Isovolumetric Regulation of Isolated S_2 Proximal Tubules in Anisotonic Media

J. W. Lohr and J. J. Grantham
Department of Medicine, Kidney and Urology Research Center, University of Kansas Medical Center, Kansas City, Kansas 66103

Abstract

Sudden alteration in medium osmolality causes an osmometric change in proximal tubule cell size followed by restoration of cell volume toward normal in hypotonic but not in hypertonic medium. We determined the capability of isolated nonperfused proximal tubules to prevent a change in cell volume in anisotonic media. The external osmolality was gradually changed over a range from 110 to 480 mosM. At 1.5 mosM/min, cell volume remained constant between 167±9 and 361±7 mosM, a phenomenon termed isovolumetric regulation (IVR). Cells lost intracellular solutes in hypotonic and gained intracellular solutes in hypertonic media. Raffinose or choline chloride substitution showed that osmolality, rather than NaCl, signalled cell volume maintenance in hyperosmotic media. Cooling (7–10°C) blocked IVR. IVR was maintained when osmolality was lowered at a rate of 27, but not at 42 mosM/min. IVR was not observed when the rate of osmolality increase exceeded 3 mosM/min. We conclude that proximal tubule cells sensitively regulate intracellular volume in an osmolality range of pathophysiologic interest by mechanisms dependent on (*a*) the rate of net water movement across basolateral membranes and (*b*) the absolute intracellular content of critical solutes.

Introduction

Volume regulation is a fundamental property of nearly all mammalian cells. In addition to meeting homocellular requirements for volume control, kidney tubule cells constantly transfer water from the luminal to the basolateral side of the epithelial layer. In proximal tubules, a volume of water one to four times the volume of the cells is reabsorbed each minute. Because most of the fluid flows through the cells of renal tubules, marked changes in cell volume could result if an inequality developed between the rates of fluid entry and exit.

Studies of volume regulatory mechanisms in mammalian kidneys have principally used nonperfused tubule segments studied in vitro (1–8). In this nonpolar preparation, volume regulation primarily reflects the steady-state transport of solutes and water across basolateral membranes (2, 3). The response of cell volume to change in medium osmolality has been used to describe some of the volume regulatory phenomena. Renal tubule cells swell rapidly when placed in hypotonic medium because the plasma membrane is more permeable to water than to crystalloid solutes (9, 10). After this rapid swelling phase, cells of both proximal and collecting tubules shrink toward their original volume. This restoration of cell size has been termed volume regulatory decrease (VRD)[1] (1, 11).

Received for publication 7 April 1986.

1. *Abbreviation used in this paper:* VRD, volume regulatory decrease.

J. Clin. Invest.

0021-9738/86/11/1165/08 $1.00
Volume 78, November 1986, 1165–1172

Conversely, when proximal renal tubules are exposed suddenly to hyperosmotic solutions, the tubule cells rapidly shrink but they remain reduced in size (4). This contrasts to other cell types that progressively restore cell volume toward normal in hypertonic medium (12–21).

Previous studies of renal volume regulation have used tissue slices, cell suspensions, isolated tubules, and single cells (1–8, 22–30). In all of these studies the medium osmolality was changed suddenly, which is uncharacteristic of osmotic changes that might be encountered in pathophysiologic states in vivo. Because cortical renal cells are rarely exposed to osmotic change rates greater than a few milliosmoles per kilogram per minute, we determined the extent to which proximal S_2 segments could prevent changes in cell size when they were gradually exposed to relatively large changes in osmolality. Our results show that S_2 proximal cells maintain normal size when osmolality is changed at relatively slow rates over a wide range of tonicities.

Methods

Female New Zealand white rabbits were anesthetized with intravenous pentobarbitol and the left kidney was removed. A thin transverse slice was immediately transferred to chilled rabbit serum (Pel-Freez Biologicals, Rogers, AR). Individual tubules were dissected at 4°C under a dissecting microscope (10–90×) using fine-tipped forceps. Straight portions of the proximal tubules were taken from the mid portion of the cortex, and trimmed to a tubule length of 0.3–0.5 mm. Tubule fragments were transferred to a sealed beaker of rabbit serum at room temperature, and incubated in a water bath at 37°C for 30 min. Individual tubule fragments were transferred to a temperature-controlled chamber (1.5 ml volume) on the stage of an inverted microscope (Unitron Instruments Inc., Plainview, NY) that contained rabbit serum at 37°C. The ends of the tubule were occluded by crimping them with micropipets (1). Fluid in the chamber was continually gassed using a mixture of 95% O_2 and 5% CO_2. After the tubule had equilibrated for 5 min in the rabbit serum, the bath was replaced with an isotonic standard medium. The tubules were kept in isotonic medium for at least 10 min.

In the experimental period, the osmolality of the tubule bath was changed by pumping mixtures of isotonic and hypo or hypertonic medium from an osmotic gradient-generating system, similar to that used to construct sucrose density gradients. The arrangement consisted of two plastic bottles of equal size connected in series by a tube inserted at the base of the containers (Fig. 1). Bath fluid was pumped out of the bottles into the tubule chamber by a roller pump (Sigmamotor, Inc., Middleport, NY) at a rate that was varied between 24 and 96 ml/h. Bath fluid was removed from the chamber by a similar pump to maintain a constant volume in the chamber. The dead space of the tubing from the mixing bottle to the chamber was 7 ml.

Osmolality of fluid withdrawn from a constant site in the tubule chamber was measured by freezing point depression with a coefficient of variation of 0.5%. The pH of tubule chamber fluid was checked periodically (Radiometer Co., Copenhagen, Denmark) and was maintained between 7.3 and 7.4. At the conclusion of some experiments, the anisotonic bath was rapidly exchanged with isotonic standard medium.

To test the cells' osmometric response, the bath osmolality was changed rapidly by flushing 2.5 ml of test medium through the chamber in a manner to ensure vigorous stirring about the tubules. Total fluid exchange time was 3.5 s. The bath was replaced with fresh anisotonic

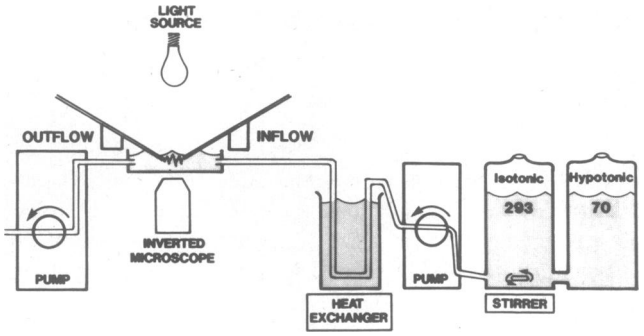

Figure 1. Gradient generating system. The bottle closest to the tubule chamber contained isotonic solution and the bottle further from the chamber contained either hypotonic or hypertonic solution. The rate of tubule bath osmolality change was altered by changing the pump speed.

medium every 5 min. After 10 min the bath was replaced with isotonic medium.

The microscope was fitted with a Panasonic television camera (model WV 1600) connected to a Panasonic monitor (model WV 5410) and a JVC video cassette recorder, model CR 60600. The tubule could be viewed at a magnification of 400 in the microscope and at a magnification of 1800–2200 on the monitor. The tubule was oriented vertically near the center of the screen. The measurement was made with a clear plastic ruler. The darkest line indicating interface between the basolateral surface and the external medium was aligned with the markers on the ruler, which were 1 mm apart. With this method we could resolve changes in tubule diameter of 0.5 μm. Outer tubule diameter was measured at three equidistant points, with care to avoid parallax. In nonperfused tubules without lumens, the volume of the cells can be estimated from the outer diameter of the tubule (d). Mean diameter for 12 representative tubules was 36.6 μm±3.0, corresponding to an average volume of 10.5×10^{-9} liters/cm of tubule length. Tubule volume, in nanoliters per centimeter of tubule length (L), was calculated from $\pi(d/2)^2L$. Tubule length did not change to an important degree and was assumed constant in the computation of tubule volume. Data is expressed as relative tubule volume V_t/V_o, where V_t is the volume at time t, and V_o is the volume in isotonic medium at zero time. Means and standard errors were determined from several tubules in each series of studies. Slopes were compared using a modified *t* test described by Steel and Torrie (31).

In some experiments the temperature of the bathing medium was reduced to 7–10°C. Bath fluid was chilled by inserting a heat exchanger (thin-walled polyethylene tubing in an ice bath) in the circuit between the osmolality mixing device and the tubule bath.

Media. The standard isotonic medium contained (in millimolars NaCl 114, K_2HPO_4 2.5, $NaC_2H_3O_2$ 5, $NaHCO_3$ 25, $MgSO_4$ 1.2, $CaCl_2$ 2.0, and glucose 5.5. The osmolality was ~293 mosM. To vary osmolality, NaCl was added to or deleted from the standard medium. In some experiments NaCl was replaced by choline chloride (114 mM) or raffinose (228 mM). Bovine serum albumin (Sigma Chemical Co., St. Louis, MO) was dialyzed extensively against standard medium and added to all media to a final concentration of 6 g/100 ml.

Results

Because the current studies are derivatives of previous work (1), we reexamined the osmometric and volume regulatory behavior of S_2 segments as a baseline for subsequent analysis of isovolumetric regulation (Fig. 2 *A*). Tubule volume was initially constant in the isotonic medium (293 mosM/kg), but rapidly increased after changing the bath to hypotonic medium, the osmometric phase. Tubule volume then decreased steadily to reach a relatively stable volume within five minutes, the VRD phase. The patterns of the osmometric and VRD phases were similar in three different hypotonic media, although the relative height of the peak volume change was inversely proportional to medium osmolality. As noted previously (1–4), when exposed to strongly hypotonic media, tubule volume decreased but did not return completely to the original baseline. This reflects incomplete VRD. When returned to isotonic medium, tubule volume decreased below the initial isotonic baseline, which reflects a net loss of intracellular solute during the VRD phase. K^+ and Na^+ are the major intracellular cations lost during the VRD phase (2), and a recent report indicates that chloride may be the principal anion accompanying the cations (6).

In contrast to the response in hypotonic medium, no volume regulatory phase was observed in hypertonic solutions (Fig. 3). The bath was replaced with isotonic medium after 10 min. Tubule volume returned to the original baseline value, but did not exceed it. These observations in hyperosmotic media are in agreement with the findings of Gagnon et al. (4).

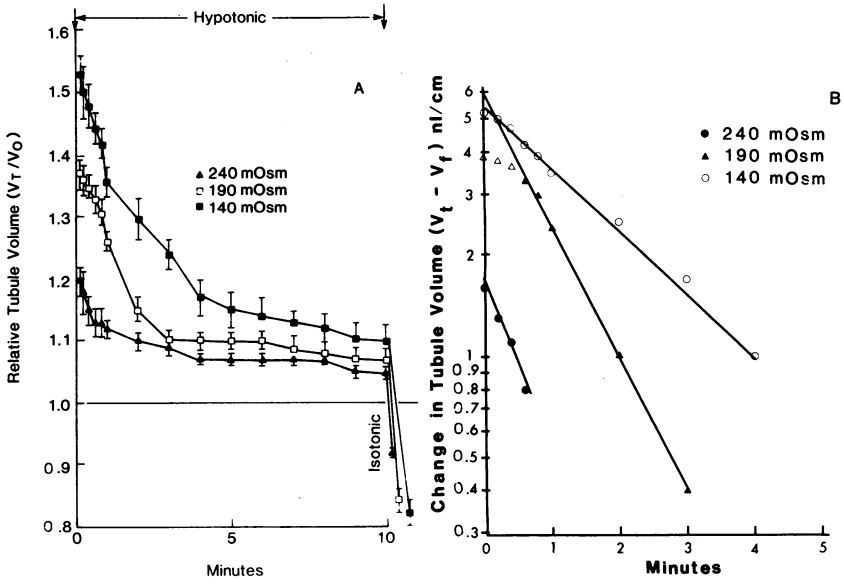

Figure 2. (*A*) Effect of different hypotonic media on relative cell volume of nonperfused S_2 segments. Cell volume in 293 mosM medium was taken as 1.0. Osmolalities of different media are indicated for each study. After 10 min, each tubule was rinsed with isotonic medium. The reduction of V_t/V_o below 1.0 reflects the loss of intracellular solute in the various hypotonic media. Mean values and SEM for four to six tubules in each experiment. (*B*). Effect of different hypotonic media on cell volume of nonperfused S_2 segments showing the difference between the volume at any time (V_t) and the steady-state volume determined at 10 min (V_f). Linear regression (solid line) for each group of experiments was calculated from points displayed. (Open triangles were not included in this analysis of maximal rates.)

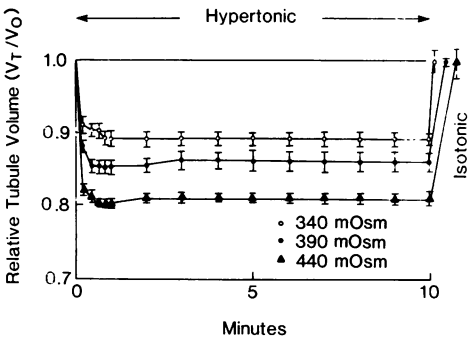

Figure 3. Effect of different hypertonic media on cell volume. See legend to Fig. 2 *A*. After 10 min, each tubule was rinsed in isotonic medium. The return of V_t/V_o to 1.0 indicates no net change in intracellular solute during prolonged incubation in hypertonic medium. Mean values and SEM for five to six tubules in each experiment.

The relation between the initial maximal volume changes and the reciprocal of medium osmolality was linear, which indicates that the cells behave as relatively good osmometers (See osmometric plot in Fig. 9). If it is assumed that cell solute does not change in the osmometric phase, the extrapolated intercept of this relationship in the current series indicates that ~45% of the intracellular volume is osmotically inactive.

The time course of the VRD phase from peak to steady-state volume of the S_2 segments revealed a first order relationship for each of the three hypotonic test solutions (Fig. 2 *B*). The slopes of the VRD response from peak to steady-state volume were compared and not found to be statistically different. The half times ($t_{1/2}$) of the VRD phases were 0.6, 0.8, and 1.6 min for medium osmolalities of 240, 190, and 140 mosM, respectively. The volume fluxes of the regulatory decrease phases of the tubules were estimated from $J_v = K\Delta V$, where K is the first order rate constant, equal to $0.693/t_{1/2}$, and ΔV is the difference between the initial peak and steady-state volume after maximal VRD. Volume flux was estimated to be 0.21, 0.34, and 0.22 nl/min per mm for the 240, 190, and 140 mosM media, corresponding to maximal solute efflux rates across basolateral membranes of 50.4, 64.6, and 30.8 $\times 10^{-12}$ osmol/min per mm, respectively.

To estimate the quantity of intracellular solute lost during VRD, we assumed that in isotonic medium ~60% of nonperfused proximal tubule cell volume was water. Based on this assumption, the calculated initial osmotically active solute content of S_2 segments was 176 $\times 10^{-12}$ osM/mm tubule length. Furthermore, if the osmolalities of cell cytoplasm and the bath remained virtually equal during VRD, net solute loss would be equal to the net change in cell volume multiplied by bath osmolality. For the three hypotonic solutions, the estimated net loss of solute was 38.4, 60.8, and 63.0 $\times 10^{-12}$ osmol/mm for medium osmolalities of 240, 190, and 140 mosM, respectively, and the fractional losses of intracellular solutes were 21.8, 34.5, and 35.8%, respectively.

The primary objective of this study was to determine the capability of proximal renal tubules to maintain constant cell volume over a large range of osmolalities developed at widely differing rates. We performed experiments in which the tubules were exposed to changes in osmolality at rates much slower than examined previously (1–8) and illustrated in Figs. 2 and 3. With the gradient system described in Fig. 1, we generated constant rates of change in bath osmolality ranging between 0.8 and 42 mosM/min (Fig. 4).

Tubules exposed to isotonic medium for 1 h revealed no significant change in tubule volume $V_t/V_o = 1.00 \pm .005$ ($n = 4$). When the osmolality was reduced from 293 to 110 mosM at a rate of 1.5 mosM/min, tubule volume did not change appreciably until the osmolality reached 167±9 mosM ($n = 5$) (Fig. 5). With our measurement technique we can confidently detect a 3% change in relative tubule volume V_t/V_o. We refer to that portion of the study in which cell volume did not change appreciably as osmolality decreased as the isovolumetric regulation phase. With a continued decrease in osmolality below 167 mosM, the tubules eventually swelled. In this low osmolality range the relation between the change in medium osmolality and the change in cell volume approached that observed in the osmometric phase of the acute swelling experiments (Figs. 2 and 3). When the osmolality reached 110 mosM, we replaced the bath with isotonic medium (Fig. 6). Tubule volume decreased rapidly to 80% of the baseline value, indicating that a significant fraction of intra-

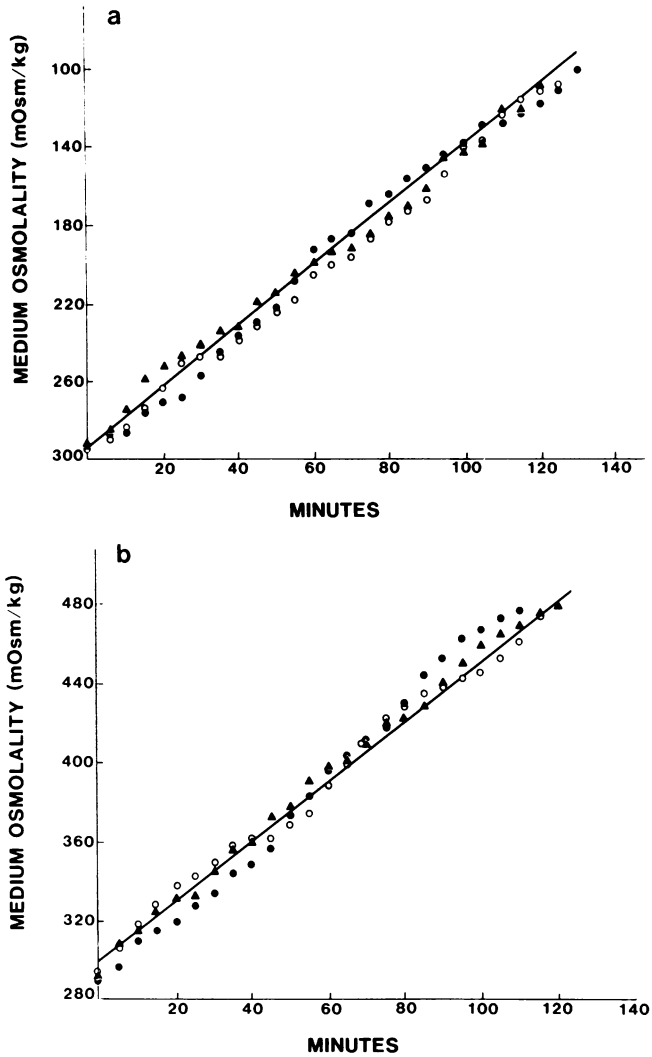

Figure 4. Linearity of medium osmolality change. Three characteristic experiments in hypotonic (*A*) and hypertonic medium (*B*) are shown. Osmolality was changed at 1.5 mosM/min in these examples. The calculated regression line was compiled from the five hypotonic and seven hypertonic experiments shown in Fig. 5.

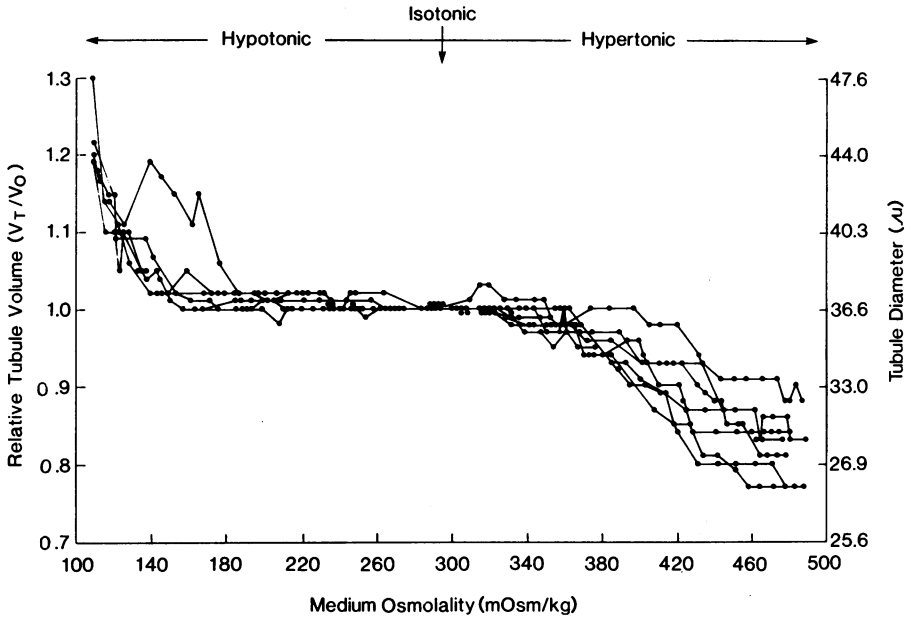

Figure 5. Response of cell volume to gradual change in medium osmolality. Osmolality was changed at 1.5 mosM/min in five hypotonic and seven hypertonic experiments. Cell volume at 293 mosM was the reference, $V_t/V_o = 1.0$.

cellular solute had been lost during the incubation in hypotonic media (16). To determine if solute was also lost from cells during the isovolumetric portion of the experiment, five tubules with mean relative volumes of 1.02 at 190 mosM were rapidly exposed to 293 mosM medium. This caused an abrupt decrease in relative tubule volume to 0.79, which is consistent with the interpretation that intracellular solutes were lost during the isovolumetric portion of the hypotonic study.

If osmotic equilibrium is assumed between cytoplasm and external bath during isovolumetric regulation, the experiments in Figs. 5 and 6 illustrate that the net flux of osmotic solute out of the cells was proportional to the rate of change in the external bath osmolality. For a bath osmolality change of 1.5 mosM/min, net osmotic solute flux from the tubule in the isovolumetric range was 0.9×10^{-12} osmol/min per mm tubule length.

When bath osmolality was increased from 293 to 480 mosM at 1.5 mosM/min in seven studies, tubule volume did not deviate significantly from the isotonic baseline until the osmolality reached 361±7 mosM (Fig. 5 and 6). Thus, S_2 tubules exhibited isovolumetric regulation in hypertonic media. Beyond this limit, cell shrinkage was observed with further increases in osmolality. When tubules gradually exposed to hypertonic medium were suddenly bathed in isotonic medium they swelled 12% above the isotonic control baseline (Fig. 6). This increase in volume indicated that the cells accumulated solute during the isovolumetric phase in hypertonic media. The gain of intracellular solute during hypertonic isovolumetric regulation at a bath osmolality change of 1.5 mosM/min was 0.9×10^{-12} osmol/min per mm tubule length.

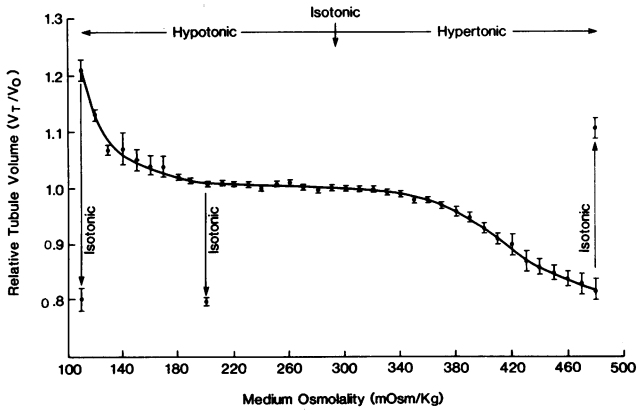

Figure 6. Response to isotonic medium of tubules adapted in anisotonic medium. Control curve gives mean values±SEM of experiments in Fig. 5 in which medium osmolality was changed at 1.5 mosM/min. Vertical arrows indicate points at which medium was rapidly changed to isotonic solution at the extremes of osmolality in the experiments shown in Fig. 5. Five additional tubules taken to 190 mosM maintained normal size, but shrank in isotonic medium.

In the next series of studies, we determined the rate of osmolality change needed to decompensate isovolumetric regulation. At rates slower than 27 mosM/min, isovolumetric regulation maintained normal cell volume over a significant osmolality range. We detected initial deviation of cell volume from the isotonic baseline value when the rate of osmolality decrease exceeded 27 mosM/min (Fig. 7). To maintain cell volume constant at the upper limit of external osmolality change, 27 mosM/min, the cells would have to lose solute at 16.2×10^{-12} osmol/min per mm. This value is approximately one-third the maximal VRD rates of solute loss noted previously.

Tubules exposed to hypertonic external medium at rates exceeding 1.5 mosM/min did not exhibit isovolumetric regulation to the same extent as in hypotonic medium. At rates exceeding 3 mosM/min, no isovolumetric regulation was detected (Fig. 8).

In a previous study (1), reduced temperature was shown to block the VRD in renal tubules. In the current study, we exposed cooled tubules (7–10°C) to slow changes in bath osmolality (1.5 mosM/min) (Fig. 9). When the bath was changed from 293 to 190 mosM, V_t/V_o rose to 1.24, a value significantly greater than that observed in experiments performed at 37°C. The effect of cooling was reversible within 5 min when the temperature was raised to 37°C (data not shown). At 7–10°C a change in bath osmolality from 293 to 390 mosM at a rate of 1.5 mosM/min caused V_t/V_o to decrease steadily without evidence of isovolumetric regulation. V_t/V_o decreased to 0.83 at a bath osmolality of 390 mosM, which is slightly lower than the V_t/V_o value observed during the acute osmometric shrinking experiments.

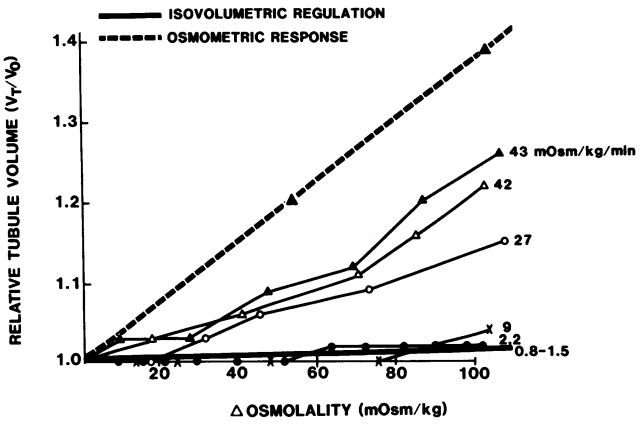

Figure 7. Effect on relative volume of altering rate of change of hypotonic bath osmolality. Osmolality change rate is shown on the right of each experiment. The osmometric response line was calculated from peak V_t/V_o values in Fig. 2.

To probe the general mechanism of isovolumetric regulation upon exposure to hypertonic medium, we performed experiments in which NaCl was replaced isosmotically with choline chloride or raffinose in the bathing medium. In four experiments, tubules were exposed to a sudden change from the standard isotonic media to isosmotic media in which choline chloride (114 mM) was substituted for the NaCl. After 10 min, relative tubule volume was slightly less than baseline volume, 0.95. This suggests a higher reflection coefficient for choline chloride than NaCl (32). We performed three experiments in which tubules were preincubated in standard isotonic medium, then exposed to medium in which the osmolality was gradually increased by adding choline chloride at a rate of 1.5 mosM/min. Isovolumetric regulation in hyperosmotic choline chloride was not statistically different from that in hyperosmotic NaCl over the range of 295 to 350 mosM (Fig. 10).

In four experiments the standard isosmotic bath was rapidly changed to isosmotic medium in which raffinose (228 mM) was substituted for NaCl. Relative tubule volume was 0.96 after 10 min in raffinose medium, consistent with a higher reflection coefficient for raffinose than for NaCl. In three experiments the bath osmolality was changed from 293 to 480 mosM by adding raffinose at a rate of 1.5 mosM/min. Isovolumetric regulation in hyperosmotic raffinose was not different from that in hypertonic NaCl over the range of 295 to 350 mosM (Fig. 10).

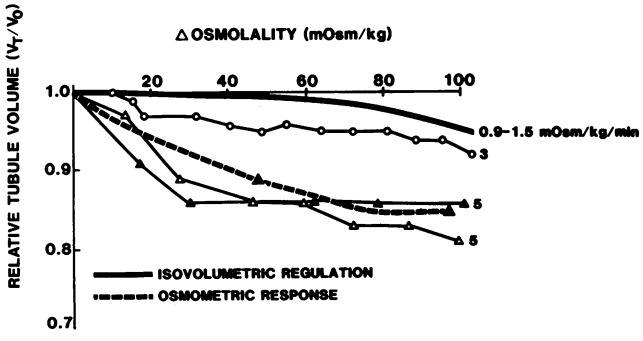

Figure 8. Effect on relative volume of altering the rate of change of hypertonic bath osmolality. Osmolality change rate is shown on the right of each experiment. The osmometric response line was calculated from the minimal V_t/V_o values in Fig. 3.

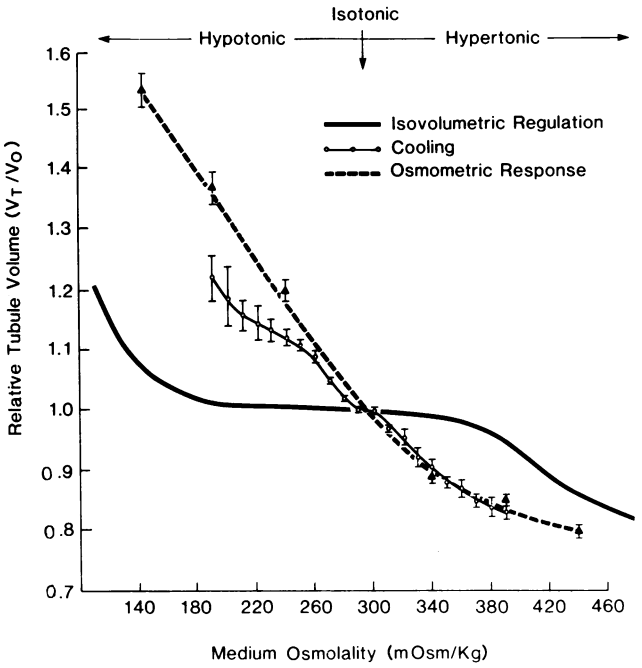

Figure 9. Effect of cooling to 7–10°C on isovolumetric regulation. Each point represents the mean and SEM of four hypotonic and four hypertonic experiments. Solid curve represents mean values for isovolumetric curves defined in Figs. 5 and 6. Dashed line represents osmometric relation between tubule volume and medium osmolality shown in Figs. 2 and 3.

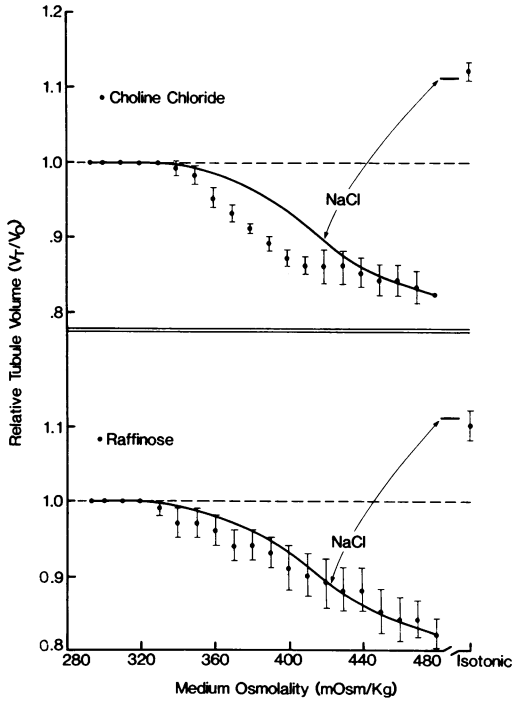

Figure 10. Effect on isovolumetric regulation of increasing osmolality with choline chloride or raffinose instead of NaCl. Choline chloride or raffinose was added to isotonic medium to increase osmolarity at 1.5 mosM/min. Points represent mean±SEM of three experiments. Solid lines represent mean response to hypertonic NaCl in Figs. 5 and 6. Points above dashed line obtained after rinse with isotonic medium.

Discussion

The importance of anisotonic volume regulatory mechanisms is seen clearly in pathophysiologic states associated with chronic progressive hyponatremia and hypernatremia (hypo and hyperosmolality) (33, 34). In these clinical conditions the anisotonic state usually develops over a matter of hours, or days and the body cells appear to maintain intracellular volume relatively efficiently, provided the osmolality change is not extreme or rapid.

To address the concern that cells exposed suddenly to changes in external osmolality might not adequately reflect the mechanisms involved in more chronic pathophysiologic states, we studied the effect of relatively slow changes in medium osmolality on the volume of proximal renal tubule cells. In this way, one determines the extent to which cells prevent changes in volume, rather than their ability to restore volume to normal. To our knowledge, this is a novel approach to studying volume regulatory behavior of cells in vitro. The results show that proximal renal tubule cells can maintain stable cell volume over a range of osmolalities extending from 167 to 361 mosM, provided that the rate of osmolality change is kept within certain limits. We refer to this phenomenon as isovolumetric cell volume regulation.

Cell volume regulation occurred over this broad range of osmolalities as evinced by the fact that a sudden return of the tubules to isotonic medium (293 mosM) caused the cells to swell or to shrink, depending on the osmolality of the anisotonic medium to which the tubules had become accustomed. The change in cell volume on return to isotonic medium gives evidence that during isovolumetric regulation solutes were removed from (hypotonic) or added to (hypertonic) intracellular fluid. The identity of the solutes is not revealed by the current study, but previous work suggests that in hypotonic media Na, K, and Cl are probably lost from the cytoplasm (2, 6). Volume regulation in hypertonic media was not observed previously in isolated proximal tubules, so there is little basis to speculate on the identity of solutes added to the cells during hypertonic isovolumetric regulation.

Cooling the tubules stopped isovolumetric regulation in hypo and hypertonic media. In earlier studies we found in tubules exposed suddenly to hypotonic medium that cooling blocked the VRD and prevented the net loss of intracellular cations (1). Because cooling prevented VRD and net cation loss, but did not prevent exchange of intracellular K for extracellular Na, we suggested that reduced temperature interfered with the VRD mechanism rather than having a nonspecific effect on the diffusion of water and solutes (2). Cooling also interfered with isovolumetric regulation, showing parallelism between the newly described process and VRD after a sudden decrease in medium osmolality.

As revealed in this study, isovolumetric regulation illustrates the exquisite sensitivity and precision with which tubule cells regulate intracellular volume. Over a 194-mosM span of concentrations in the physiologic range we could not detect a significant change in tubule cell volume when the osmolality change was 1.5 mosM/min or less. Repetitive measurements indicate that we can precisely detect relative changes in cell volume > 3%. It is possible that as osmolality is gradually changed to lower or higher levels that the cells undergo transient cycling of cell volume, i.e., extremely small changes of cell volume followed by compensatory volume regulation. Stated another way, if changes in cell volume activate the volume regulatory mechanisms on a continuing basis, the intensity of the signal must be <3% of the baseline volume in isotonic medium. The first step in the activation of isovolumetric regulation is a change in extracellular osmolality, not a change in NaCl concentration, as addition to isotonic medium of raffinose or choline chloride were as effective as NaCl in promoting cell volume maintenance in hyperosmotic medium.

In previous studies, proximal tubules cells exposed suddenly to hypotonic media did not return cell volume to control levels (1–6). This incomplete volume regulatory behavior in vitro is typical of other types of cells as well (35, 36). The more gradual osmotic gradient change used in this report seems, therefore, to reveal a capability for more precise regulation of cell volume in hypotonic medium than was observed previously. Moreover, a regulatory increase in cell size was never observed in mammalian proximal renal cells exposed suddenly to relatively hypertonic medium, whereas the capability for volume regulation in hypertonic media was readily demonstrated in the current study. The reasons for the different response of renal cells to sudden, as opposed to gradual increases in extracellular osmolality, are not revealed in this study.

The study of isovolumetric behavior did reveal some limitations in the ability of proximal tubule cells to maintain constant volume in anisotonic media. Tubule cell volume maintenance was clearly sensitive to the rate at which the medium osmolality was changed. Although we could not determine precise limits, we found that hypotonic changes > 27 and hypertonic changes > 3 mosM/min exceeded the capability of tubules to keep cell volume constant. Because isovolumetric regulation probably depends on the loss or gain of intracellular solutes, principally Na, K, and Cl, we can estimate the solute flux rates above which volume maintenance breaks down and the cells swell or shrink as osmolality is changed further. Assuming that intracellular water is osmotically equilibrated with extracellular fluid, and is 60% of total tubule cell volume, the limiting solute flux in hypotonic medium is 16.2×10^{-12} osM/min per mm and in hypertonic medium 1.8×10^{-12} osM/min per mm. Thus, isovolumetric regulation proceeds at a faster rate in hypotonic than in hypertonic media. The relative magnitudes of hypo and hypertonic regulation can be judged in comparison with the movements of solute from cytoplasm to bath during isosmotic transepithelial fluid absorption. Isolated S_2 proximal tubules reabsorb fluid at a rate ranging between 0.2 and 0.4 nl/min per mm, depending on the nature of the experiment (37). At 0.4 nl/min per mm, the solute absorption rate is 117×10^{-12} osM/min per mm. Thus, during isovolumetric regulation the maximal rate at which solute can move from the cells to the bath to maintain cell volume constant in hypotonic medium is about one-seventh as great as the steady-state movement of solutes across the basolateral plasma membrane during isosmotic fluid reabsorption. Thus, the maximal rates of isovolumetric regulation in proximal tubules are not quantitatively inconsequential.

Cell volume maintenance is also limited by the absolute change in extracellular osmolality. The lower limit of 167 mosM in hypotonic medium indicates that cells can lose ~43% of their diffusible intracellular solutes before cell volume maintenance is disrupted and the cells swell osmometrically. If we assume that intracellular water is ~60% of tubule volume, the cells lose $\sim 79.5 \times 10^{-12}$ osM/mm as they keep cell volume constant up to the limiting osmolality of 167 mosM. This is equivalent to 39.7×10^{-12} mol/mm of monovalent salt (cations and anions). Previous measurements in these tubule segments show there are

twice as many intracellular cations (Na + K) than needed for isovolumetric regulation to proceed below a medium osmolality of 167 mosM (2); however, anions may be limiting volume regulation in these segments (6). Explicit measurements of chloride and bicarbonate have not been reported in isolated nonperfused S_2 proximal tubules. Recent estimates in rat proximal tubule cells revealed that sodium and chloride concentrations were approximately equal (38). In the rat, the ratio of intracellular Na:K was similar to that previously found in rabbit nonperfused straight tubules (2); thus, we assume that intracellular chloride is approximately equal to the measured sodium content of 35 $\times 10^{-12}$ eq/mm tubule. Studies of isolated perfused rabbit proximal tubules (39) and cortical suspensions (40, 41), reveal intracellular pH values between 7.22 and 7.40, corresponding to intracellular HCO_3^- concentrations of 16–25 mM. Thus, the upper limit of HCO_3 content is 15×10^{-12} eq/mm tubule length. By this account, there are $\sim 50 \times 10^{-12}$ osM/mm of diffusible intracellular anions, which is barely sufficient to account for isovolumetric regulation in hypotonic medium. Thus, the limitation in isovolumetric regulation in hypotonic media (167 mosM) may be dependent on the quantity of diffusible intracellular anions.

Although there is no limit on the amount of extracellular solutes that can enter the cells during hypertonic isovolumetric regulation, tubular volume declined when medium osmolality exceeded 361 mosM despite osmolality change rates as slow as 0.8 mosM/min. This limitation may reflect the depletion of one or more intracellular solutes critical for the movement of extracellular solutes into the cells.

The current studies provide evidence to indicate that proximal renal cells can maintain intracellular volume constant when the change in extracellular osmolality is relatively slow. More than likely, isovolumetric regulation mechanisms are used by cells in chronic hypo and hypernatremic states, and in this sense, the current studies provide a new model to determine mechanisms for the control of cell volume in anisotonic patho-physiologic states. The sensitivity to changes in osmolality also suggests that the same mechanisms operating in anisotonic media may be useful in buffering small differences in transmembrane osmolality that may occur in the course of coupled transepithelial solute and water absorption in proximal tubules.

Acknowledgments

We thank Vicki Donoso and Bonnie Danley for assistance.

This work was supported by a grant from HHS-AM 13476.

References

1. Dellasega, M., and J. J. Grantham. 1973. Regulation of renal tubule cell volume in hypotonic media. *Am. J. Physiol.* 224:1288–1294.

2. Grantham, J. J., C. M. Lowe, M. Dellasega, and B. R. Cole. 1977. Effect of hypotonic medium on K and Na content of proximal renal tubules. *Am. J. Physiol.* 232:F42–F49.

3. Grantham, J. J., M. Linshaw, and L. Welling. 1981. Volume regulation in isotonic and hypotonic media in isolated rabbit renal proximal tubule. *In* Epithelial Ion and Water Transport. A. D. C. MacKnight and J. P. Leader, editors. Raven Press, New York. 339–347.

4. Gagnon, J., D. Ouimet, H. Nguyen, R. Laprade, C. LeGrimellec, S. Carriere, and J. Cardinal. 1982. Cell volume regulation in the proximal convoluted tubule. *Am. J. Physiol.* 243:F408–F415.

5. Welling, P., M. A. Linshaw, and L. Sullivan. 1985. Effect of barium on cell volume regulation in rabbit proximal straight tubules. *Am. J. Physiol.* 249:F20–F27.

6. Welling, P. A., and M. A. Linshaw. 1984. Role of Cl^- in hypotonic cell volume regulation of proximal straight tubules (PST). *Fed. Proc.* 43: 892.

7. Linshaw, M. A., and J. J. Grantham. 1980. Effect of collagenase and ouabain on renal cell volume in hypotonic media. *Am. J. Physiol.* 238:F491–F498.

8. Carpi-Medina, P., B. Lindemann, E. Gonzalez, and G. Whittembury. 1984. The continuous measurement of tubular volume changes in response to step changes in contraluminal osmolality. *Pfleugers Arch.* 400:343–348.

9. Welling, L. W., D. J. Welling, and T. J. Ochs. 1983. Video measurement of basolateral membrane hydraulic conductivity in the proximal tubule. *Am. J. Physiol.* 245:F123–F129.

10. Whittembury, G., and J. J. Grantham. 1976. Cellular aspects of renal sodium transport and cell volume regulation. *Kidney Int.* 9:103–120.

11. Kregenow, F. M. 1971. The response of duck erythrocytes to nonhemolytic hypotonic media. *J. Gen. Physiol.* 58:372–395.

12. Siebens, A. W., and F. M. Kregenow. 1985. Volume regulatory responses of Amphiuma red cells in anisotonic media. The effect of amiloride. *J. Gen. Physiol.* 86:527–564.

13. Kregenow, F. M., T. Caryk, and A. W. Siebens. 1985. Further studies of the volume regulatory response of Amphiuma red cells in hypertonic media. Evidence for amiloride sensitive Na^+/H^+ exchange. *J. Gen. Physiol.* 86:565–584.

14. Cala, P. M. 1977. Volume regulation by flounder red blood cells in anisotonic media. *J. Gen. Physiol.* 69:537–552.

15. Cala, P. M. 1980. Volume regulation by Amphiuma red blood cells: the membrane potential and its implications regarding the nature of the ion-flux pathways. *J. Gen. Physiol.* 76:683–708.

16. Fisher, R. S., B. E. Persson, and K. R. Spring. 1981. Epithelial cell volume regulation: bicarbonate dependence. *Science (Wash. DC).* 214:1357–1359.

17. Ericson, A.-C., and K. R. Spring. 1982. Coupled NaCl entry into Necturus gallbladder epithelial cells. *Am. J. Physiol.* 243:C140–C145.

18. Ericson, A.-C., and K. R. Spring. 1982. Volume regulation by Necturus gallbladder: apical Na^+-H^+ and Cl^--HCO_3^- exchange. *Am. J. Physiol.* 243:C146–C150.

19. Fisher, R. S., and K. R. Spring. 1984. Intracellular activities during volume regulation by Necturus gallbladder. *J. Membr. Biol.* 78:187–189.

20. Foskett, S. K., and K. R. Spring. 1985. Involvement of calcium and cytoskeleton in gallbladder epithelial cell volume regulation. *Am. J. Physiol.* 248:C27–C36.

21. Ussing, H. H. 1982. Volume regulation of frog skin epithelium. *Acta Physiol. Scand.* 114:363–369.

22. Kleinzeller, A., J. Nedvidkova, and A. Knotkova. 1967. Effect of saline osmolarity on the steady-state level of water and electrolytes in kidney cortex cells. *Biochim. Biophys. Acta.* 135:286–289.

23. Law, R. O. 1975. Volume adjustment by renal medullary cells in hypo- and hyper- osmolal solutions containing permeant and impermeant solutes. *Am. J. Physiol.* 247:55–70.

24. Gilles, R., C. Duchene, and I. Lambert. 1983. Effect of osmotic shocks on rabbit kidney cortex slices. *Am. J. Physiol.* 244:F696–F705.

25. Lechene, C., L. Larsson, P. Chang Sing, and G. Boseck. 1985. Cytoplasmic gel may allow cellular volume maintenance. *Fed. Proc.* 44: 740.

26. Savin, V. J., R. C. Harris, J. L. Seifter, B. M. Brenner, and C. Lechene. 1986. Hypotonicity causes net efflux of K and Cl in normal rat renal proximal tubular cells (RPTC) and net efflux of Na and Cl in Na loaded RPTC. *Kidney Int.* 29:406.

27. Hebert, S. C. 1986. Basolateral Na^+/H^+ and Cl^-/HCO_3^- exchangers mediate hypertonic cell volume regulation in mouse medullary thick limbs. *Kidney Int.* 29:398.

28. Gyory, A. Z., G. Kweifio-Okai, and Y. Ng. 1981. Hypo- and

hyperosmolal saline and raffinose on kidney cortical cell volume at 37°C. *Am. J. Physiol.* 240:F180–F184.

29. Hughes, P. M., and A. D. C. McKnight. 1976. The regulation of cellular volume in renal cortical slices incubated in hypoosmotic medium. *Am. J. Physiol.* 257:137–154.

30. Paillard, M., F. Leviel, and J. P. Gardin. 1979. Regulation of cell volume in separated renal tubules incubated in hypotonic medium. *Am. J. Physiol.* 236:F226–F231.

31. Steel, R. G., and J. H. Torrie. 1960. *In* Principles and Procedures of Statistics. McGraw-Hill Book Company, New York. 173.

32. Welling, L. W., and D. Welling. 1984. Paired studies of the response of renal tubules to hypotonic raffinose and sodium chloride solutions. *Fed. Proc.* 43:892.

33. Epstein, F. H. 1980. Signs and symptoms of electrolyte disorders. *In* Clinical Disorders of Fluid and Electrolyte Metabolism. M. H. Maxwell and C. L. Kleeman, editors. McGraw-Hill Book Company, New York. 499–530.

34. Arieff, A., and R. Guisado. 1976. Effects on the central nervous system of hypernatremic and hyponatremia states. *Kidney Int.* 10:104–116.

35. Grinstein, S., A. Dupre, and A. Rothstein. 1982. Volume regulation by human lymphocytes. Role of calcium. *J. Gen. Physiol.* 79:849–868.

36. Hoffman, E. K., L. O. Simonsen, and I. H. Lambert. 1984. Volume-induced increase of K^+ and Cl^- permeabilities in Ehrlich ascites tumor cells. Role of internal Ca^{2+}. *J. Membr. Biol.* 78:211–222.

37. Schafer, J. A., and D. W. Barfuss. 1982. The study of pars recta function by the perfusion of isolated tubule segments. *Kidney Int.* 22: 434–448.

38. Sauberman, A. J., V. L. Scheid, D. C. Dobyan, and R. E. Bulger. 1986. Simultaneous comparison of techniques for x-ray analysis of proximal tubular cells. *Kidney Int.* 29:682–688.

39. Sasaki, S., T. Shiigari, and J. Takeuchi. 1985. Intracellular pH in the isolated perfused rabbit proximal straight tubule. *Am. J. Physiol.* 249:F417–F423.

40. Kleinman, J., W. Brown, R. Ware, and J. Schwartz. 1980. Cell pH and acid transport in renal cortical tissue. *Am. J. Physiol.* 739:F440–F444.

41. Balaban, R. S. 1982. Nuclear magnetic resonance studies of epithelial metabolism and function. *Fed. Proc.* 41:42–47.