J. Membrane Biol. 117, 113–122 (1990)

The Journal of
Membrane Biology

Effect of Potassium on Cell Volume Regulation in Renal Straight Proximal Tubules

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Summary. The present study was designed to assess for the influence of extracellular potassium and of inhibitors of potassium transport on cell volume regulatory decrease in isolated perfused straight proximal tubules of the mouse kidney. Volume regulatory decrease is virtually unaffected when bath potassium concentration is elevated from 5 to 20 mmol/liter, and still persists, albeit significantly retarded, in the presence of the potassium channel blocker barium on both sides of the epithelium and during virtually complete dissipation of the transmembrane potassium gradient by increasing extracellular potassium concentration to 40 mmol/liter. As evident from electrophysiologic observations, barium blocks the potassium conductance of the basolateral cell membrane. Reduction of bicarbonate concentration and increase of H^+ concentration in the bath solution cannot compensate for enhanced potassium concentration and cell volume regulatory decrease is not affected in the presence of the K/H exchange inhibitor omeprazole. Similarly cell volume regulatory decrease is not affected by ouabain. In conclusion, potassium movements through potassium channels in the basolateral cell membrane are important determinants of cell volume and may participate in cell volume regulatory decrease. However, a powerful component of cell volume regulatory decrease in straight proximal tubules of the mouse kidney is apparently independent of potassium conductive pathways, K/H exchange and Na^+/K^+-ATPase.

Key Words cell volume regulation · potassium conductance · intracellular potassium concentration · proximal renal tubule · cell membrane potential · microelectrodes · ouabain · omeprazole · barium

Introduction

Cell volume regulatory decrease involves loss of potassium in a variety of tissues [1, 3, 5–10, 12–17, 19–23, 25–28, 33–35]. In most of these tissues, including proximal renal tubules, potassium is thought to be lost via barium-sensitive potassium channels [7, 12, 14, 15, 20, 27, 28, 32–34]. Potassium loss is generally believed to be concomitant with loss of chloride [3, 5, 7, 12, 14, 16, 19, 23, 26–28]. The low intracellular chloride activity in proximal renal tubules [4], however, renders a crucial contribution of chloride rather unlikely. Accordingly, cell volume regulatory decrease in proximal straight tubules of the mouse kidney depends on the presence of bicarbonate and sodium, but not of chloride [32]. This observation is suggestive of involvement of sodium bicarbonate cotransport in volume regulation. In *Amphiuma* red cells, on the other hand, potassium is thought to be lost in exchange for hydrogen ions [3]. In either case, increase of potassium concentration in the bath should eventually impair cell volume regulatory decrease, but the concentration of potassium in the bath necessary to impair cell volume regulatory decrease may allow for discrimination between these systems. Furthermore, the persistence of some volume regulatory decrease at high extracellular potassium concentrations or after inhibiting the basolateral membrane potassium conductance may point to a potassium-independent component of cell volume regulation.

The present study was carried out to define the influence of extracellular potassium concentration on cell volume regulatory decrease in proximal straight tubules of the mouse kidney and to test for cell volume regulatory decrease in the presence of inhibitors of potassium transport.

Materials and Methods

Swiss mice weighing 20–25 g were killed by cervical dislocation and the left kidney was removed. Proximal straight tubule segments of 0.2 to 0.4 mm length were dissected and perfused following principally the method of Burg et al. [2]. Modifications of the technique, including track system, pipette arrangement and use of a dual-channel perfusion pipette have been described in previous publications [11, 29]. The luminal perfusion rate was ~10 nl/min. The bath was perfused continuously at a rate of 20 ml/min and thermoregulated with a dual-channel feedback system (W. Hampel, Frankfurt, FRG). The bath temperature was kept constant at 38°C. The composition of the solutions is listed in Table 1. Ouabain (0.1 mmol/liter), barium (1 or 2 mmol/liter) or omeprazole (0.1 mmol/liter) were added to solutions 1, 2, 3, 4, or 17 in the respective experiments.

Table 1. Composition of solutions (solutions 1–16 for bath, solutions 17 and 18 for lumen)[a]

Solution	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
NaCl	70	70	65	65	55	55	45	45	35	35	45	45	35	35	40	40	120	85
KCl	5	5	10	10	20	20	30	30	40	40	40	40	20	20	40	40	5	40
$NaHCO_3$	20	20	20	20	20	20	20	20	20	20	10	10	40	40	20	20	20	20
$CaCl_2$	1.3	1.3	1.3	1.3	1.3	1.3	1.3	1.3	1.3	1.3	1.3	1.3	1.3	1.3	1.3	1.3	1.3	1.3
$MgCl_2$	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
Na_2HPO_4	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2
Na-acetate	10	10	10	10	10	10	10	10	10	10	10	10	10	10	—	—	—	—
Glucose	5	5	5	5	5	5	5	5	5	5	5	5	5	5	3	3	—	—
Na-lactate	—	—	—	—	—	—	—	—	—	—	—	—	—	—	5	5	—	—
L-glutamine	—	—	—	—	—	—	—	—	—	—	—	—	—	—	2	2	—	—
Mannitol	80	—	80	—	80	—	80	—	80	—	80	—	80	—	80	—	5	5
Σ	308	228	308	228	308	228	308	228	308	228	308	228	308	228	308	228	308	308

[a] All solutions were equilibrated with 5% CO_2 and 95% O_2. Concentrations are expressed in mmol/liter (Σ = sum of concentrations).

The potential difference across the basolateral membrane (PD_{bl}) was measured with a high input-impedance electrometer (FD 223, WPI, Science Trading, Frankfurt, FRG) connected to the electrode via an Ag/AgCl half-cell. The electrodes used for recording PD_{bl} were pulled from filament capillaries (1.5 mm o.d., 1.0 mm i.d., Hilgenberg, Malsfeld, FRG) with a Narishige PE 2 vertical puller, which was adjusted to deliver electrodes with tip resistances between 100 and 200 MΩ. The microelectrodes were filled with 1 mol/liter KCl solution immediately before use. For impalement, the electrodes were advanced rapidly with a piezoelectric stepper (M. Frankenberger, Germering, FRG) mounted on a Leitz micromanipulator (E. Leitz, Wetzlar, FRG). A recording was accepted only when the penetration of the cell membrane resulted in a rapid deflection of the reading. Furthermore, PD had to be more negative than −50 mV and stable (±2 mV) for at least 1 min.

As shown in a previous paper [29], impalements most frequently result in a PD between −60 and −70 mV. Impalements below −50 mV were rarely stable and thus considered to be leaky. Withdrawal of the electrode had to be followed by an immediate return of the electrode reading to the baseline value (±2 mV). The resistance of the electrodes was checked by passing short current pulses and had to be constant during the impalement (±20%). In one series intracellular potassium activity was assessed utilizing ion-selective microelectrodes with a slope of >52 mV/decade K^+ concentration in mixed extracellular solutions [34].

Before, during and after exposure to hyposmotic solutions, photographs were taken at a magnification of 400× using interference-(Nomarski) contrast (ICM 405 microscope, Zeiss, Oberkochen, FRG) focused on the center of the tubule. For evaluation, tubule segments were selected in which the borders of the cells were in focus throughout the experiment. The negatives were enlarged 50-fold and mean outer radius (r) and cell height (h) were determined in each print of one cycle. The evaluation was made by the experimenter and, as a control, by a person not knowing the experimental protocol (blind). The respective results did not differ significantly. Apparent cell volume per unit tubule length (V) was calculated from:

$$V = \pi[r^2 - (r - h)^2] = \pi(2rh - h^2).$$

The data are expressed in fractions of the apparent volume (V_a) calculated for tubules prior to hypotonic swelling. No significant difference in cell volume changes were observed between tubule segments close to the perfusion pipette and tubule segments close to the collecting pipette.

Mean values are given ± SEM, statistical analysis was made by t test, $P < 0.05$ was accepted as a statistically significant difference.

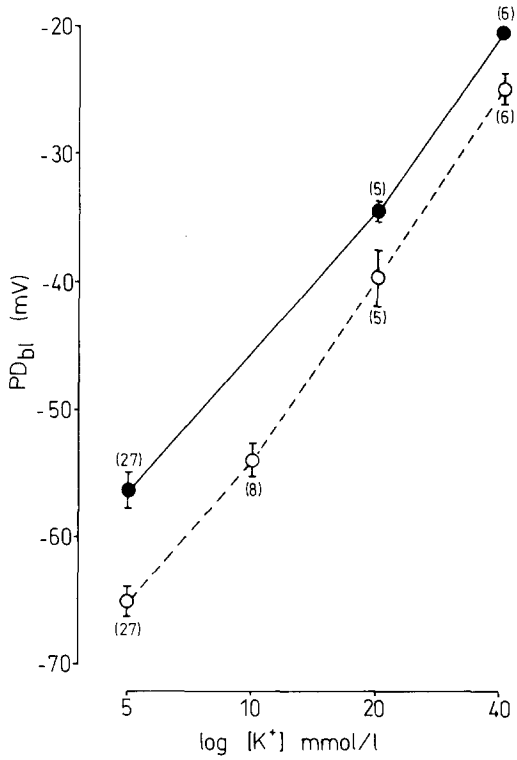

Fig. 1. The potential difference across the basolateral cell membrane (PD_{bl}) of isolated perfused proximal straight tubules as a function of bath potassium concentration in isosmotic (open symbols, broken line) and hyposmotic (filled symbols, solid line) bath (means ± SEM of steady-state values, numbers in parenthesis indicate numbers of experiments)

Results

During control conditions (solution 1) the potential difference across the basolateral cell membrane (PD_{bl}) is −65 ± 1 mV (n = 38).

As illustrated in Figs. 1 and 2, an increase of bath potassium concentration from 5 to 10 (solution 3), 20 (solution 5), or 40 mmol/liter (solution 9) depo-

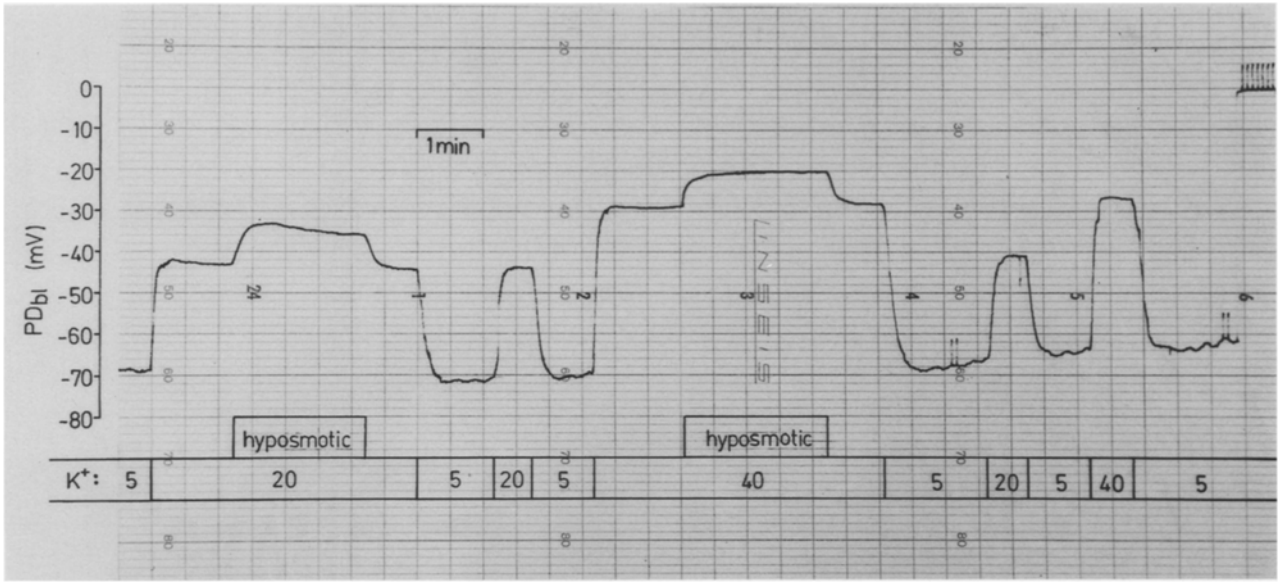

Fig. 2. Effect of hyposmotic bath perfusates on the potential difference across the basolateral cell membrane (PD_{bl}) of isolated perfused straight proximal tubules at 20 and 40 mmol/liter bath potassium concentration (original tracing)

larizes the basolateral cell membrane by +11 ± 1 mV (n = 8), +28 ± 1 mV (n = 5), or +43 ± 1 mV (n = 6), respectively.

Decrease of bath osmolarity by 80 mosmol/liter by omission of mannitol depolarizes the basolateral cell membrane within 30 sec by +9 ± 1 mV (n = 27) at 5 mmol/liter bath potassium concentration (solution 2), by +6 ± 2 mV (n = 5) at 20 mmol/liter bath potassium concentration (solution 6), and by +6 ± 1 mV (n = 6) at 40 mmol/liter bath potassium concentration (solution 10) (*see* Figs. 1–3).

Reduction of bath osmolarity by omission of 20, 40, 60, 80 and 100 mmol/liter mannitol (Fig. 3) leads to depolarizations by 2 ± 1, 5 ± 1, 7 ± 1, 9 ± 1 and 11 ± 2 mV, respectively (n = 5).

A 60-sec exposure of the tubule segments to increased bath potassium concentration under isosmotic conditions leads to significant swelling of the cells (Table 2). Continued exposure to a 40 mmol/liter [K^+] bath leads to further cell swelling, followed by partial volume decrease. At 1, 2, and 5 min of exposure to a 40-mmol/liter potassium concentration in the bath, the respective cell volumes were 110 ± 2, 115 ± 3, or 109 ± 3% (n = 5) of control cell volume (*see* Fig. 4).

The photograph series in Fig. 5 and Table 2 demonstrate that isolated segments of straight proximal tubules are still able to regulate cell volume when bathed in 20-mmol/liter potassium (solutions 5 and 6).

As illustrated in Fig. 6 and Table 2, some impairment of cell volume regulation is observed at 30 mmol/liter [K^+] (solutions 7 and 8). Cell volume regulation is markedly retarded but not abolished by increasing bath [K^+] to 40 mmol/liter in bath (solutions 9 and 10) or in bath and lumen (solutions 15, 16, and 18).

Increasing bath bicarbonate concentration from 20 to 40 mmol/liter (at constant P_{CO_2}), in the presence of 20-mmol/liter potassium (solutions 13 and 14), leads to some impairment of volume regulatory decrease (Fig. 7, Table 3). However, a reduction of bath bicarbonate concentration (solutions 11 and 12) from 20 to 10 mmol/liter (at constant P_{CO_2}) does not accelerate volume regulatory decrease in the presence of 40-mmol/liter potassium (Fig. 7, Table 3). Thus, the bicarbonate or H^+ gradients across the basolateral cell membrane do not appear to be critical for volume regulatory decrease.

As shown in Fig. 8 and Table 4, 0.1 mmol/liter omeprazole does not alter cell volume in isotonic or hypotonic bath perfusates. Similarly, cell volume regulatory decrease is virtually unchanged in the presence of ouabain, provided that the drug is applied 1 min before exposure of the tubules to hyposmotic perfusate (Fig. 9, Table 4).

The K^+ channel blocker barium (1 mmol/liter) depolarizes the cell membrane to −34 ± 6 mV (n = 4) and abolishes the basolateral potassium conductance. In the presence of barium, an increase of bath potassium concentration from 5 to 10 mmol/liter leads to a slight significant hyperpolarization by −2 ± 1 mV (n = 4) in isosmotic and to no significant change (−1 ± 1 mV, n = 4) in hyposmotic perfusate (solutions 3 and 4).

Exposure of proximal renal tubules to barium in

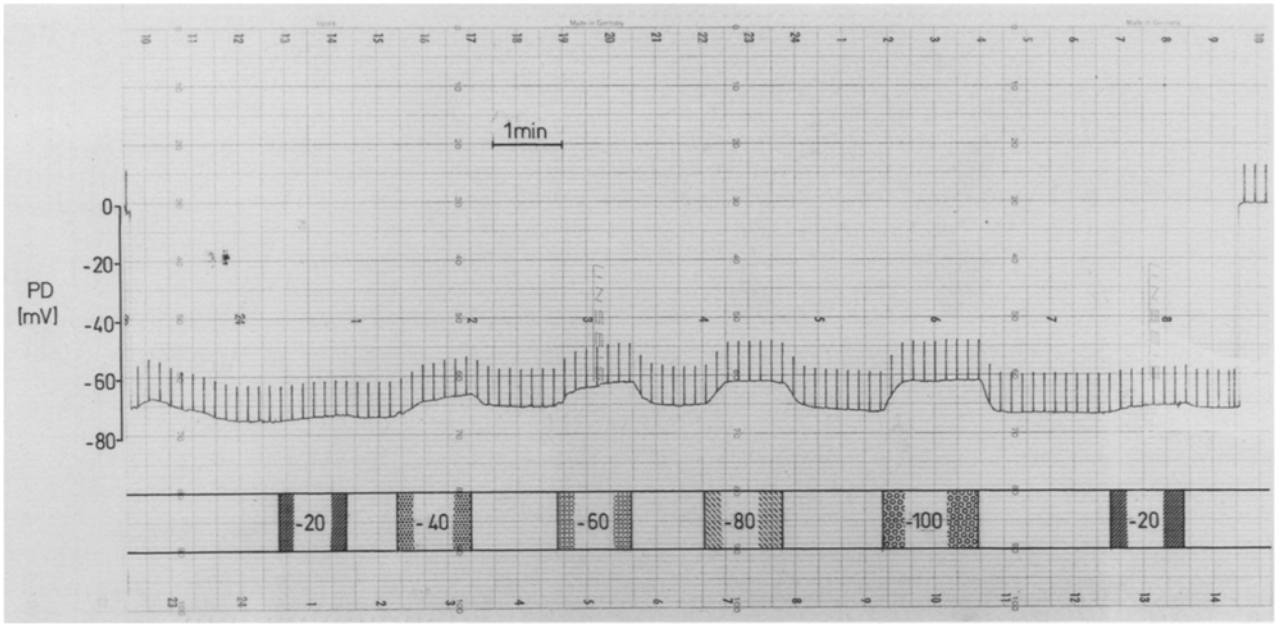

Fig. 3. Effects of graded alterations of extracellular osmolarity on the potential difference across the basolateral cell membrane (PD). In the isosmotic bath solution 50 mmol/liter NaCl were replaced by 100 mmol/liter mannitol. Alterations of osmolarity (−20, −40, −60, −80 and −100 mmol/liter) were achieved by reducing bath mannitol

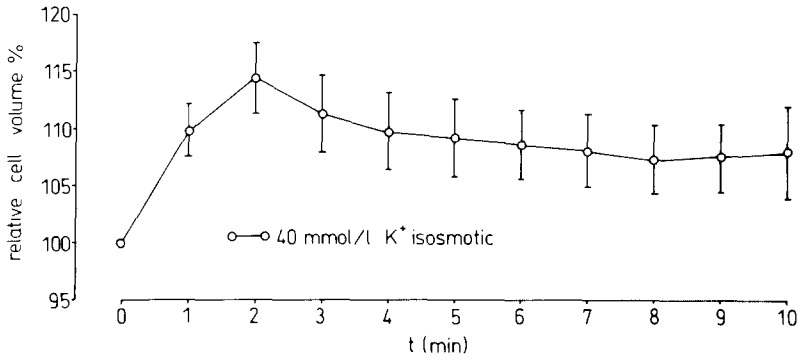

Fig. 4. Effect of increasing bath potassium concentration from 5 to 40 mmol/liter on cell volume in isolated perfused proximal tubule segments (means ± SEM, $n = 5$)

Table 2. Influence of extracellular K^+ concentration on cell volume regulatory decrease in proximal renal tubules[a]

Condition (solutions[b])	A Isosmotic, (1 min)	B Hyposmotic			C Isosmotic, (0.5 min)
		0.5 min	2 min	5 min	
Control (1, 2)	100	116.4 ± 0.9 (8)[c]	105.7 ± 1.3 (8)[c]	—	94.0 ± 1.3 (8)[c]
20 mM K (5, 6)	105.8 ± 1.6 (18)[d]	120.2 ± 1.9 (18)[c]	107.7 ± 2.1 (18)	—	94.7 ± 1.8 (18)[c]
30 mM K (7, 8)	103.7 ± 0.9 (7)[d]	126.3 ± 2.7 (7)[cd]	115.6 ± 2.4 (7)[cd]	—	95.9 ± 3.0 (7)[c]
40 mM K (9, 10)	110.6 ± 1.6 (15)[d]	134.5 ± 2.9 (15)[cd]	123.8 ± 2.4 (15)[cd]	117.8 ± 3.1 (15)	99.8 ± 2.2 (15)[cd]
40 mM K bath+lu (15, 16)	107.5 ± 1.8 (6)[d]	133.0 ± 3.7 (6)[cd]	124.0 ± 2.7 (6)[cd]	108.4 ± 2.6 (6)	92.8 ± 1.6 (6)[c]

[a] Cell volume (% of control, i.e., exposure to solutions 1 and 17) 1 min following treatment with respective isosmotic solution (A), 0.5, 2 and 5 min following subsequent exposure to respective hyposmotic perfusate (B), and 0.5 min following re-exposure to respective isosmotic solution (C). Numbers of observations in parentheses.

[b] Bath solutions (bath) as listed in Table 1; luminal solution (lu) is 17 (Table 1) except in series 40 mM K bath+lu, where the luminal solution is 18.

[c] Significantly different from value before exposure to hypotonic perfusate.

[d] Significantly different from respective value at 5 mmol/liter K^+.

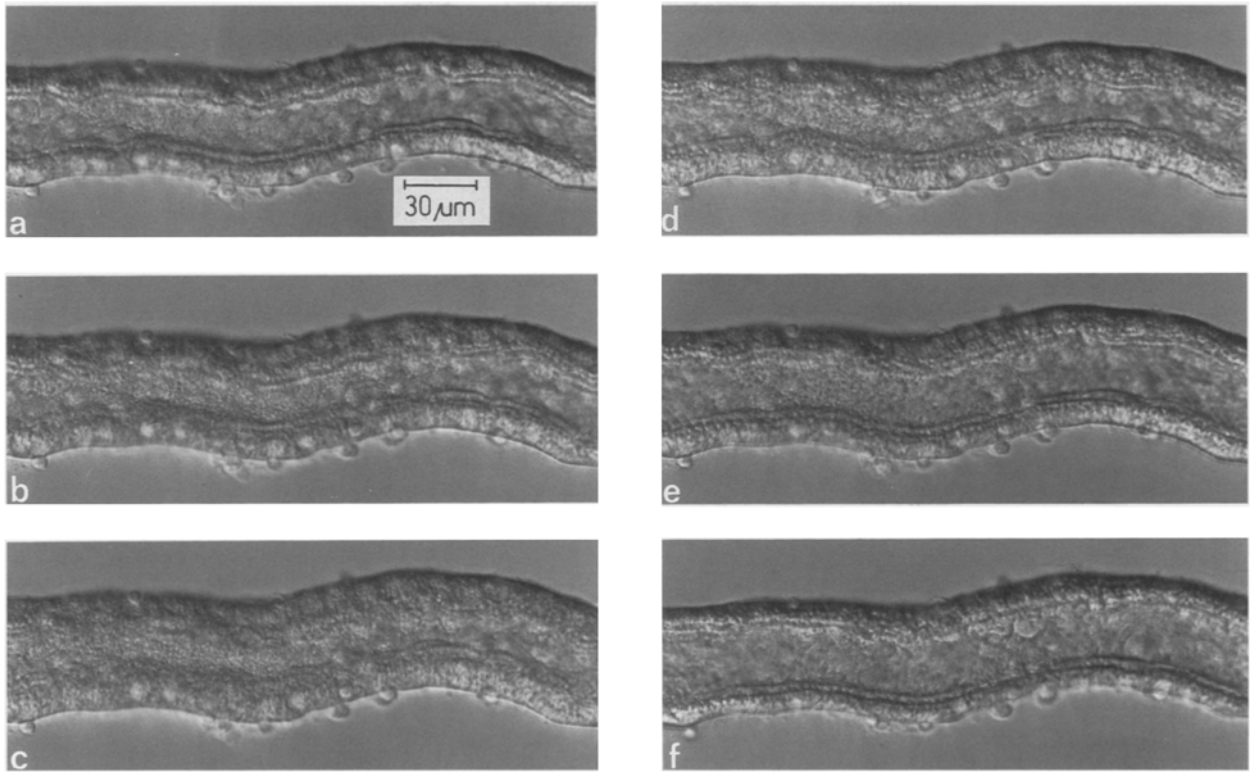

Fig. 5. Effect of hyposmotic bath perfusate on straight proximal tubule at increased bath potassium concentration (*a*) Tubule exposed to isosmotic bath solution containing 5 mmol/liter potassium. (*b*) 60 sec after exposure to isosmotic bath solution containing 20 mmol/liter potassium. (*c*) 30 sec after exposure to hyposmotic bath solution containing 20 mmol/liter potassium. (*d*) 60 sec after exposure to hyposmotic bath solution containing 20 mmol/liter potassium. (*e*) 120 sec after exposure to hyposmotic bath solution containing 20 mmol/liter potassium. (*f*) 30 sec after re-exposure to isosmotic bath solution containing 20 mmol/liter potassium

both, luminal perfusate and bath leads to cell swelling by 7 ± 1% (n = 7) within 1 min. Subsequent exposure of the tubules to hyposmotic bath leads to an additional cell swelling followed by retarded cell volume regulatory decrease (Figs. 10 and 11, Table 4).

Intracellular K^+ activity is 72 ± 6 mmol/liter at 5 mmol/liter extracellular K^+ concentration (Fig. 12). Increase of extracellular [K^+] to 40 mmol/liter leads within 1 min to an increase of intracellular [K^+] by 14 ± 6 mmol/liter and virtually abolishes the K^+ gradient across the basolateral cell membrane (0 ± 2 mV). Subsequent reduction of bath osmolarity decreases intracellular K^+ activity by 15 ± 3 mmol/liter, which remains almost constant (+3 ± 4 mmol/liter) throughout exposure to hyposmotic perfusates (up to 5 min).

Discussion

At bath K^+ concentration in the range of 5 to 40 mmol/liter, exposure of proximal straight tubules to hyposmotic media depolarizes the cell membrane. This finding parallels similar observations made in our laboratory in Madin Darby Canine Kidney cells and in Ehrlich ascites tumor cells [18]. In intestine [20] and in early proximal tubule of *Necturus* kidney [24], on the other hand, hyposmotic perfusates have been shown to hyperpolarize the basolateral cell membrane.

One factor contributing to the change in cell membrane potential is certainly the dilution of intracellular electrolytes and the respective shifts of equilibrium potentials. Intracellular potassium activity decreases due to both entry of water and loss of cell potassium during volume regulatory decrease. The decrease of cell potassium activity reduces the chemical gradient and thereby the equilibrium potential for potassium. As a result, the cell membrane tends to depolarize. Dilution of intracellular anions, on the other hand, increases the equilibrium potentials for the respective conductive pathways and tends to hyperpolarize the cell membrane. The net result of the alterations of equilibrium potentials depends on the selectivity of the cell membrane to the respective ions: if potassium selectivity is predomi-

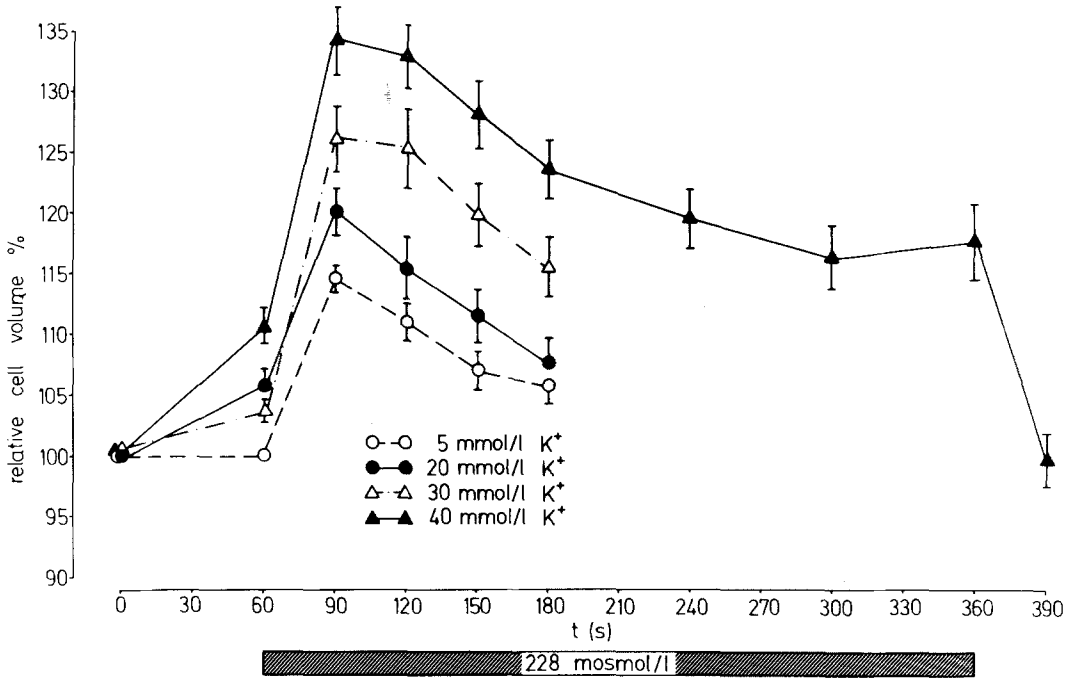

Fig. 6. Effect of hyposmotic bath perfusates on cell volume in isolated perfused proximal tubule segments at various bath potassium concentrations, i.e., at 5 mmol/liter (open circles, broken line, $n = 8$), at 20 mmol/liter (filled circles, solid line, $n = 18$), at 30 mmol/liter (open triangles, broken line, $n = 7$) and at 40 mmol/liter (filled triangles, solid line, $n = 15$). Means ± SEM

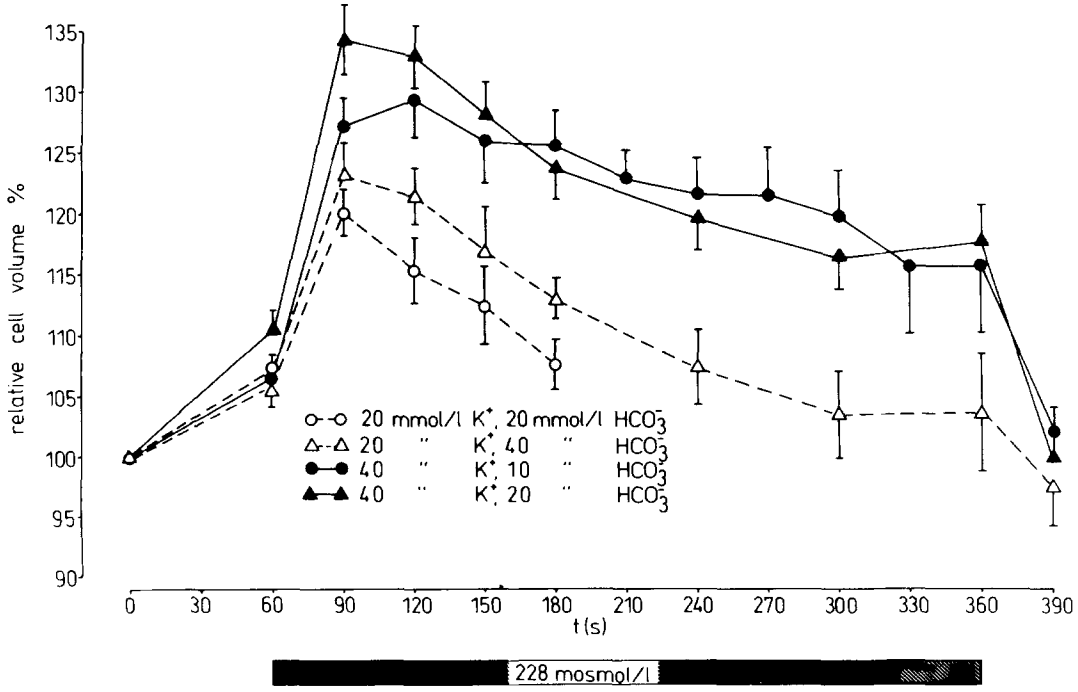

Fig. 7. Effect of hyposmotic bath perfusates on cell volume in isolated perfused proximal tubule segments at 20 mmol/liter potassium concentration (open symbols, broken lines) and either 20 (open circles, $n = 18$) or 40 (open triangles, $n = 8$) mmol/liter bicarbonate, as well as, at 40 mmol/liter potassium (filled symbols, solid lines) and either 10 (filled circles, $n = 12$) or 20 (filled triangles, $n = 15$) mmol/liter bicarbonate in the bath. Means ± SEM

Table 3. Influence of bath HCO_3^- on cell volume regulatory decrease in proximal renal tubules at increased extracellular K^+ concentration[a]

Condition (solutions[b])	A Isosmotic, 1 min	B Hyposmotic			C Isosmotic, 0.5 min
		0.5 min	2 min	5 min	
40 mM K, 20 mM HCO_3^- (9, 10)	110.6 ± 1.6 (15)	134.5 ± 2.9 (15)[c]	123.8 ± 2.4 (15)[c]	117.8 ± 3.1 (15)	99.8 ± 2.2 (15)[c]
40 mM K, 10 mM HCO_3^- (11, 12)	106.6 ± 0.8 (12)	127.3 ± 2.4 (12)[c]	125.8 ± 2.9 (12)[c]	115.8 ± 5.5 (3)	102.0 ± 2.1 (3)
20 mM K, 20 mM HCO_3^- (5, 6)	105.8 ± 1.6 (18)	120.2 ± 1.9 (18)[c]	107.7 ± 2.1 (18)	—	94.7 ± 1.8 (18)[c]
20 mM K, 40 mM HCO_3^- (13, 14)	107.2 ± 1.3 (8)	123.2 ± 2.8 (8)[c]	113.2 ± 1.7 (8)[c]	103.7 ± 4.9 (4)	97.6 ± 3.4 (8)[c]

[a] Cell volume (% of control, i.e., exposure to solutions 1 and 17) 1 min following treatment with respective isosmotic solution (A), 0.5, 2 and 5 min following subsequent exposure to respective hyposmotic perfusate (B), and 0.5 min following re-exposure to respective isosmotic solution (C). Numbers of observations in parentheses.
[b] Bath solutions (bath) as listed in Table 1; luminal solution is 17.
[c] Significantly different from value before exposure to hypotonic perfusate.

Table 4. Effect of inhibitors on cell volume regulatory decrease in proximal renal tubules

Condition (solutions[b])	A Isosmotic, 1 min	B Hyposmotic			C Isosmotic, 0.5 min
		0.5 min	2 min	5 min	
Control (1, 2)	100	116.4 ± 0.9 (8)[c]	105.7 ± 1.3 (8)[c]	—	94.0 ± 1.3 (8)[c]
1 mM Barium (1, 2)	106.2 ± 1.0 (11)[d]	125.5 ± 2.3 (11)[cd]	120.0 ± 2.6 (11)[cd]	102.3 ± 2.1 (10)	88.7 ± 1.6 (10)[c]
2 mM Barium bath + lu (1, 2)	107.0 ± 1.4 (7)[d]	125.9 ± 2.5 (7)[cd]	117.8 ± 4.0 (7)[cd]	103.4 ± 3.1 (7)	92.7 ± 2.0 (7)[c]
0.1 mM Omeprazole (1, 2)	100.6 ± 1.5 (5)	118.7 ± 1.5 (5)[c]	106.7 ± 3.3 (5)[c]	—	91.7 ± 2.2 (5)[c]
0.1 mM Ouabain (1, 2)	100.1 ± 0.8 (8)	116.4 ± 1.1 (8)[c]	105.5 ± 1.3 (8)[c]	—	94.8 ± 1.4 (8)[c]

[a] Cell volume (% of control, i.e., exposure to solutions 1 and 17) 1 min following treatment with respective isosmotic solution (A), 0.5, 2 and 5 min following subsequent exposure to respective hyposmotic perfusate (B), and 0.5 min following re-exposure to respective isosmotic solution (C). Numbers of observations in parentheses.
[b] Bath solutions (bath) as listed in Table 1; luminal solution is 17.
[c] Significantly different from value before exposure to hypotonic perfusate.
[d] Significantly different from respective value in the absence of the inhibitor.

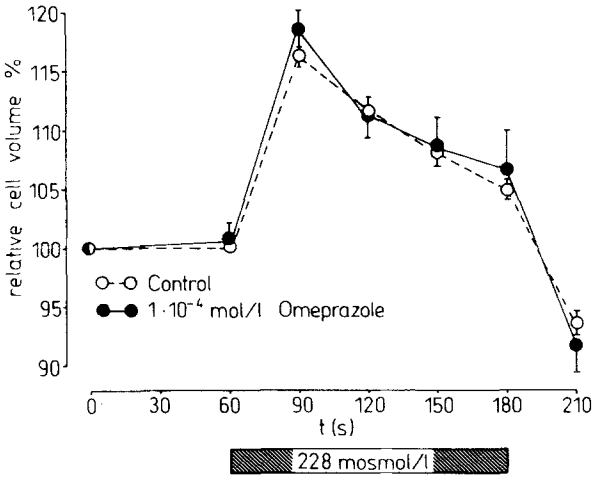

Fig. 8. Effect of hyposmotic bath perfusates on cell volume in isolated perfused proximal tubule segments in the absence (control, open circles, broken line, $n = 8$) and in the presence of 0.1 mmol/liter omeprazole (filled circles, solid line, $n = 5$). Means ± SEM

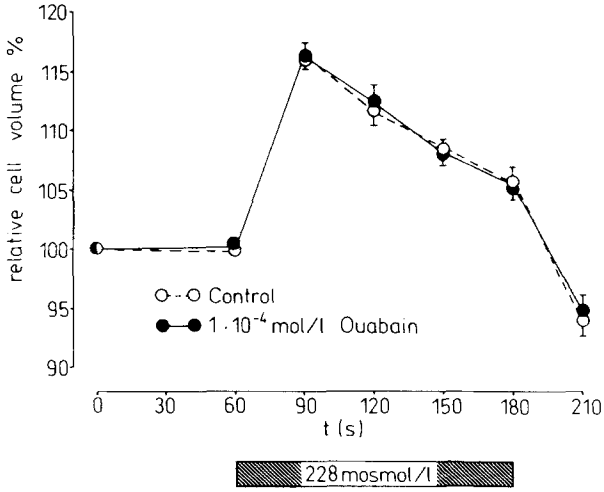

Fig. 9. Effect of hyposmotic bath perfusates on cell volume in isolated perfused proximal tubule segments in the absence (control, open circles, broken line, $n = 8$) and in the presence of ouabain (filled circles, solid line, $n = 8$). Means ± SEM

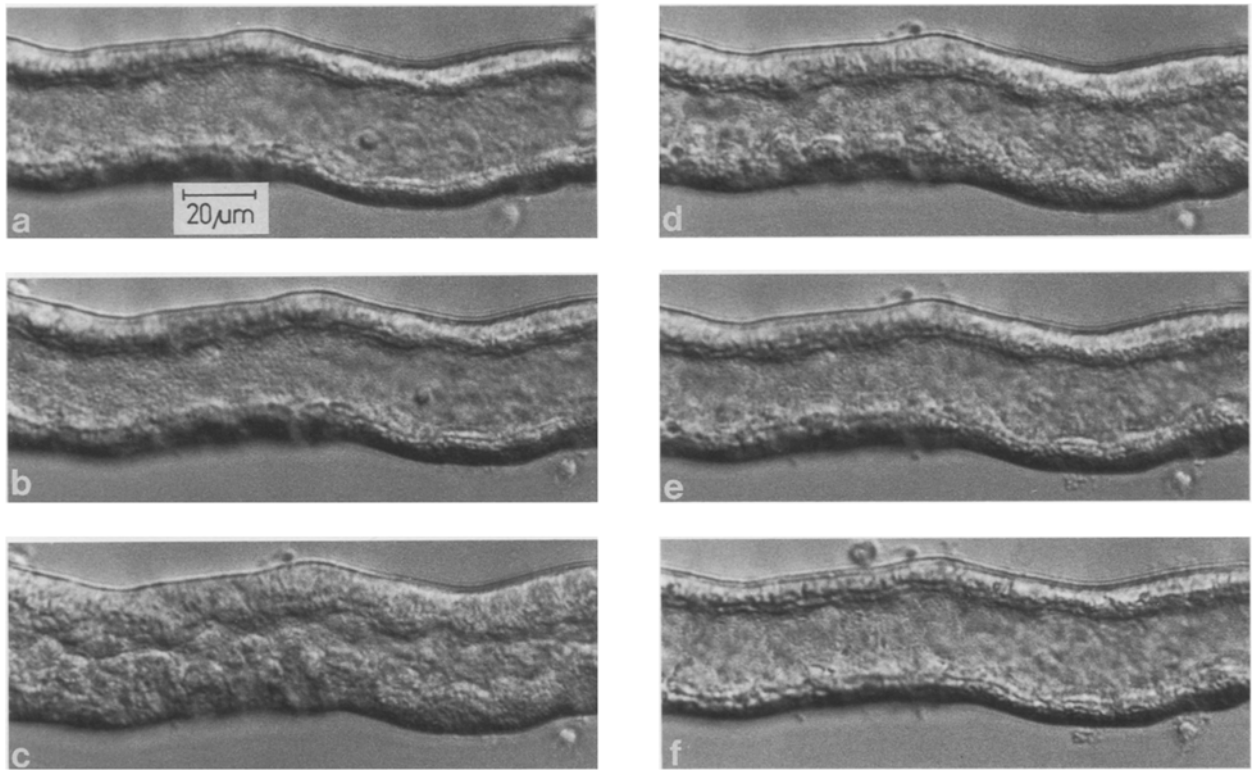

Fig. 10. Effect of hyposmotic bath perfusate on straight proximal tubule in the presence of 2 mmol/liter barium in both, luminal and bath perfusate. (*a*) Tubule exposed to isosmotic bath solution without barium. (*b*) 60 sec after exposure to isosmotic bath solution with 2 mmol/liter barium. (*c*) 60 sec after exposure to hyposmotic bath solution containing 2 mmol/liter barium. (*d*) 180 sec after exposure to hyposmotic bath solution containing 2 mmol/liter barium. (*e*) 300 sec after exposure to hyposmotic bath solution containing 2 mmol/liter barium. (*f*) 30 sec after re-exposure to isosmotic bath solution containing 2 mmol/liter barium

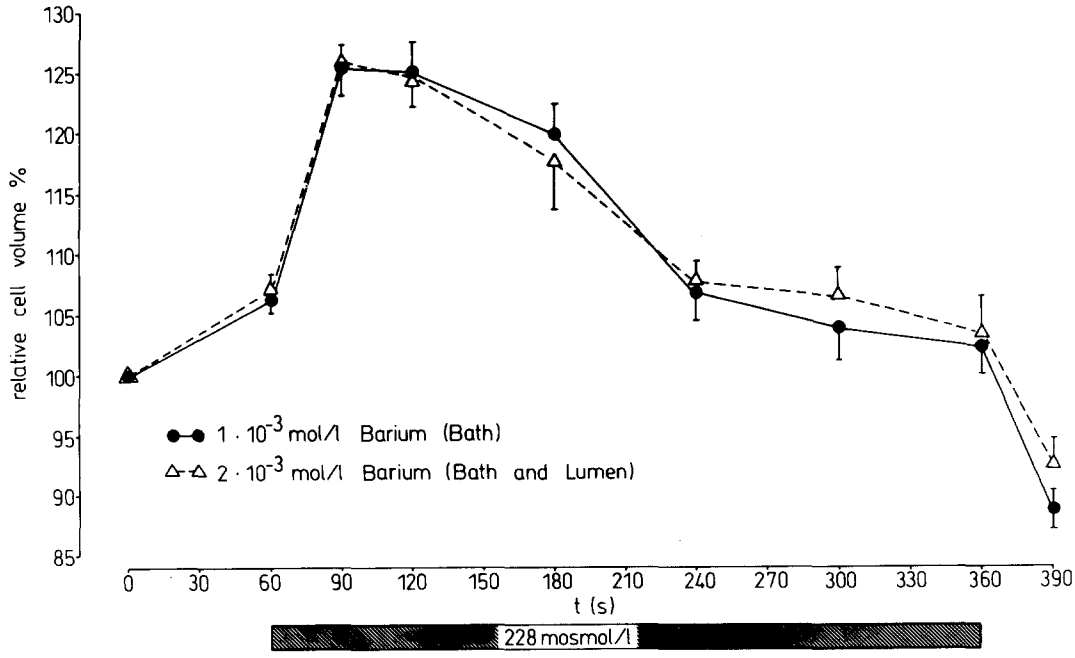

Fig. 11. Effect of hyposmotic bath perfusates on cell volume in isolated perfused proximal tubule segments in the presence of 1 mmol/liter barium in the bath (open symbols, broken line, $n = 11$) and in the presence of 2 mmol/liter barium in both, the bath and luminal perfusate (filled symbols, solid line, $n = 7$). Means ± SEM

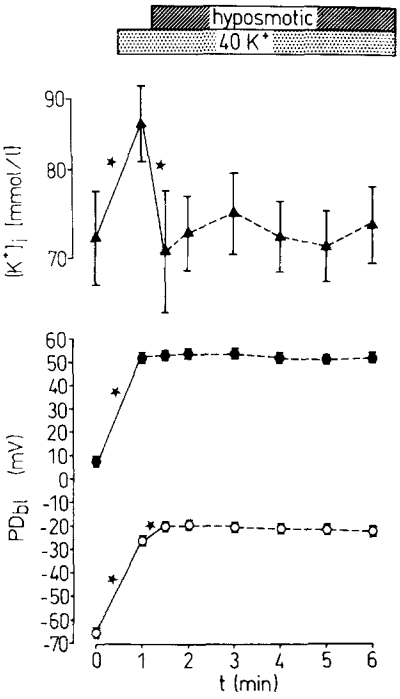

Fig. 12. Effects of hyposmotic bath perfusate on the potential difference across the basolateral cell membrane (PD_{bl}) determined with conventional (open circles, $n = 10$) and potassium-selective (filled circles, $n = 7$) microelectrodes, as well as, on intracellular K^+ activity ($[K^+]_i$). Means ± SEM. asterisk indicates significant change

nant, the cells will depolarize, whereas anion selectivity causes hyperpolarization. In mouse proximal tubules, potassium conductance amounts to more than $\frac{2}{3}$ of the cell membrane conductance and thus clearly dominates [30].

Another factor possibly influencing cell membrane potential during cell swelling is a change in ion selectivity of the cell membrane due to stimulation or inhibition of conductive pathways during volume regulatory decrease. In a previous study we observed a decrease of potassium selectivity and an increase of bicarbonate selectivity of the cell membrane during exposure of isolated perfused proximal straight tubules to hyposmotic perfusates [31]. Potassium selectivity was similarly reduced by swelling of MDCK- and Ehrlich ascites tumor cells [18]. The reduction of potassium selectivity certainly contributes to the observed depolarization. The hyperpolarization upon exposure to hyposmotic perfusates observed in intestine [20], *Necturus* gall bladder [19] and in early proximal tubule of *Necturus* kidney [24] has been explained by stimulation of potassium conductance. The decrease in potassium selectivity observed in our laboratory (*see above*) appears to contradict this assumption. The lack of cellular cable analysis, however, precludes safe predictions of alterations of any particular conductance.

Obviously, potassium cannot leave the cell without being exchanged for some other cation or being accompanied by some anion. In previous studies, we have shown that cell volume regulatory decrease is unaffected in the absence of chloride but is impaired in the absence of bicarbonate and sodium [32]. Furthermore, the bicarbonate selectivity increases during exposure to hyposmotic solutions [31]. These observations preclude a crucial role for chloride and are compatible with involvement of sodium-bicarbonate cotransport in cell volume regulatory decrease. The present observation that cell volume regulatory decrease is virtually unaffected at 20 mmol/liter bath potassium concentration despite a depolarization of the cell membrane below −35 mV strongly suggests that a potent cell volume regulatory mechanism is operative in straight proximal tubules of the mouse kidney, which does not depend on potassium channels and is not likely to be mediated by conductive pathways at all.

To test for the possible involvement of K/H exchange, which mediates cell volume regulatory decrease in *Amphiuma* red cells [3], we have altered bath bicarbonate concentration in parallel to bath potassium concentration. Although increase of bath bicarbonate concentration to 40 mmol/liter leads to some increase of cell volume and to some slowing down of cell volume regulatory decrease, reciprocal alterations of bicarbonate and H^+ concentrations do not compensate for the effects of altered potassium concentration. Thus, cell volume regulatory decrease is apparently not mediated by passive K/H exchange or a thermodynamically equivalent system. At 40 mmol/liter $[K^+]$ in the bath, intracellular $[K^+]$ remains virtually constant during regulatory volume decrease. Thus, cellular $[K^+]$ is lost in parallel to the loss of volume, and potassium remains close to passive equilibrium across the basolateral cell membrane. If volume regulatory decrease were accomplished by loss of potassium salts or exchange of K^+ for H^+, intracellular $[K^+]$ should decrease below passive equilibrium.

In conclusion, cell volume regulatory decrease in proximal straight tubules is paralleled by cell membrane depolarization, is virtually unaffected at 20 mmol/liter bath potassium concentration, and only retarded by raising bath $[K^+]$ to 40 mmol/liter, or by complete inhibition of potassium conductance with barium. These observations strongly suggest the involvement of a nonconductive volume regulatory mechanism, which is not likely to be K/H exchange, considering the limited influence of changes in extracellular bicarbonate and H^+ concentrations and the constancy of intracellular K^+ concentration.

The authors gratefully acknowledge the valuable discussions with P. Deetjen; the skilled technical assistance of H. Heitzenberger, A. Grundnig, G. Siber and S. David; and the excellent mechanic and electronic support by K.H. Streicher and M. Hirsch. The study has been supported by the Fonds zur Förderung der wissenschaftlichen Forschung: Grant No. P5813 and P6792M.

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Received 12 January 1990; revised 30 March 1990