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Hypertonicity Decreases Basolateral K^+ and Cl^- Conductances in Rabbit Proximal Convoluted Tubule

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Abstract. Collapsed proximal convoluted tubules (PCT) shrink to reach a volume 20% lower than control and do not exhibit regulatory volume increase when submitted to abrupt 150 mOsm/kg hypertonic shock. The shrinking is accompanied by a rapid depolarization of the basolateral membrane potential (V_{BL}) of 8.4 ± 0.5 mV, with respect to a control value of −54.5 ± 1.9 mV ($n = 15$). After a small and transient hyperpolarization, V_{BL} further depolarizes to reach a steady depolarization of 19.5 ± 1.5 mV ($n = 15$) with respect to control. In the post-control period, V_{BL} returns to −55.8 ± 1.5 mV. The basolateral partial conductance to K^+ (t_K) which is 0.17 ± 0.01 ($n = 5$) in control condition, decreases rapidly to nonmeasurable values during the hypertonic shock and returns to 0.23 ± 0.03 in the post-control period. The basolateral partial conductance to Cl^- (t_{Cl}), which is 0.05 ± 0.02 ($n = 5$) in control, also decreases in hypertonicity to a nonmeasurable value and returns to 0.03 ± 0.01 in post control. The partial conductance mediated by the Na-HCO_3 cotransporter (t_{NaHCO3}), which is 0.48 ± 0.06 ($n = 5$) in control condition, remains the same at 0.44 ± 0.05 ($n = 5$) during the hypertonic period. Similarly, the membrane absolute conductance mediated by the Na-HCO_3 cotransporter ($G_{Na\text{-}HCO3}$) does not vary appreciably. Concomitant with cell shrinkage, intracellular pH (pH_i) decreases from a control value of 7.26 ± 0.01 to 7.13 ± 0.02 ($n = 12$) and then remains constant. Return to control solution brings back pH_i to 7.28 ± 0.03. From these results, we conclude that in collapsed PCT, a sustained decrease in cellular volume leads to cell acidification and to inhibition of K^+ and Cl^- conductances.

Key words: Membrane potential — Ionic conductances — Hypertonic shock — Isolated collapsed proximal tubule — pH — Cellular volume

Introduction

The proximal convoluted tubule is responsible for the reabsorption of the major portion of the glucose and amino acids filtered by the glomerula. This process involves cotransport with Na^+ at the apical membrane followed by passive efflux of the substrates and active pumping out of Na^+ by the Na^+/K^+ ATPase across the basolateral membrane. As recognized in previous reviews on epithelial transport [13, 34], intrinsic regulatory mechanisms must be present to allow solute efflux at the basolateral membrane to closely match solute influx at the apical membrane, when the latter is altered to minimize changes in cell volume and maintain cell homeostasis. Of particular interest in this cross-talk between the apical and basolateral membranes is the upregulation of the K^+ conductance which accompanies increased transepithelial transport and which allows recycling towards the peritubular milieu of the K^+ that has entered the cell through the increased activity of the Na^+/K^+ pump. In parallel with this increase in K^+ conductance in the proximal tubule, a substantial cellular swelling is observed [2, 3, 4, 10], which precedes the increase in K^+ conductance [22]. The latter observation strongly suggests that the volume change and the ensuing membrane deformation or stretch [33] could act as a trigger or a modulator to increase the K^+ conductance.

In relation with this, cell volume regulation, which takes place following cell swelling induced by a hypotonic shock has been shown to involve loss of K^+ and Cl^- ions in response to activation of K^+ and Cl^- conductances [4, 12, 14, 15, 17, 19, 26, 32, 37, 38]. In a recent study, we compared the temporal behavior of the individual basolateral ionic conductances to that of the vol-

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ume, following hypotonic cell swelling on a nonperfused, and thus nontransporting, proximal tubule [26]. Interestingly, the delay observed between the increase in cell volume and that of K^+ and Cl^- conductances was comparable to that obtained during transport stimulation, demonstrating that the change in volume *per se* can activate the conductances.

In the light of these results and given that inhibition of transepithelial transport leads to a sustained cell volume reduction [22], we further explored the correlation between ionic membrane conductances and cellular volume changes by extending our studies to cell shrinkage from a control value, for which no electrophysiological data were available. We proceeded to study the changes in basolateral membrane potential, intracellular pH as well as K^+, Cl^- and Na-HCO_3 partial conductances which accompany a reduction in cellular volume induced by a rapid hypertonic shock.

Our results show that the cellular shrinkage which follows a rapid hypertonic shock, induces cellular acidification, depolarization of the basolateral membrane, and important decreases in partial conductances to potassium and chloride which reach unmeasurable values, whereas the partial conductance mediated by the Na-HCO_3 cotransporter remains unchanged.

Altogether the results of our previous work [26] and the present one support the notion that in the PCT, changes in the cellular volume lead to changes in the same direction in basolateral membrane potential, potassium and chloride conductances, as well as intracellular pH. Therefore, cell volume can be considered as an important modulator involved in the membrane cross-talk that takes place during physiological variations in proximal transepithelial reabsorption.

Materials and Methods

Proximal convoluted tubules (S1 and S2 segments) were dissected from the midcortex of New Zealand white rabbit kidneys at 4°C with fine forceps under 40× magnification in cold preservation solution (Na_2HPO_4: 56 mM; NaH_2PO_4: 13 mM; sucrose: 140 mM). Experiments were performed using non perfused isolated tubules, as described previously [26].

Peritubular perfusion and solution exchange were accomplished as described previously [26]. Briefly, the tubules were transferred into the perfusion chamber and positioned near the end of three parallel glass tubes with a common exit, laying on a thermostated plate, which allowed rapid solution exchange (<200 msec) at constant temperature. Bathing solutions, when reaching the perfusion chamber, were at the temperature of 38°C.

The composition of the bathing solutions used in this study is listed in Table 1. The pH for all solutions was adjusted to 7.4 after bubbling with a gas mixture of 95% O_2 and 5% CO_2, except for solutions C and G which were bubbled with 97% O_2 and 3% CO_2. The osmolality was 300 mOsm/Kg H_2O for control solutions and the experimental solutions were made hypertonic at 450 mOsm/Kg H_2O by addition of mannitol.

The basolateral membrane potential (V_{BL}) was measured using conventional glass microelectrodes as described previously [26]. The apparent partial conductance of the basolateral membrane to a given ion *i*, t_i, was estimated by means of potential measurements, according to the following approximation:

$$t_i \approx \frac{\Delta V_{BL}}{\Delta E_i} \tag{1}$$

where ΔV_{BL} is the instantaneous change in basolateral membrane potential induced by a step change in the concentration of the ion, corresponding to a change in its equilibrium potential, ΔE_i. All ΔV_{BL} values have been corrected for the change in the liquid junction potential at the bath bridge (1 M KCl)-bath solution interface according to Laprade and Cardinal [20]. The step changes in ionic concentrations used to alter the equilibrium potential for an ion were: (i) from 5 to 15 mM for potassium, leading to a change in the K^+ equilibrium potential (ΔE_K) of +29.4 mV; (ii) from 118.6 to 21 mM for chloride, giving a ΔE_{Cl} of +46.5 mV; (iii) from 25 to 15 mM for the HCO_3^- (keeping pH_o constant), which produced a $\Delta E_{Na\text{-}HCO3}$ of +20.5 mV, assuming a stoichiometry of 1 Na^+ to 3 HCO_3^- [39]. These step concentration changes at 18-sec intervals were of short duration (1.5 sec) in order not to change appreciably intracellular contents, but yet long enough to allow a plateau value for ΔV_{BL} to be reached.

pH_i of proximal tubules was measured fluorometrically as described previously [1, 26] using the pH-sensitive dye 2,7-biscarboxyethyl-5(6)-carboxyfluorescein (BCECF). The dye was excited alternatively at 450 and 500 nm. Fluorescent light was filtered at 530 nm and was measured with a photomultiplier tube by averaging for 300 msec the signal at both wavelengths. The ratio of the intensities of emitted light from the two excitation wavelengths was computed after correction for background fluorescence. Calibration of the dye was performed using 10 μM nigericin in presence of 120 mM potassium at pH 7.0, 7.25 and 7.5.

The values of t_i, V_{BL} and pH_i reported in our results are the averages of at least 5 measurements taken from stable portions of the signal in control, hypertonic and post-control conditions.

Bicarbonate absolute conductance was measured as described previously [26] using the following equation:

$$G_{NaHCO3} = \frac{V \cdot \beta_T}{(t_{NaHCO3} - 1) \cdot \Delta E_{NaHCO3}} \cdot \frac{dpH_i}{dt} \cdot \frac{(n-1) \cdot F}{n \cdot A} \tag{2}$$

where dpH_i/dt is the initial rate of change in intracellular pH (pH_i) during the step change in peritubular bicarbonate concentration when returning from low (15 mM) to control (25 mM) bicarbonate. β_T is the total intracellular buffering power determined from the buffering power of the CO_2-HCO_3^- pair and the intrinsic buffering power calculated from the change in pH_i when CO_2 is changed from 5% to 3%. *A* is the basolateral membrane area, *V*, the tubular volume, *F* the Faraday constant, n, the stoichiometry of HCO_3^- to Na^+ (3 in the present case) and $(n - 1)/n$ represents the number of Faradays transported per mole of bicarbonate. Equation 2 assumes that all HCO_3^- flux is mediated by the Na-HCO_3 cotransporter.

Tubules were magnified 400× using an Olympus IMT-2 microscope and each image was recorded by a monochrome CCD-72 camera (DAGE-MTI, Michigan City, IN) in conjunction with an IBM 486 DX2 computer equipped with a IP8/AT videographics system board (Matrox, Dorval, Qc, Canada). Cellular volume is expressed relative to the initial control volume. Images were analyzed with a homemade software and cellular volume was estimated from the outside diameter of the tubules for a given length, using a cylindrical configuration as described previously [7].

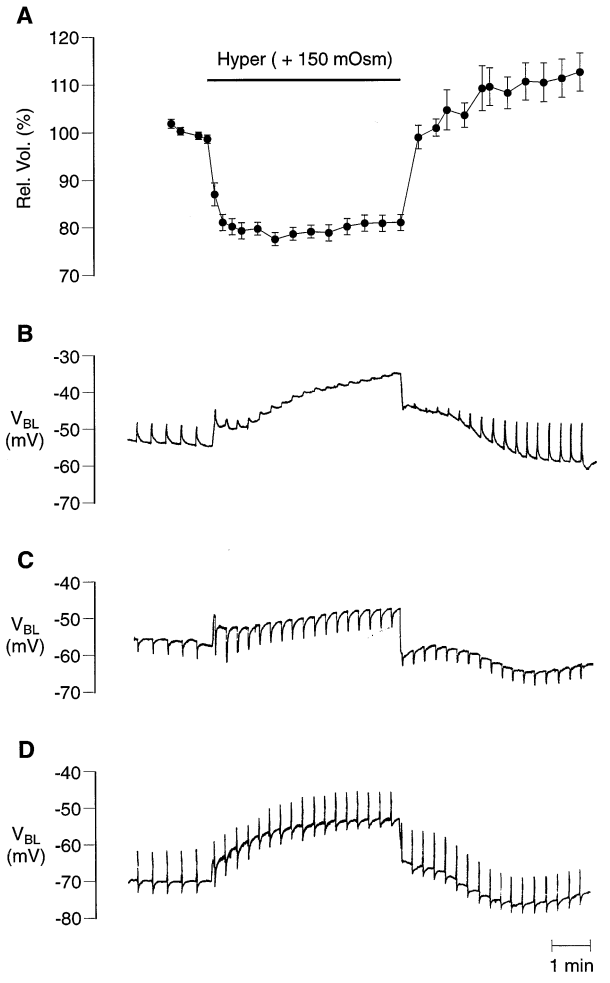

Fig. 1. Effect of osmolality increase from 300 to 450 mOsm/Kg. Rel. vol. represents relative tubular volume and 100% indicates control volume (*A*). The plots of V_{BL} represent typical recordings of basolateral potential with superimposed potential changes due to rapid changes of potassium concentration from 5 to 15 mm (ΔE_K = 29.4 mV) (*B*), of chloride concentration from 118.6 to 21 mm (ΔE_{Cl} = 46.5 mV) (*C*) and of bicarbonate concentration from 25 to 15 mm ($\Delta E_{Na\text{-}HCO3}$ = 20.5 mV (*D*).

Statistics

Data are means ± SE. Two-tailed paired *t*-test was used with InStat software (Graph PAD Software).

Results

Tubular Volume

The effect of hypertonic shock on tubular volume is shown in Fig. 1*A*. An abrupt increase in bath osmolality induced a rapid decrease of volume of 20%. After 5 min in hypertonicity the tubule did not display regulatory volume increase. When the tubule was re-exposed to the original osmolality, volume increased rapidly to a value close to the starting volume and then gradually increased to reach a value 15% greater than the starting volume after 5 min.

Table 1. Composition of bath solutions

	Isotonic solutions			
	A Control	B High K^+	C Low HCO_3^-	D Low Cl^-
NaCl	100.0	100.0	110.0	2.4
KCl	5.0	15.0	5.0	5.0
$MgSO_4$	1.2	1.2	1.2	1.2
NaH_2PO_4	1.0	1.0	1.0	1.0
Glucamine-Cl	10.0		10.0	10.0
Mannitol				
$CaCl_2$	1.8	1.8	1.8	1.8
Na-cyclamate				97.6
Na-acetate	4.0	4.0	4.0	4.0
Na_3-citrate	1.0	1.0	1.0	1.0
Glucose	5.5	5.5	5.5	5.5
Alanine	6.0	6.0	6.0	6.0
Na_2HPO_4	3.0	3.0	3.0	3.0
$NaHCO_3$	25.0	25.0	15.0	25.0
	Hypertonic solutions			
	E Control	F High K^+	G Low HCO_3^-	H Low Cl^-
NaCl	100.0	100.0	110.0	2.4
KCl	5.0	15.0	5.0	5.0
$MgSO_4$	1.2	1.2	1.2	1.2
NaH_2PO_4	1.0	1.0	1.0	1.0
Glucamine-Cl	10.0		10.0	10.0
Mannitol	150.0	150.0	150.0	150.0
$CaCl_2$	1.8	1.8	1.8	1.8
Na-cyclamate				97.6
Na-acetaate	4.0	4.0	4.0	4.0
Na_3-citrate	1.0	1.0	1.0	1.0
Glucose	5.5	5.5	5.5	5.5
Alanine	6.0	6.0	6.0	6.0
Na_2HPO_4	3.0	3.0	3.0	3.0
$NaHCO_3$	25.0	25.0	15.0	25.0

Solution composition in mM. All solutions, except solutions C and G were bubbled with 5% CO_2 − 95% O_2 gas.

Basolateral Membrane Potential

Typical recordings of the basolateral membrane potential are shown in Fig. 1*B*, *C* and *D*. Tubules were exposed to the control solution (*A*, Table 1) for at least 5 min before they were submitted to the hypertonic solution (*E*, Table 1) for 5 min. after which the control solution was reintroduced. The increase in bath osmolality from 300 to 450 mOsm/Kg produced a rapid depolarization of the basolateral membrane followed by a small and transient hyperpolarization and finally by a slow depolarization,

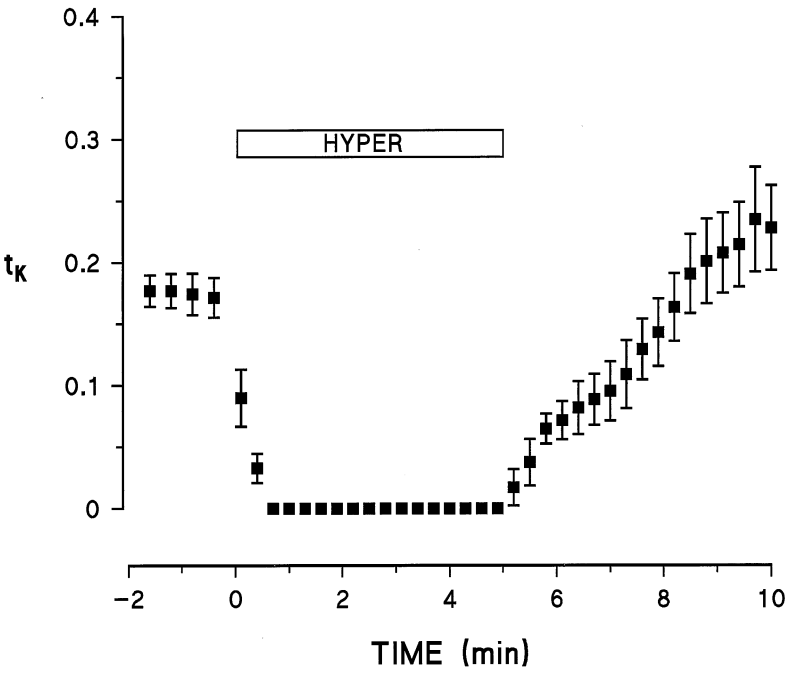

Fig. 2. Effect of hypertonicity on partial K^+ conductance. t_K ($n = 5$) rapidly decreases to nonmeasurable values following the hypertonic shock and slowly reaches a value above control at the end of the post-control period.

the membrane potential reaching a relatively stable value at the end of the experimental period. The average basolateral potential for 15 tubules under control conditions was −54.5 ± 1.9 mV. The rapid initial depolarization under hypertonic conditions was 8.4 ± 0.5 mV and the final depolarization 19.5 ± 0.5 mV ($n = 15$). During the post-control period, a rapid hyperpolarization was observed, followed by a transient depolarization and by a final gradual repolarization to reach −55.8 ± 2.5 mV ($n = 15$), a value not significantly different from that in control.

Partial Conductance to Potassium

Figure 1*B* shows a typical tracing of the V_{BL} depolarizing spikes induced by step changes of bath K^+ from 5 to 15 mM ($\Delta E_K = 29.4$ mV) under control and hypertonic conditions. The average t_K under control conditions was 0.17 ± 0.01 ($n = 5$). Following the hypertonic shock, t_K decreased to nonmeasurable values within 30 sec ($P < 0.05$), and in the post-control period, gradually recovered to 0.23 ± 0.03, a value not significantly different from the control value (Fig. 2).

Partial Conductance to Chloride

Step reductions of Cl^- concentration in the bath solution from 118.6 to 21 mM ($\Delta E_{Cl} = 46.5$ mV) induced hyperpolarizing spikes instead of the expected basolateral membrane depolarizations (Fig. 1*C*). This was due to the change in the bath-bridge liquid junction potentials as discussed in previous studies [20, 26]. However, after correction of the data for these liquid junction potentials, the spikes in the isotonic solution were in the depolarizing direction. Under control conditions the average t_{Cl} value was 0.05 ± 0.02 ($n = 5$). Figure 1*C* shows that during hypertonicity the magnitude of the hyperpolarizing spikes augmented. The amplitude of the spikes at the end of the hypertonic period was identical to the measured change in bath-bridge liquid junction potential, implying that t_{Cl} was negligibly small at the end of the hypertonic period. Upon return to the isotonic solution, t_{Cl} increased rapidly to 0.03 ± 0.01, a value not significantly different from that in control.

Partial Conductance to Bicarbonate

To investigate the partial conductance associated with the Na-HCO_3 cotransporter we reduced bath HCO_3^- from 25 to 15 mM ($\Delta E_{Na\text{-}NHO3} = 20.5$ mV) at a constant pH_o of 7.4. We considered a $3HCO_3^-/1Na^+$ stoichiometry for the cotransporter [38]. Under control and hypertonic conditions, the step changes in bath HCO_3^- induced V_{BL} depolarizing spikes (Fig. 1*D*). The average value of bicarbonate partial conductance estimated from these spikes under control conditions was 0.48 ± 0.06 ($n = 5$). The hypertonic shock induced a very small decrease in the amplitude of the depolarization spikes leading to a

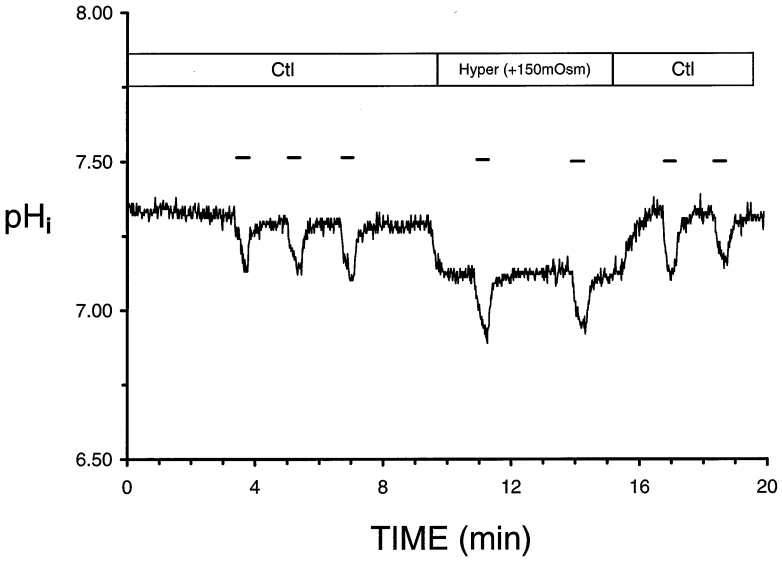

Fig. 3. Effect of hypertonicity on intracellular pH. Are also shown, the changes in pH_i produced by the step changes in HCO_3^- from 25 to 15 mM, indicated by horizontal bars, (keeping peritubular pH constant), in control, hypertonic and post-control conditions. The latter are used to calculate G_{NaHCO3} in Eq. 2.

nonsignificant reduction of $t_{Na\text{-}HCO3}$ to 0.44 ± 0.05. In the post-control period, t_{NaHCO3} slightly increased to reach 0.50 ± 0.07 ($n = 5$), a value not significantly different from that in control.

Absolute Conductances

To determine the behavior of the absolute conductance mediated by the Na-HCO_3 cotransporter and consequently the absolute conductance to potassium and chloride during the hypertonic shock, we used Eq. 2 and followed the procedure we had developed in a previous paper [26]. Figure 3 shows the changes in pH_i produced by the step changes in peritubular bicarbonate concentration from 25 to 15 mM. As shown in Table 2, dpH_i/dt decreased approximately by a factor 1.35 during the hypertonic shock and the intrinsic buffering power β_i increased by approximately a factor 1.22 as expected from the concentrating effect of cell shrinkage. However, total buffering power, β_T did not change due to the reduction in intracellular HCO_3^- (decrease in pH_i). The calculated values of the ratio of bicarbonate conductances in experimental and control conditions show that G_{NaHCO3} decreases slightly although not significantly during the hypertonic period. Therefore, the decrease in t_{Cl} and t_K in hypertonic conditions can be attributed solely to decreases in G_K and G_{Cl}.

Intracellular pH

Figure 3 also shows the behavior of pH_i. Under control conditions, pH_i was 7.26 ± 0.01 ($n = 12$). Following the hypertonic shock, pH_i decreased within one minute to 7.13 ± 0.02 ($P < 0.01$) and then remained constant. pH_i, which was 7.12 ± 0.03 at the end of the hypertonic period, came back to 7.28 ± 0.03 in post-control conditions, a value not significantly different from control.

To investigate the mechanism responsible for the acidification observed during the hypertonic shock, we have repeated the same experiment, but 5 min after blocking the Na-HCO_3 cotransporter by the addition of 0.5 mM SITS (Fig. 4). Following SITS addition, in all six experiments performed, pH_i decreased transiently to come back close to its original value in 5 min. This is in contrast with what has been observed in the perfused tubule where the same maneuver lead to an alkalinization, a behavior that was attributed to inhibition of basolateral HCO_3^- exit (1). Compatible with this, the present results would then suggest that the observed acidification in the collapsed tubule would be due to blockade by SITS of basolateral entry of HCO_3^-. Also, in all of the present experiments, and in contrast to what is seen in absence of SITS, pH_i initially alkalinized following the hypertonic shock (mean ΔpH_i: 0.08 ± 0.008, $P < 0.05$) and gradually returned to its original value (7.19 ± 0.08 at the end of the hypertonic period compared to 7.17 ± 0.08 before the shock, a nonsignificant change).

Discussion

In a previous paper, we have shown that the volume regulation observed following cell swelling caused by a hypotonic shock was correlated with increases in basolateral potential, potassium and chloride conductances,

Table 2. Intracellular buffering power and ratio of bicarbonate conductances

	$\frac{dpH_i}{dt}$	β_i(M)	β_T(M)	$\frac{(G_{NaHCO3})^{EXP}}{(G_{NaHCO3})^{CTL}}$
Control	0.023 ± 0.002	0.032 ± 0.005	0.052 ± 0.008	1.00
Hypertonic 1	0.017 ± 0.001*	0.039 ± 0.003	0.063 ± 0.006	0.67 ± 0.15
Hypertonic 2	0.019 ± 0.003	0.038 ± 0.006	0.068 ± 0.01	0.79 ± 0.32
Post-control	0.022 ± 0.004	0.035 ± 0.006	0.065 ± 0.01	1.25 ± 0.49

Values ($n = 6$) of the instantaneous variations in pH_i during the step changes in bicarbonate concentration (dpH_i/dt, pH unit/s), of intrinsic cellular buffering power (β_i), total intracellular buffering power (β_T) and the ratio of bicarbonate conductances, $(G_{NaHC3})^{EXP}/(G_{NaHCO3})^{CTL}$ (*see* Materials and Methods). The measurements were done in control condition, 1 min after the hypertonic shock (Hypertonic 1), 5 min after the shock (Hypertonic 2) and in post-control (*see* Fig. 3). *Values significant with respect ot control ($P < .05$).

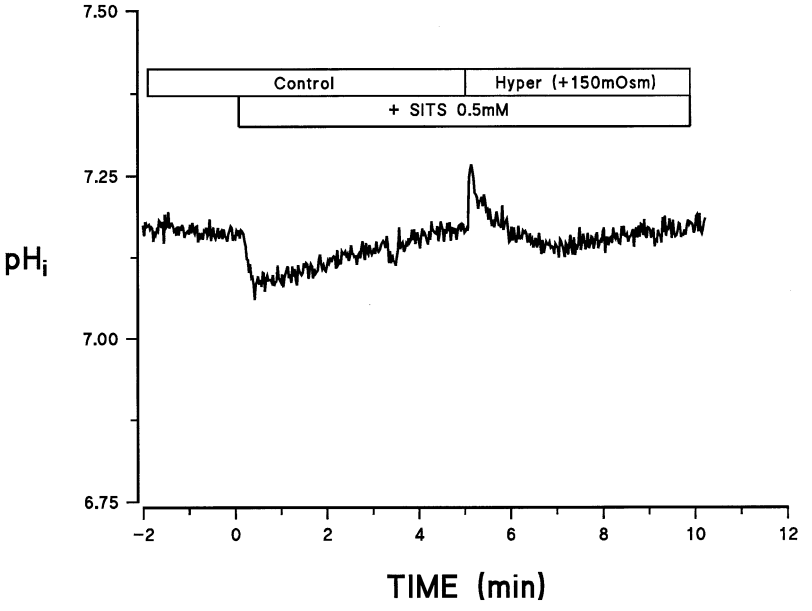

Fig. 4. Effect of hypertonicity on intracellular pH in the presence of SITS.

and in intracellular pH [26]. In the case of hypertonic shock we now report a sustained cellular volume decrease which is accompanied by an acidification of intracellular milieu and a reduction in potassium and chloride conductances i.e., a mirror image of what is observed in hypotonicity.

Hypertonic Shock and Basolateral Membrane Potential

In parallel to tubular volume reduction, we observed that the basolateral membrane depolarized first, transiently, and then gradually, to reach a final depolarization of 19.5 ± 1.5 mV. It is interesting to note again that this behavior of the membrane potential following hypertonicity is the mirror image of what happens in hypotonicity where, after a rapid hyperpolarization (blockable by SITS, ref. 26) followed by a transient depolarization, we observed a gradual hyperpolarization. The rapid initial depolarization observed following the hypertonic shock is compatible with Na-coupled exit of HCO_3^-, due to the outwardly increased chemical gradient for Na^+ and HCO_3^- following exit of water, while the gradual depolarization is quite probably linked to the gradual decrease in K^+ conductance. Indeed, due to the large reduction in potassium and chloride conductances together with the nonsignificant change in sodium-bicarbonate conductance, the basolateral potential would then approach under these conditions, the equilibrium potential of the $Na\text{-}HCO_3$ cotransport. However, opening of a nonspecific conductive pathway could also be responsible for the observed depolarization since it has been previously reported that hypertonic shrinkage activates a large non selective cation conductance in airway epithelial [11] and renal epithelial [36] cells. To investigate this, we submitted the basolateral membrane of PCT to step changes in Na (from 164 to 64 mM) by replacement of Na by N-methyl-D-glucamine, in the absence of HCO_3^- (re-

placed by Cl^-) so as to eliminate the Na-HCO_3 conductance. In all of the five experiments performed (*results not shown*), we could not detect any measurable hyperpolarization in response to the step changes in Na, both in control and hypertonic conditions, indicating nonsignificant nonselective cationic conductance at the basolateral membrane of the PCT.

Ionic Conductances and Tubular Shrinking

The present study shows that a hypertonic shock induces a sustained shrinkage of proximal tubule cells that is not followed in the short term by a regulatory volume increase (Fig. 1*A*). This result is in agreement with previous data reported in the literature [14, 19, 25, 31]. In parallel with this decrease in cellular volume, the magnitude of partial basolateral conductances to potassium and chloride rapidly decreased to nonmeasurable values (Fig. 1*B* and *C* and Fig. 2). Indeed, t_K and t_{Cl} are already undetectable 30 sec after the beginning of the hypertonic period. The dramatic reduction in t_K and t_{Cl}, together with the nonsignificant change in G_{NaHCO3} (Table 2) during the hypertonic period, clearly indicate that potassium and chloride absolute conductances decreased to negligible values. The fact that t_K and t_{Cl} decreased while t_{NaHCO_3} did not increase may appear somehow paradoxical. However, although the sum of G_k, G_{Cl} and G_{NaHCO_3} certainly constitute the major portion of the basolateral membrane conductance, we cannot exclude the possible presence of additional, yet unidentified conductances, in control or hypertonic conditions, which could then largely mask the otherwise expected increase in t_{NaHCO_3}. It is interesting to point out that the present finding of a reduction in basolateral PCT K^+ conductance is in agreement with those of Ritter et al. [30] and those of Simmons & Tivey [35] in MDCK cells, who observed that mannitol-induced hypertonicity induced a reduction of basolateral K^+ conductance.

Given the mirror behavior of membrane potential and conductance as well as pH_i between hypertonicity and hypotonicity, we may inquire why the volume does not recuperate following the initial shrinking. We may also inquire about the possible significance of the accompanying decrease in G_K and G_{Cl} with respect to transepithelial transport regulation and cell homeostasis, especially in the light of the numerous studies dedicated to RVD following an hypotonic shock. The latter studies were conducted largely with the underlying rationale that the mechanisms responsible for RVD likely operated in minimizing volume changes during alterations of transport. They have shown that an enhanced exit of K^+ and Cl^- following increases in G_K and G_{Cl} [26, 38] is largely responsible for the observed RVD. So, in the particular case where transepithelial transport is stimulated, an initial increase in cellular volume occurs, due to the latency of the basolateral transport mechanisms to adapt to the increased rate of entry of solutes at the apical membrane. As the increase in cell volume triggers an increase in G_K and G_{Cl} and thus an exit of these solutes through the basolateral membrane, further increase in cell volume is then hindered and so, changes in volume minimized. Conversely, if transepithelial transport is decreased, a decrease in cell volume will rapidly take place. The resultant increase in intracellular K^+ and Cl^- concentrations due to exit of water following preferential exit of Na^+ by the Na/K pump would then promote their enhanced exit and hence a further decrease in cell volume. Therefore, a reduction in G_K and G_{Cl} will prevent this exit of K^+ and Cl^- and thus prevent further volume reduction. On the other hand, contrary to the situation in hypotonic conditions, volume recovery cannot take place in hypertonicity simply because the electrochemical gradients for K^+ and Cl^- ions can only favour their exit, not their entry, so volume recuperation cannot take place through this osmolyte pathway. It is interesting to note that the RVI, observed in hypertonicity in the presence of butyrate [31], was associated with an increase in Na^+ and Cl^- indicating that part of the RVI was due to increased uptake of these ions promoted by butyrate. So, in summary, our findings, both in hypotonic and hypertonic conditions, would be in agreement with the concept that a change in G_K and G_{Cl} in the same direction as that of the volume contributes to minimize changes in volume during alterations in the rate of transepithelial transport.

Intracellular pH and Volume Changes

Despite the dramatic decreases in G_K and G_{Cl}, cell shrinking during the hypertonic shock did not induce significant changes in G_{NaHCO3} (Table 2). This is consistent with our previous observations that hypotonicity, although it increased importantly K^+ and Cl^- conductances had negligible effects on bicarbonate conductance [26] indicating that specific mechanisms are involved in the modulation of G_K and G_{Cl}. It is interesting to recall that while in the present study, hypertonicity leads to an acidification, hypotonicity induced an alkalinization [26]. This suggests that the changes in intracellular HCO_3^- are likely induced by the movement of HCO_3^- through the Na-HCO_3 cotransporter via the volume-induced changes in intracellular Na concentration. Indeed, the exit of HCO_3^- in hypertonicity cannot be produced by the changes in membrane potential since it is in the direction opposite to that expected. Such a movement of HCO_3^- in a direction opposite to that expected from the change in membrane potential had also been observed by Beck et al. following an hypotonic shock (1). In the latter study, inhibition of the Na-HCO_3 cotransport with SITS prior to the hypotonic shock prevented the alkalinization, hence demonstrating the implication of this cotransporter in the observed alkalinization. The finding in the present study that SITS prevents

the acidification observed in hypertonicity further confirms the role of the Na-HCO_3 cotransporter in the changes of pH_i during changes in cellular volume.

The mechanisms by which cell shrinking is able to inhibit so dramatically G_K and G_{Cl} while not affecting G_{NaHCO3} are presently unknown. Ca^{++} is unlikely to be involved. Indeed, we have shown that the PCT K^+ conductance is Ca^{++}-independent and moreover, that although hypotonicity triggers increases in intracellular Ca^{++}, these play no role in the RVD process [2, 6]. Although the decrease in pH_i might in principle contribute to the decrease in G_K, it could only be to a very modest extent, given the known pH-dependence of G_K [1] and the relatively small decrease in pH_i observed here. Although we have shown previously that the basolateral G_K of the PCT is ATP sensitive [18], ATP unlikely played a role in the activation of G_K in hypotonicity, given that intracellular ATP should have then been modulated mainly by cell water content so that the temporal behavior of the increase in G_K should have paralleled that of volume, which was not the case [26]. A role for ATP is even less likely here, considering that the changes in volume are quite small, especially when compared to the total inhibition of G_K. On the other hand, the cytoskeleton, in a way still to be uncovered, might be involved in this process as it has been shown that actin filaments are implicated in volume-activated Cl^- channels in cultured distal tubule cells [29]. This aspect is certainly worth investigating.

In summary, a 20% reduction in cellular volume reduces intracellular pH and decreases K^+ and Cl^- conductances to nonmeasurable values but does not affect the Na-HCO_3 cotransporter. From this and our previous findings on cellular swelling [26], we conclude that in the proximal convoluted tubule, changes in cellular volume induce changes in the same direction in intracellular pH, and in potassium and chloride conductances. These findings clearly demonstrate that cell volume plays an important role in the regulation of transepithelial transport in the PCT.

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