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Intracellular Solute Gradients During Osmotic Water Flow: An Electron-Microprobe Analysis

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Summary. In an attempt to quantify possible intracellular water activity gradients during ADH-induced osmotic water flow, we employed energy dispersive X-ray microanalysis to thin, freeze-dried cryosections obtained from fresh, shock-frozen tissue of the toad urinary bladder. The sum of all detectable small ions (Na + K + Cl) in the cellular water space was taken as an index of the intracellular osmolarity. Presuming that all ions are osmotically active, they comprise about 90% of the cellular solutes. When the cells were exposed to dilute serosal medium, the reduction in the sum of the ions agreed well with the expected reduction in osmolarity. After inducing water flow by addition of ADH and dilution of the mucosal medium, all epithelial cells showed a fall in osmolarity. The change was more pronounced in granular cells than in basal or mitochondria-rich cells, consistent with the notion that granular cells represent the main transport pathway. Most significantly, intracellular osmolarity gradients, largely caused by an uneven distribution of K and Na, were detectable in granular cells. The gradients were not observed after ADH or mucosal dilution alone, or when the direction of transepithelial water flow was reversed. We conclude from these results that there is a significant cytoplasmic resistance to water flow which may lead to intracellular gradients of water activity. Concentration gradients of diffusible cations can be explained by a flow-induced Donnan-type distribution of fixed negative charges. With regard to transepithelial Na transport, the data suggest that ADH stimulates transport by increasing the Na permeability of the apical membranes of granular cells specifically.

Key Words ADH · osmotic water flow · transepithelial Na transport · toad urinary bladder epithelium · X-ray microanalysis · intracellular ion concentrations

Introduction

Previous investigations of the cellular response to ADH-induced, osmotic movement of water across the toad urinary bladder have revealed that extensive cell swelling may accompany the brisk transepithelial movement of water (Pechey & Rasmussen, 1961; DiBona, Civan & Leaf, 1969). Comparable results have been noted for mammalian collecting tubule (Ganote et al., 1968; Kirk, Schafer & DiBona, 1984). At the level of transmission electron microscopy, images of cells fixed at or near the peak of water flow suggest, as well, that the swelling is asymmetrically distributed: granular cells are predominantly swollen at their apical poles while the organelle-rich basal portion of the cell is only marginally distended (DiBona et al., 1969). The possibility has been suggested (DiBona, 1981*a*,*b*) that the cytoplasmic swelling profile is indicative of a comparable asymmetric distribution of suspended solutes and that a gradient of water activity is established within the cell. The two major implications of this proposition are that there is a significant cytoplasmic resistance to transcellular movement of water and that the hydraulic flow is sufficiently high to offset the randomizing effects of solute diffusion. In fact, because straightforward dilution of the granular cell interior through dilution of the serosal bathing medium does not result in this distinctive cellular geometry, it would appear that the shape changes seen are dependent on water flow from mucosa to serosa *per se*.

The application of electron-microprobe analysis to freeze-dried cryosections seemed to us a particularly useful tool for further investigation of this issue because, with this approach, direct examination of the solute composition of toad bladder epithelial cells is feasible, even down to a subcellular level (Rick, Dörge & Thurau, 1982). In this study, we attempted first, to establish the validity of approximating intracellular osmolarity with electron-microprobe analysis, second, to verify whether or not the flow of water results in an intracellular concentration gradient for solutes, and third, to evaluate the cellular specificity of the response to ADH. The results show that the concentrations of small ions

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Fig. 1. Scanning transmission electron micrograph of a 1-μm-thick freeze-dried cryosection of the toad urinary bladder epithelium. The structure on top of the epithelium corresponds to the albumin standard layer used for quantification. Note the differences in height of the granular cells

such as Na,K and Cl can be used to reliably gauge intracellular osmolarity changes. Furthermore, we were able to demonstrate the existence of intracellular gradients of diffusible ions during water flow. Changes in intracellular ion concentration were most pronounced in granular cells consistent with the role of these cells as the main target of ADH action. We conclude that the cytoplasm of epithelial cells offers significant hydraulic resistance and, therefore, should be considered as an additional barrier to transepithelial water flow.

Materials and Methods

Large female toads of the species *Bufo marinus*, obtained from the Dominican Republic through National Reagents (Bridgeport, Conn.), were used. The animals were kept dry in plastic troughs but bathed in tap water for about 20 min on a daily basis. Toads were doubly pithed and each dissected urinary hemibladder was mounted over a Pexiglas® ring. The apical surface was jet-washed with amphibian Ringer's solution to remove the mucous layer. After initial equilibration with Ringer's solution on both sides for about 30 min, the incubation was continued according to the experimental regime described in Results. The bladders were kept open-circuited throughout. Normal Ringer's solution contained (in mM): 110 NaCl, 2.5 $KHCO_3$, and 1 $CaCl_2$. Normal osmolarity was 220 mosmol/liter. Solutions of reduced osmolarity were obtained by reducing the NaCl concentration. All solutions were bubbled with air and had a pH ranging between 8.1 and 8.3. Antidiuretic hormone (Pitressin, Parke Davis) was added to the serosal bath at an activity of 200 mU/ml. Amiloride (a gift from Merck, Sharp, and Dohme) was added at 10^{-4} M concentration to the mucosal bath. The composition of solutions and standards was checked by flame photometry, chloridometry, and vapor pressure osmometry.

After incubation, the mucosal surface of the epithelium was coated with a thin layer of a standard solution containing 20 g% bovine albumin (Behringwerke) dissolved in the mucosal bathing solution. The tissue was then shock-frozen by plunging the rings into a propane/isopentane mixture (−196°C). Approximately 5 sec elapsed between layering with the standard solution and freezing. Sections of about 1-μm thickness were cut from the frozen material at −90°C in a modified cryoultramicrotome (Reichert OmU 3) and freeze-dried at −80°C and 10^{-6} Torr in a custom-made freeze-dryer based on a turbomolecular pumping unit (Balzers BAE 080T). Energy dispersive X-ray microanalysis of the freeze-dried sections was performed in a scanning electron microscope (Cambridge S150) to which a solid-state X-ray detecting system (LINK Systems) was attached. The measuring conditions were 20 kV acceleration voltage, between 0.2 and 0.5 nA beam current (determined in a Faraday cup at the level of the specimen), and 100 sec analysis time. Analyses were obtained in reduced raster mode, scanning areas of between 0.1 and 0.5 μm^2. The emitted X-rays were analyzed in the energy range from 0 to 10 keV, encompassing the Kα lines of the biologically relevant light elements Na, Mg, P, S, Cl, K and Ca. Quantification of the cellular element concentrations and cellular dry weight fraction was achieved by a comparison of the cellular X-ray spectra with those obtained in the adherent albumin layer. A detailed description of the methods has been given previously (Bauer & Rick, 1978; Dörge et al., 1978; Jehl et al., 1981).

Concentrations are expressed as mmol/kg wet weight or, after recalculation based on the cellular dry weight fraction, as

Table 1. Effect of serosal dilution (half-strength Ringer's, about 10 min) on the intracellular Na, Cl, K and P concentrations and dry weight fraction (d.w.) of granular cells[a]

	Na	K	Cl	P	d.w.
	(mmol/kg wet weight)				(g%)
Control	12.5 ± 5.2	116.7 ± 14.9	33.5 ± 8.3	129.1 ± 13.6	19.5 ± 1.6
Serosal dilution	5.4[b] ± 1.1	73.9[b] ± 17.4	13.1[b] ± 1.7	93.4[b] ± 17.5	13.9[b] ± 1.5

[a] Mean ± SD of 59 (Control) and 60 (Serosal dilution) measurements obtained from three pairs of hemibladders

[b] Superscripts indicate the level of significance ($b = 2P < 0.001$).

mmol/liter cell water or mmol/kg dry weight. Cellular dry weight fraction is given as g% (g dry matter/100 ml). Statistical significance was evaluated by Student's *t*-test. If not stated otherwise, data were taken from paired hemibladders.

Results

Specimen Preparation and X-ray Microanalysis

The measurements were performed on fresh, 1-μm-thick, freeze-dried cryosections which were neither chemically fixed, nor coated, nor stained. Compared to conventional electron micrographs such preparations afford much lower spatial resolution, of course. Nevertheless, as shown in Fig. 1, the image quality obtained in the scanning transmission mode was sufficient to allow discrimination between different epithelial cells and different intracellular compartments. Analyses were performed in granular cells, basal cells and mitochondria-rich cells. Goblet cells were too infrequently observed to be included in this study. As noted previously (Rick et al., 1978*a*), separate measurements in the nucleus and cytoplasm showed small, systematic differences in the P and Cl concentration as well as in the dry weight fraction. The sum of the intracellular ion concentrations, however, was generally the same since the lower nuclear Cl value was balanced by a slightly higher K value. Care was taken to stay at least 0.5 μm away from cell boundaries to avoid stray excitation of extracellular spaces.

The experimental perturbations employed in this study generally did not lead to gross, easily recognizable changes in epithelial morphology with the exception of the marked increase in cell size observed after serosal dilution. After mucosal dilution and ADH some granular cells showed a somewhat lighter appearance of the cytoplasm, indicating some swelling. The degree of swelling, however, was judged by the change in the cellular dry weight fraction, assuming that the total dry matter of the cell was unchanged over the experimental period.

Table 2. Effect of serosal dilution on the intracellular concentrations of Na, K and Cl of granular cells expressed per liter cell water or per dry kg cellular dry matter[a]

		Na	K	Cl	Σ ions
Control	mmol/liter	15.5	145.0	41.6	202.1
	mmol/kg d.w.	64.1	598.4	171.8	834.4
Serosal dilution	mmol/liter	6.3	85.8	15.2	107.3
	mmol/kg d.w.	38.8	531.7	94.2	664.7

[a] Data are from Table 1. Water concentrations are calculated by multiplying the wet weight concentration with 100/(100-d.w.). Dry weight concentrations are calculated by multiplying the wet weight concentration with 100/(d.w.).

Serosal Dilution

In a first series of experiments, the effect of serosal dilution was examined in order to test the hypothesis that intracellular osmolarity changes can be gauged by measuring the intracellular ion concentrations. For this purpose we exposed the serosal aspect of three hemibladders to half-strength Ringer's solution for about 10 min. Table 1 compares the mean intracellular Na, K, Cl and P concentrations and dry weight fraction of granular cells under control conditions (isosmotic bathing media) and after serosal dilution. It is apparent that serosal dilution leads to a drop in the concentration of all three diffusible ions. In addition, significant decreases in largely structurally bound elements such as P and in the cellular dry weight fraction were detectable. The observed change in dry matter corresponds to a cellular swelling of about 40%.

Table 2 lists the Na, K and Cl values when these are computed as mmol/liter of cell water. In control tissues, the sum of the concentrations of the

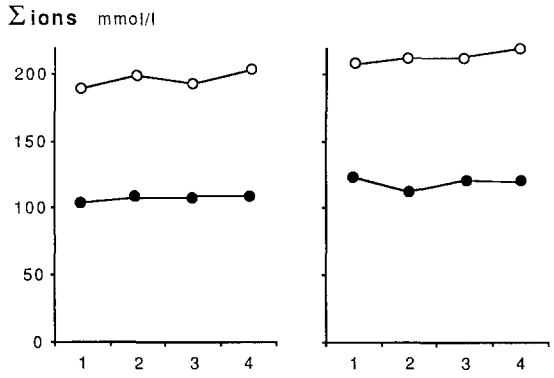

Fig. 2. Intracellular profile of the sum of the intracellular ion concentrations (Na + K + Cl) in granular cells under isosmotic conditions (open circles) and after replacing the serosal bath with half-strength Ringer's solution (filled symbols). The left panel shows an experiment without ADH, the right panel the results with ADH. The points 1 through 4 correspond to four equally spaced measuring positions along the apical-to-basal axis of the cells

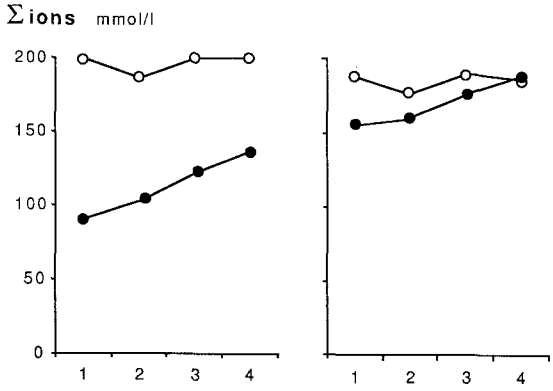

Fig. 3. Intracellular profile of the sum of the intracellular ion concentrations (Na + K + Cl) in granular cells in the absence (open circles) and presence (filled symbols) of mucosal-to-serosal water flow. In the left experiment, both hemibladders were stimulated with ADH. Osmotic water flow was initiated by replacing, in one hemibladder, the mucosal bath with 1/10-strength Ringer's solution. In the right experiment, both hemibladders were first exposed to mucosal dilution and, afterwards, one additionally received ADH. For further details *see* Fig. 2

three ions in the water space was only slightly lower than the total salt concentration of the Ringer's solution (228 mmol/liter), suggesting that these three ions account for most of the intracellular osmolarity. After replacing the serosal bath with half-strength Ringer's solution, the sum of the ion concentrations fell by 47%, closely approximating the theoretical change predicted by assuming that the epithelial cells are in osmotic equilibrium with the serosol bath. Table 2 also lists the concentrations of Na, K and Cl as mmol/kg cellular dry weight. Provided that the epithelial cells do not lose dry matter in the course of the experiment, a change in the dry weight concentration of an ion could only be caused by a change in the total intracellular amount of the ion. The observed reduction in the sum of the ion concentrations after serosal dilution, therefore, is consistent with a loss of cellular solutes. The loss is largely attributable to an efflux of KCl but some loss of Na is detectable as well. We conclude from this result that toad urinary bladder epithelial cells are capable of regulatory volume decrease.

Measurements performed in different regions of the sampled cells showed that the sum of the ion concentrations per liter cell water was constant throughout the intracellular space. This was found to be true for control conditions as well as after serosal dilution, as illustrated in Fig. 2 by two apical-to-basal concentration profiles detected in granular cells. In an additional pair of hemibladders, the effect of serosal dilution was tested after enhancement of water permeability with ADH. As also shown in Fig. 2 (right panel), the results are hard to distinguish from those obtained in nonstimulated bladders. The average fall in intracellular ion concentrations was slightly attenuated, however, and corresponded to a reduction in osmolarity by only 43%.

Basal cells and mitochondria-rich cells showed Na and K concentrations which, under control conditions, were very similar to those observed in granular cells. As noted before (Rick et al., 1978*a*), the Cl concentration in mitochondria-rich cells was significantly lower than in basal or granular cells. After serosal dilution, in the presence as well as in the absence of ADH, a marked fall in the Na, K, Cl and P concentration and dry weight fraction was apparent. Judged by the change in the sum of the intracellular ion concentrations per liter cell water, the intracellular osmolarity fell by between 42 and 51%, suggesting that, like the granular cells, basal and mitochondria-rich cells are in osmotic equilibrium with the serosal bath.

Mucosal Dilution

The effect of mucosal dilution was examined in ADH-stimulated hemibladders by exposing the mucosal side to 1/10-strength Ringer's solution for about 20 min. The results were compared to hemibladders that were exposed to mucosal dilution but did not receive ADH (three experiments) or to hemibladders which were stimulated by ADH but not exposed to mucosal dilution (four experiments). Figure 3 shows typical intracellular profiles of the

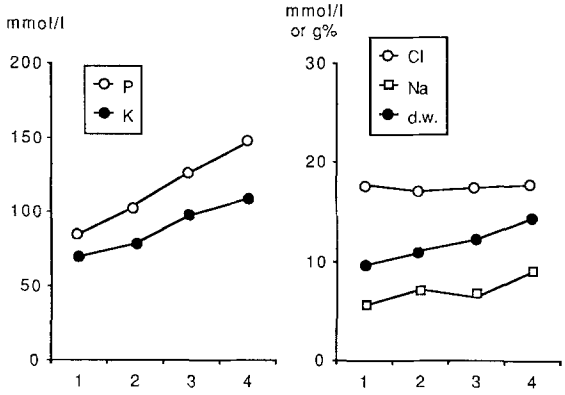

Fig. 4. Intracellular concentration profiles in a granular cell during ADH-induced osmotic water flow (same experiment as in Fig. 3, left panel). Measuring position 3 was in the nucleus and, therefore, values were corrected for systematic differences between the cytoplasm and nucleus. For further details *see* Fig. 2

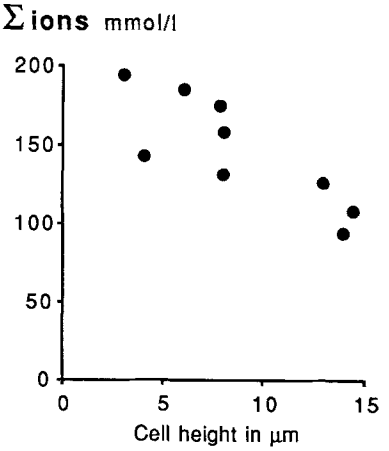

Fig. 5. Correlation between the sum of the intracellular ion concentrations (Na + K + Cl) and epithelial cell height of granular cells. Data from an individual hemibladder in which osmotic water flow was induced by ADH and mucosal dilution

sum of the ion concentrations obtained in granular cells. Using either protocol, mucosal dilution in the presence of ADH caused a reduction in the sum of the intracellular ion concentrations that was most pronounced in the apical cytoplasm and gave rise to an intracellular, basal-to-apical ion concentration gradient. In contrast (and as expected), mucosal dilution alone or application of ADH alone did not result in intracellular gradients. Figure 4 demonstrates that the gradient in the sum of the ion concentrations was largely due to a nonuniform distribution of Na and K. The P concentration and the cellular dry weight fraction also showed a basal-to-apical gradient. A reversed concentration gradient for Cl was observed occasionally. However, as illustrated in Fig. 4, Cl was evenly distributed in most cases.

The slope of the gradients and the apparent degree of intracellular dilution found were highly variable (*see* Fig. 3). For the most part, the scatter of the data can be accounted for by differences in the epithelial thickness rather than differences in the experimental protocol. Figure 5 shows the relationship between the mean intracellular ion concentration and the height of granular cells, as observed in a single hemibladder exposed to mucosal dilution and ADH. The sum of the ion concentrations varied inversely with the epithelial thickness; the taller the cell, the larger was the reduction in ion concentration. Similarly, the slope of the intracellular ion gradients was directly correlated with epithelial cell height.

Figure 6 summarizes the results obtained after mucosal dilution for all three epithelial cell types. Using either experimental protocol, the fall in intracellular ion concentrations was highly significant in granular cells as well as in basal cells. Measurements in mitochondria-rich cells also showed a slight reduction of the ion concentrations. However, presumably due to the large scatter in the data and the relatively small number of cells, this change did not exhibit statistical significance. Apparently, mucosal dilution *per se* results in some reduction of the intracellular osmolarity as the sum of the intracellular ion concentrations under this condition was slightly lower than in control bladders (*compare* Ta-

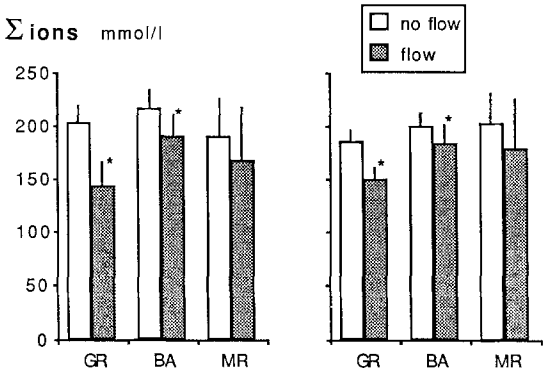

Fig. 6. Effect of mucosal-to-serosal water flow on the sum of the intracellular ion concentrations (Na + K + Cl) in granular (GR), basal (BA), and mitochondria-rich (MR) cells. On the left, both hemibladders were first stimulated with ADH and, then, in one hemibladder osmotic water flow was initiated by mucosal dilution. On the right, both hemibladders were first exposed to mucosal dilution and, afterwards, one additionally received ADH. Mean values ± 2 SEM of four and three paired experiments, respectively. The asterisk denotes values which are statistically different at $2P < 0.05$ or better

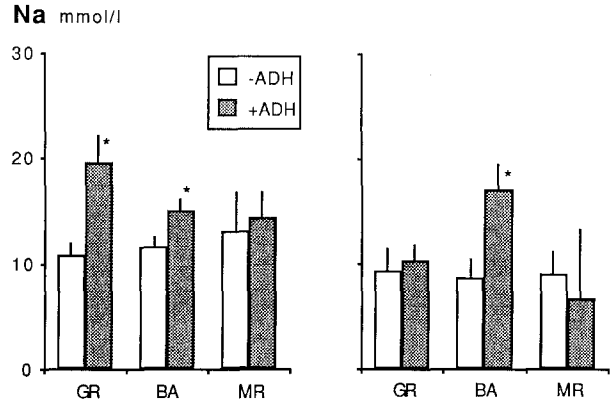

Fig. 7. Effect of ADH on the intracellular Na concentrations in granular (GR), basal (BA), and mitochondria-rich (MR) cells. On the left, results obtained under isosmotic conditions; on the right, after mucosal dilution. For further details *see* Fig. 6

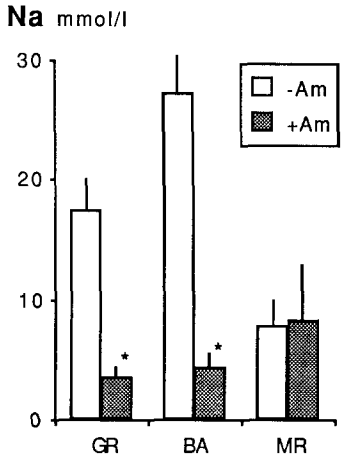

Fig. 8. Effect of amiloride on the intracellular Na concentrations in granular (GR), basal (BA), and mitochondria-rich (MR) cells. In both hemibladders osmotic water flow was induced by ADH and mucosal dilution. For further details *see* Fig. 6

ble 2). Based on the change in the dry weight fraction, mucosal dilution in the presence of ADH resulted in an overall swelling of granular cells by about 20%. The change in volume was accompanied by a small loss of intracellular ions (approximately 10%), largely K and Cl. The dry weight fraction and P concentration of basal cells and mitochondria-rich cells were not significantly altered.

Natriferic Effect of ADH

The effect of ADH on transepithelial Na transport was not an intended primary focus of this investigation. Nevertheless, we noted that ADH produced a significant increase in the intracellular Na concentration in both granular cells and basal cells. In contrast, mitochondria-rich cells generally showed no changes in Na concentration after stimulation by ADH. Figure 7 summarizes the effect of ADH on the intracellular Na concentration when both sides of the bladder were bathed in normal Ringer's solution (left panel), or when the mucosal bath was replaced with 1/10-strength Ringer's (right panel). Surprisingly, in the presence of a transepithelial osmotic gradient, the Na increase in basal cells was more pronounced than in granular cells. Under isosmotic conditions, the increase in the intracellular Na concentration was accompanied by an equivalent decrease in the K concentration.

In an additional experiment, we investigated whether the increase in the intracellular Na concentration after ADH could be abolished by the Na channel blocker amiloride. A pair of hemibladders was stimulated by ADH and exposed to mucosal dilution with only one of the pair simultaneously receiving amiloride. As shown in Fig. 8, amiloride produced a striking decrease in Na concentration in both granular and basal cells. Notably, the Na concentration remained essentially unchanged in mitochondria-rich cells. Other effects of mucosal dilution were apparently unaffected by amiloride; ion concentration gradients in the amiloride-treated hemibladder were even more pronounced, if only slightly, than in the untreated hemibladder.

Discussion

The most significant result of the present study is the demonstration of large intracellular ion concentration gradients and, consequently, gradients in water activity during ADH-induced osmotic water flow and implications of this finding for our understanding of the mechanisms in transepithelial water transport are discussed below. We will begin, however, with a discussion of the feasibility of our initial premise that the sum of intracellular ion concentrations detectable by electron-microprobe analysis is a valid index of intracellular osmolarity. Separately, we will deal with those results that refer to the natriferic action of the hormone.

Estimation of Intracellular Osmolarity

Our results suggest that the sum of the ion concentrations in the cellular water space can be used as a reliable measure of intracellular osmolarity. This view is based mainly on two observations. First, under strictly isosmotic conditions, the sum of the ion concentrations in all cell types closely approxi-

mated the salt concentration of the Ringer's solution, leaving very little to be accounted for by additional intracellular osmolytes. The important supposition here, of course, is that all of the measured ions are osmotically active. Electron-microprobe analysis of the Na, K and Cl concentrations in comparable epithelia such as the frog skin (Rick et al., 1978*b*) or frog cornea (Rick et al., 1985) provided values which were found to be in close agreement with ion activity measurements by others (Nagel, Garcia-Diaz & Armstrong, 1981; Reuss et al., 1983), suggesting that this, indeed, is the case. Second, under all of the experimental conditions employed in this study, the sum of the ions displayed a behavior consistent with a valid indicator of intracellular osmolarity. After exposing the bladder to a dilute serosal solution, the measured change in intracellular ion concentrations closely approximated the change in osmolarity predicted by assuming that the cells were in osmotic equilibrium with the inner bath. Conversely, when ADH was applied in the absence of an osmotic gradient, the sum of the ion concentrations remained unchanged despite significant changes in the intracellular Na and K concentrations.

Although other solutes may represent only a small fraction of the intracellular osmolarity, a diametrically opposed distribution of these solutes could offset, at least partially, any osmolarity gradient created by ions. However, we cannot propose a mechanism that would readily explain such a distribution pattern. It is conceivable as well, that the osmolarity gradient is balanced by a hydrostatic pressure differential, resulting in a constant water activity throughout the cell. To offset an osmolarity gradient of about 50 mOsm/liter, however, a hydrostatic pressure difference of more than one atmosphere would be required between the basal and apical part of the cell.

On the Origin of Intracellular Gradients

Considering the high mobility of small ions, the finding of a significant intracellular concentration gradient for Na and K is, at least at first glance, very surprising. Published values for ADH-induced osmotic water flow in the toad urinary bladder (Civan, 1970) are far too low to explain these gradients simply by solvent drag. Also, there are no transepithelial ion fluxes to account for the gradients by diffusion. A possible explanation for the apparently fixed (standing) gradient of ions is a flow-induced intracellular electrical potential difference. To maintain a distribution of cations as displayed in Fig. 4, a potential difference of 8 mV (basal cytoplasm negative) would be required; a voltage gradient of this magnitude might be generated by a Donnan-type distribution of fixed negative charges. The cellular profile for P and dry matter does, indeed, suggest an uneven distribution of polyanions (e.g. nucleic acids and proteins). Consistent with a Donnan effect, a reversed Cl gradient, opposite to that for Na and K, was observed occasionally. However, in most granular cells the Cl profile was flat which might best be explained by a slight contamination of the most basal measurements by an intra- or extracellular compartment with a high Cl concentration.

Reductions in the P concentration and dry weight fractions of the apical cytoplasm agree well with the preferential swelling and distension of the apical pole of granular cells demonstrated in conventional electron micrographs (DiBona et al., 1969).[1] Probably the most straightforward explanation for the apical swelling is an elevation of the hydrostatic pressure induced by the water movement. The distension of the apical cytoplasm could well be facilitated by an effect of ADH on the compliance of the cytoskeletal network (DiBona, 1981*b*). In addition, large intracellular solutes, such as free cytoplasmic proteins, may be dragged along with the water flow, adding to the basal-to-apical density gradient of the cellular matrix.

Barriers and Pathways in Transepithelial Water Transport

The establishment of a transepithelial osmolarity or water activity gradient provides the necessary driving force for the transcellular movement of water. In the absence of ADH, this activity gradient should be largely expressed across the rate-limiting apical membrane whereas, after increasing the conductivity of the apical membrane with ADH, a significant fraction of the transepithelial gradient may be expressed across the cellular pathway and the basolateral membrane as well. In agreement with this expectation, intracellular concentration gradients were only detectable in ADH-stimulated bladders. In fact, neglecting possible hydrostatic pressure differences, the relative hydraulic resistances can be gauged directly by the magnitude of the osmolarity drop across each barrier. Analyzing the data in this way, the cytoplasmic pathway accounts for be-

[1] The degree of swelling observed by DiBona et al. (1969) appears to be significantly higher than that calculated from our data. A factor which might well account for the discrepancy is differential stretch of the tissue. In the present study, a relatively high degree of stretching was required to obtain a sufficiently thin albumin layer for freezing.

tween 20 and 50% of the total resistance in bladders exposed to ADH and mucosal dilution. Similarly, the ratios of the hydraulic resistances of apical and basolateral membrane were found to be highly variable, ranging from 1 : 2 in cells with large gradients to about 8 : 1 in cells with small gradients.

The scatter of the data may reflect an inherent variability in the cellular response to ADH. Some cells may respond to the hormone with a larger increase in hydraulic conductivity than others and, therefore, will experience a larger transcellular water flow, swelling and dilution. In part, this mechanism may account for the observed correlation between epithelial cell height and the height of the intracellular gradient. Another, probably more relevant factor is a primary difference in the stretch of the tissue. The taller the epithelial cell, the longer will be the transcellular pathway for water flow and, hence, the larger the cytoplasmic contribution to the total resistance. Moreover, tall cells should experience more water flow because of a higher density of apical and basolateral membranes per cross-sectional area. Notably, entirely different values for the hydraulic resistances are obtained when the calculation is based on results obtained with serosal dilution and ADH. Under these conditions, cytoplasmic resistance is scarcely detectable and the apical-to-basal resistance ratio appears to be about 10 : 1. The difference between the two sets of data suggests that the hydroosmotic effect of ADH is attenuated when the osmotic gradient is reversed.

The evaluation of the barriers to water flow as performed above provides, at best, an estimate of the effective resistances involved.[2] Clearly, because of the structural complexity of the transport pathway, a more detailed, distributed transport model seems appropriate (*see* Fig. 9). The large intracellular dilution and gradients observed in granular cells are consistent with the now generally held tenet that this cell type is the major pathway for ADH-induced osmotic water movement (DiBona et al., 1969). The mitochondria-rich cells showed a slight fall in mean intracellular ion concentrations but with no detectable change in their volume. In principle, the data do not exclude the possibility

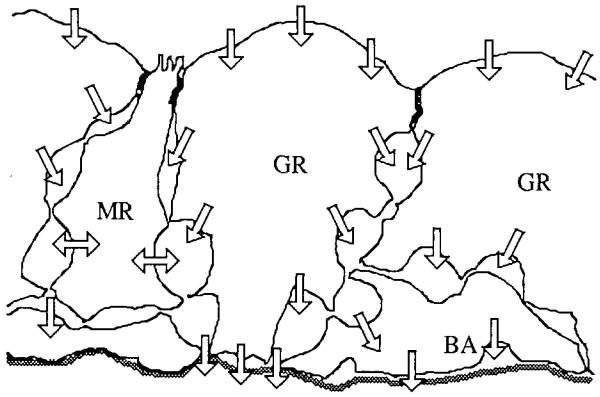

Fig. 9. Distributed model for ADH-induced osmotic water flow in the toad urinary bladder epithelium. For details *see* text

that mitochondria-rich cells participate in the transport of water. We find it more likely, however, that the observed modest dilution of these cells is attributable to a reduction in the osmolarity of the fluid in the surrounding lateral spaces. In fact, the comparable dilution of basal cells (more reliably estimated from a larger number of measurements) can only be explained if there is an at least equivalent reduction in lateral space osmolarity.

It is necessary to consider as well that the flow of water across the granular cells involves preferential intracellular pathways. Evaluation of the actual extent of cytoplasmic involvement in the overall hydraulic resistance will require more detailed information on the subcellular distribution of water flow and, of course, improved spatial resolution of intracellular osmolarity measurements. Analyses on a much finer scale will be needed to avoid contamination of most apical measurements by endocytic vesicles (Harris, Wade & Handler, 1986). However, since the vesicles are probably in osmotic equilibrium with the surrounding cytoplasmic fluid, their presence should not significantly change the sum of the ion concentrations. Similar considerations apply to quantification of the inner membrane resistance. Obviously, a large portion of the inner membrane is lining the intercellular channels and is, therefore, not in direct communication with the serosal bath. It is conceivable that the attenuated spaces between cells at the level of the basal lamina provide sufficient hydraulic resistance to prevent full equilibration of the lateral spaces with the inner bath, in particular in the presence of large convective flows. In fact, our results call for additional barriers along the length of the intercellular channels which, if wide and open, might be expected to collapse any osmolarity gradient in adjacent cells.

[2] One uncertainty is the exact osmolarity of the fluid bathing the apical surface at the time of freezing since the dilute albumin standard solution had a somewhat higher osmolarity than the 1/10-strength Ringer's (50 to 60 mOsm/liter as compared to 25 mOsm/liter). For the purpose of the above calculation, we assumed that the exposure time of 5 sec was sufficient for the albumin to reach the apical surface of the cells. It remains debatable, however, whether the time was sufficient to affect intracellular solute concentrations or cellular volume.

Implications for Transepithelial Na Transport

ADH, in addition to its effect on water transport, stimulates transepithelial Na reabsorption in the toad urinary bladder (Frazier, Dempsey & Leaf, 1962). There is good evidence from electrical noise analysis that the short-term stimulatory effect of the hormone is due to an increased apical Na permeability, apparently caused by an increase in the number of active Na channels (Li et al., 1982). In agreement with this presumed mechanism of action we observed a significant increase in the intracellular Na concentration after stimulating the bladder with ADH. This increase was most pronounced in granular cells and virtually absent in mitochondria-rich cells, consistent with the notion that the granular cells, but not the mitochondria-rich cells, are involved in transepithelial Na transport (Rick et al., 1978*a*; Bobrycki et al., 1981; DiBona et al., 1981). In addition, the basal cells seem to be part of the transepithelial transport pathway as indicated by a significant increase in the Na concentration. During osmotic water flow the increase in the Na concentration of the basal cells even exceeded the increase observed in granular cells (*see* Fig. 7).

Our results confirm our previous conclusion that the granular and basal cells in the toad urinary bladder form a syncytial Na transport compartment (Rick et al., 1978*a*). In particular, the fact that the Na influx into basal cells could be completely abolished by mucosal addition of amiloride provides direct evidence for the existence of cell-to-cell communication between the two cell types. After amiloride, the intracellular Na concentrations in the transport syncytium reaches extremely low values, supporting the view that there is very little, if any, recirculation of Na across the basolateral membranes. The lack of an effect of amiloride on the Na concentration in mitochondria-rich cells is, again, consistent with our view that this cell type does not participate in transepithelial Na transport.

Quite remarkably, in the presence of transepithelial water flow, Na transport through granular cells appears to proceed against rather than along an intracellular Na concentration gradient (*see* Fig. 4). Nevertheless, Na transport in the forward direction is still possible if driven by an electrical potential gradient (and perhaps some solvent drag). The intracellular concentration profile for Na may explain why the Na increase in granular cells is attenuated during osmotic water flow whereas the Na increase in basal cells is even slightly enhanced (*see* Fig. 7). Probably, the basal cells equilibrate mainly with the basal cytoplasm of granular cells (Wade, 1978) which has a higher Na concentration. Moreover, the Na influx into basal cells might be facilitated by a favorable electrical potential difference across the cell-to-cell junction.

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