosmotic either in the presence or absence of HCO_3^- from all perfusion solutions or in the presence of basolateral Ba^{2+}. *P* values are referenced to cells exposed to hyperosmotic solutions in the presence of HCO_3^- and in the absence of Ba^{2+}. HCO_3^- removal was at constant pH.

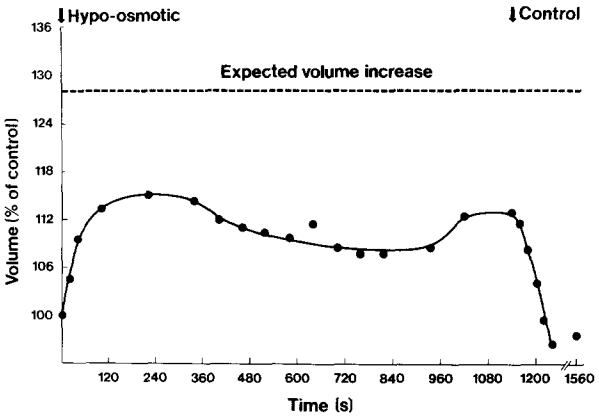

Fig. 2. Cell volume relative to control following a decrease in osmolality of the perfusion solutions. The perfusion solution osmolality was lowered at time zero

bert, 1986*a*, as an example). Given the following relationship:

$$O_1 \times (V_1 - b) = O_2 \times (V_2 - b) \quad (3)$$

where O_1 is the initial osmolality, O_2 is the final osmolality, V_1 is the initial volume, V_2 is the final volume, and b is the nonsolvent volume. Although we did not measure b directly, Spring and Giebisch (1977) determined that the nonsolvent volume of *Necturus* proximal tubule cells is about 15%. Using O_1 = 200 mOsmol, O_2 = 249 mOsmol and b = 15%, the predicted change in volume is about 17%. An examination of Fig. 1 shows that the cells never attain the volume predicted for an osmometer (dotted line in Fig. 1), suggesting they are able to regulate volume in hyperosmotic solutions. Table 3 gives the summary data that confirm the experiment shown in Fig. 1. On average, *Necturus* proximal tubule cells shrink about half as much as predicted for an osmometer, indicating clearly that *Necturus* proximal tubule cells regulate volume when exposed to hyperosmotic solutions.

To determine the involvement of HCO_3^--transport pathways in this regulation, perfusion fluid osmolality was increased in the absence of HCO_3^- from all perfusion solutions. Table 3 gives the results. In HCO_3^--free solutions increasing perfusion osmolality causes the cells to shrink about twice as much as in the presence of HCO_3^-. In this circumstance the cells shrink as osmometers. Thus, volume regulation in hyperosmotic solutions is a HCO_3^--dependent process similar to volume regulation in the *Necturus* gallbladder (Fisher, Persson & Spring, 1981; Ericson & Spring, 1982; and Fisher & Spring, 1984), the mouse medullary thick ascending limb (Hebert, 1986*a*,*b*), and the *Amphiuma* red cell (Cala, 1980; Kregenow, Caryk & Siebens, 1985; Siebens & Kregenow, 1985).

In addition, we also tested the effect of reducing the K^+ conductance of the basolateral cell membrane with Ba^{2+}. Addition of Ba^{2+} to the basolateral solution has been shown to considerably decrease the basolateral K^+ conductance (Matsumura et al., 1984*a*) but has no effect (Table 3) on the cell's ability to regulate its volume.

Volume Regulation in Hyposmotic Solutions

The influence of hyposmotic solutions on cell volume is shown in Fig. 2. Reducing osmolality causes the cells in this experiment to swell. Furthermore, upon return to normal osmolality there is an "undershoot" in cell volume below control volume, suggesting that volume regulation in hyperosmotic media probably involves a decrease in intracellular solute content. Using Eq. (3) with O_2 = 145 mOsmol, the change in volume for an osmometer would be about 28% but the observed one is 15%. Again, this cell never attains the volume predicted for an osmometer. Thus, in both hyper- and hyposmotic solutions, cell volume changes are less than if the cells were behaving as osmometers. These observations are confirmed in Table 4; on average cell swelling induced by a reduction in osmolality is only about one half that predicted for an osmometer. Thus, proximal tubule cells can also regulate volume in hyposmotic solutions in this case, by reducing intracellular solute content.

The effect of HCO_3^- removal on cell swelling in hyposmotic solutions is also given in Table 4. In the absence of HCO_3^- the cells swell about twice as great as in its presence, reaching the volume predicted for an osmometer. Likewise, the effect of

Table 4. Influence of hyposmotic solutions

	% Change in volume	*n*	*P*
Hyposmolality	+15 ± 2	12	—
Hyposmolality minus HCO_3^-	+28 ± 5	5	<0.01
Hyposmolality plus 2 mM Ba^{2+}	+37 ± 5	5	<0.001

P values are referenced to cells exposed to hyposmolality in the presence of HCO_3^- and without Ba^{2+}. HCO_3^- was removed at constant pH.

Table 5. Effects of osmolality on the electrical properties

	V_{bl} (mV)	R_a/R_{bl}
Control	−49 ± 3	4.9 ± 1.1
Hyperosmolality	−43 ± 3	2.6 ± 0.4
n	23	12
P	<0.05	<0.05
Control	−50 ± 2	5.0 ± 0.7
Hyposmolality	−53 ± 3	7.2 ± 1.4
n	17	9
P	<0.001	<0.01

basolateral Ba^{2+} is also dramatic, inducing the cells to swell beyond the volume predicted for an osmometer. Thus it appears that both HCO_3^--dependent pathways and the basolateral K^+ conductance are important for volume regulation in hyposmotic solutions. The observation that after Ba^{2+} cell swelling is 37%, greater than expected for an osmometer, may suggest that if the basolateral K^+ conductance is blocked cells may indeed accumulate ions in hyposmotic solutions.

Electrical Characteristics

In order to examine the effects of osmolality on the steady-state electrical properties of the basolateral cell membrane, V_{bl} and R_a/R_{bl} were measured both before and between 5 and 10 min after a change in perfusion osmolality. Exposing the cells to hyperosmotic solutions depolarizes V_{bl} and causes a 47% decrease in the resistance ratio, R_a/R_{bl} (Table 5). On the other hand, exposing the cells to hyposmotic solutions hyperpolarizes V_{bl} slightly and increases R_a/R_{bl} by about 44%. To determine the ionic nature of these changes in R_a/R_{bl}, V_{bl} was monitored following an increase in basolateral K^+ to 20 mM in control, hyper-, and hyposmotic solutions. The results are given in Table 6. In all solutions, raising basolateral K^+ depolarizes V_{bl}, indicating that, as

Table 6. Effects on V_{bl} (mV) of increasing basolateral K^+

	Control osmolality	Hyperosmolality
3K	−55 ± 4	−58 ± 4
20K	−37 ± 3	−45 ± 4
n	7	7
P	<0.001	<0.01
	Control osmolality	Hyposmolality
3K	−53 ± 9	−53 ± 3
20K	−41 ± 3	−34 ± 5
n	7	7
P	<0.05	<0.05

In control experiments the basolateral solution was increased to 20 mM K^+; the apical solution remained at 3 mM K^+. Hyper- and hyposmotic solutions were perfused in both apical and basolateral solutions, and the K^+ concentration was changed at either increased or decreased osmolality. Control solution in the first set of experiments is solution 1 and in the second set it is solution 7, Table 1.

Table 7. Basolateral T_K

	T_K
Control	0.44 ± 0.06
Hyperosmolality	0.29 ± 0.05
n	7
P	<0.05
Control	0.28 ± 0.09
Hyposmolality	0.41 ± 0.08
n	6
P	<0.05

The lower T_K in the hyposmotic control compared to the hyperosmotic control may reflect the lower Na^+ concentration in the former solution (*compare* solutions 7 and 1, Table 1). Reducing extracellular fluid Na^+ concentration is known to reduce basolateral K^+ permeability (Giebisch et al., 1973).

has been shown previously (i.e., Boulpaep, 1976; Matsumura et al., 1984*a,b*), the basolateral cell membrane of the proximal tubule is conductive to K^+. Examining the magnitude of the K^+-induced depolarization given in Table 6 shows that in hyperosmotic solutions the magnitude of the depolarization is smaller and in hyposmotic solutions the depolarization is much larger than the same maneuver at control osmolality. This phenomenon is shown more clearly in Table 7 which gives the partial conductance of the basolateral cell membrane for K^+, T_K. Again comparing control to experimental osmolality the T_K in hyperosmotic solutions is much less and that in hyposmotic solutions is much greater than at control osmolality. The data are con-

Table 8. Influence of HCO_3^- removal on V_{bl}

	Control osmolality	Hyperosmolality
10 HCO_3^-	−58 ± 3	−56 ± 5
0 HCO_3^-	−43 ± 4	−39 ± 4
n	9	8
P	<0.001	<0.001
	Control osmolality	Hyposmolality
10 HCO_3^-	−63 ± 4	−52 ± 4
0 HCO_3^-	−50 ± 4	−39 ± 1
n	3	3
P	<0.001	<0.05

sistent with an decreased R_a/R_{bl} in hyperosmotic solutions and a increased R_a/R_{bl} in hyposmotic solutions, suggesting that exposing the proximal tubule cell to hyperosmotic solutions may decrease while treatment with hyposmotic solutions may increase the K^+ conductance of the basolateral cell membrane. It is important to note that these osmotically-induced alterations in V_{bl}, R_a/R_{bl}, and T_K are not transient but remain between 5 and 30 min following the change in osmolality. These observations demonstrate that changes in extracellular fluid osmolality can have profound effects on transepithelial transport by affecting ionic conductances in both apical and basolateral cell membranes.

Reductions in basolateral HCO_3^- are also known to depolarize V_{bl}, suggesting that the movement of HCO_3^- across the basolateral cell membrane involves the net movement of charge (Lopes et al., 1987; Matsumura et al., 1984*b*). The effects of osmolality on the depolarization of V_{bl} induced by removing HCO_3^- from the basolateral solution are given in Table 8. As has been shown previously (Lopes et al., 1987; Matsumura et al., 1984*b*), removing basolateral HCO_3^- depolarizes V_{bl}. However, neither hyperosmotic nor hyposmotic solutions significantly alter this depolarization.

Discussion

Volume Measurements

Necturus early proximal tubule cells can regulate volume when exposed to either hyper- or hyposmotic solutions. This is similar to the *Necturus* gallbladder, which also can regulate volume changes in both hyper- and hyposmotic solutions (Fisher et al., 1981). However, the *Necturus* proximal tubule is different from the rabbit proximal tubule, which can regulate volume very well in hyposmotic solutions (Dellasega & Grantham, 1973) but not in hyperosmotic solutions (Gagnon et al., 1982). Clearly, rabbit proximal tubule cells are unable to increase intracellular ion content above normal osmolality, a phenomenon essential to volume regulate in hyperosmotic solutions.

Necturus proximal tubule cells exposed either to hyper- or hyposmotic solutions never respond biphasically by first attaining a volume close to that expected for an osmometer followed by a gradual decline toward a lower steady volume. This response is observed in some cells that regulate their volume (*see* Dellasega & Grantham, 1973; Fisher et al., 1981). One explanation for this phenomenon is that the water permeability of the *Necturus* cell membranes is low enough and the volume regulatory mechanisms fast enough to maintain volume without a biphasic change in volume. Although in the present study we did not determine the water permeability, from an examination of Figs. 1 and 2 it is clear that volume regulation is very rapid with most of the regulation occurring within 1 min following the osmolality change, perhaps rapid enough to keep up with the water movement induced by a change in osmolality. A rapid volume regulatory response was also observed in the *Necturus* gallbladder (Fisher et al., 1981) with almost complete volume regulation occurring within 90 sec. However, another explanation is that volume regulatory mechanisms are faster than we can resolve with our fluid exchange rates. Therefore, more experiments using even faster fluid exchange rates will be necessary to determine precisely why the cells do not behave as osmometers.

Mechanism of Volume Regulation in Hyperosmotic Solutions

In sharp contrast to *Necturus* proximal tubules in the presence of HCO_3^-, in its absence the cells lose their ability to regulate volume. This dependence of volume regulation on solution HCO_3^- has also been observed in the *Necturus* gallbladder (Fisher et al., 1981; Ericson & Spring, 1982; Fisher & Spring, 1984), in the mouse medullary thick ascending limb (Hebert, 1986*a,b*), and in the *Amphiuma* red cell (Cala, 1980; Kregenow et al., 1985; Siebens & Kregenow, 1985). In those systems, hyperosmotic volume regulation occurs by parallel Na-H and Cl-HCO_3 exchangers, which function to transport NaCl into the cell.

In the *Necturus* proximal tubule, Cl^- is transported across the apical cell membrane via a Na-Cl cotransporter (Spring & Kimura, 1978) which at normal osmolality is not HCO_3^- dependent (Guggino

et al., 1983). Cl^- leaves across the basolateral cell membrane by means of an exchanger which moves Na^+ and $2HCO_3^-$ into the cell in exchange for Cl^- (Guggino et al., 1983). A Cl^--independent Na-HCO_3 cotransporter also present in the basolateral cell membrane (Lopes et al., 1987) provides an alternate route for HCO_3^- exit. At normal osmolality the Cl^- conductance of apical and basolateral cell membranes is low (Shindo & Spring, 1981; Guggino, Boulpaep & Giebisch, 1982). If an increase in intracellular Cl^- content, either by an increased transport via the apical cotransporter or decreased efflux via the basolateral exchanger, is involved in volume regulation in hyperosmotic solutions, intracellular Cl^- activity must increase approximately two times. It was shown that increasing perfusion fluid osmolality by about 80 mOsmol does not change intracellular Cl^- activity (London et al., 1983). Since in the present study we have shown a decrease in volume by about 11% following an increase in osmolality of about 50 mM, this means that intracellular Cl^- content must have fallen in hyperosmotic solutions. Since neither intracellular Cl^- activity nor content rise in hyperosmotic solutions, it is clear that Cl^- is not an osmotic force in volume regulation. A decrease in the intracellular content of Cl^-, Na^+ and K^+ was also noted in *Necturus* gallbladder cells shortly after exposure to hyperosmotic solutions (Fisher & Spring, 1984). The mechanism of solute loss in hyperosmotic solutions in the gallbladder is unknown, but in the *Necturus* proximal tubule it was shown that Cl^- leaves the cell across the basolateral cell membrane via Na/$2HCO_3$-Cl exchanger (London et al., 1983).

In the *Necturus* proximal tubule hyperosmotic volume regulation is HCO_3^- dependent; one possible mechanism for this volume regulation could involve solute entry via a HCO_3^--dependent Cl^--entry pathway such as the ones in the gallbladder, thick ascending limb, and the *Amphiuma* red cell with subsequent removal of Cl^- across the basolateral cell membrane via the Na/$2HCO_3$-Cl. The net effect would be an increase in intracellular Na^+ and HCO_3^- content, but the answer for this must await future experiments.

Volume Regulation in Hyposmotic Solutions

The regulation of cell volume in *Necturus* proximal tubules exposed to hyposmotic solutions depends both on the presence of a K^+ conductance in the basolateral cell membrane and on the presence of HCO_3^- in the perfusion solutions. The dependence of hyposmotic volume regulation on the efflux of K^+ from cells is well known (*see* Kregenow (1981) and Law (1985) for reviews). For example, recently it was shown in the rabbit proximal tubule that Ba^{2+} addition to the basolateral solution slowed, but did not completely inhibit, volume regulatory decrease (VRD) associated with hyposmotic volume regulation (Welling, Linshaw & Sullivan, 1985), suggesting that the efflux of K^+ via the basolateral K^+ conductance was an important component in the VRD but that other pathways including the basolateral Na/K pump may be involved.

The anion that usually accompanies K^+ during VRD is Cl^- (*see* Kregenow, 1981; Larson & Spring, 1983; Law, 1985). However, in the *Amphiuma* red cell VDR is also HCO_3^- dependent, caused primarily by the net effect of K/H and Cl/HCO_3 exchangers (Cala, 1980). In the *Necturus* proximal tubule, hyposmotic volume regulation is inhibited in HCO_3^--free solutions. Since it is known that the T_K of the basolateral cell membrane increases in HCO_3^--free solutions (Matsumura et al., 1984*a*), the inhibition of volume regulation cannot occur via an effect on the K^+-conductive pathway but instead indicates a direct role for HCO_3^- in hyposmotic volume regulation. Thus it is possible that *Necturus* proximal tubule cells could volume regulate by losing K^+ via the basolateral K^+ conductance accompanied by HCO_3^- via a basolateral Na-HCO_3 cotransporter, a transporter that transports ions (Lopes et al., 1987) in isotonic conditions.

Electrical Aspects of Volume Regulation

It is well known since the early work of Reuss and Finn (1977) on the gallbladder epithelium that changes in extracellular fluid osmolality can have profound effects on cellular conductances. For example, Lau, Hudson and Schultz (1984) showed that swelling increases a Ba^{2+}-sensitive K^+ conductance in the basolateral cell membrane of the *Necturus* small intestine. In the present study we have demonstrated in the *Necturus* proximal tubule a similar increase in the relative K^+ conductance of the basolateral cell membrane induced by cell swelling. In addition, we demonstrated that cell shrinking can reduce this same relative conductance. Both hypo- and hyperosmolality do not significantly affect the depolarization of V_{bl} induced by HCO_3^- removal. Because we are only estimating T_K from the change in V_{bl}, several factors could be responsible for the change. For example, T_K would be affected either by changes in the resistance of the paracellular shunt pathway or by alterations in the conductance of another ion. We argue that the paracellular pathway is not a factor in the response of T_K to bath

osmolality since the depolarization of V_{bl} induced by HCO_3^- removal is unaffected by solution osmolality. Alterations in shunt resistance would result in similar changes in the magnitude of both HCO_3^- and K^+-induced depolarizations. Furthermore, the observations that the change in the magnitude of R_a/R_{bl} parallels the change in basolateral T_K (*compare* Tables 5 and 7) is additional evidence that the osmotic effect might be specific to the basolateral K^+ conductance.

The effects of these changes in basolateral K^+ conductance are significant because they could contribute to volume regulation. For example, in hyperosmotic solution a reduction in basolateral K^+ conductance could limit the exit of ions from the cell while in hyposmotic solution an increased K^+ permeability would favor efflux of ions.

The effects of osmolality on membrane conductances are caused by differences in specific ion channels. For example, Zeiske and Van Driessche (1984) showed, using noise analysis, that the number of Na^+ channels was reduced when frog skin cells regulate volume in hyperosmotic solutions. Thus, they attributed the changes in Na^+ transport induced by osmolality to be directly related to functioning Na^+ channels. Similarly, when cells of the turtle colon are exposed to high K^+ a new set of K^+ channels in the basolateral cell membrane are induced which do not participate in net ion transport across the epithelium in normal osmolality (Richards & Dawson, 1986), but may be involved in volume regulation. Thus, osmolality can exert an influence by either modulating channels that normally participate in transepithelial transport or special volume-sensitive channels.

Finally, it is known that the relative basolateral K^+ permeability of several tissues is sensitive to the magnitude of net ion reabsorption (Grasset, Gunter-Smith & Schultz, 1983; Lau et al., 1984, 1986; Matsumura et al., 1984*b*; Messner, Oberleithner & Lang, 1985). Thus, the magnitude of the basolateral K^+ conductance in these tissues parallels the magnitude of net Na^+ reabsorption, a process which maintains intracellular K^+ activity and cell volume in isotonic media during variations in the net transport of ion. Our data shows that the relative K^+ conductance of the basolateral cell membrane of the *Necturus* proximal tubule is also sensitive to differences in extracellular osmolality, suggesting that it plays a role in volume maintenance in both isotonic and anisotonic media.

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