Absence of Significant Cellular Dilution During ADH-Stimulated Water Reabsorption

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Water reabsorption across many "tight" urinary epithelia is driven by large transepithelial osmotic gradients and is controlled by antidiuretic hormone (ADH). Numerous investigators have concluded that ADH-induced water reabsorption causes large apparent increases in cell volume with concomitant cytoplasmic dilution. A central question in renal physiology has been how cellular homeostasis is maintained in tight urinary epithelia during antidiuresis. Previous direct measurements of cell membrane permeability to water and the present direct measurements of cell volume in collecting tubules of rabbit kidney cortex by quantitative light microscopy show that cell volume does not change significantly during transcellular water flow. Fluid transported across the epithelium accumulated in lateral and basal intercellular spaces; the effect was an increase in cell height and tubule wall thickness accompanied by maintenance of nearly constant cell volume. The stability of cell volume is a consequence of the relatively high water permeability of the blood-facing cell membrane.

"TIGHT" URINARY EPITHELIA SUCH as the renal cortical collecting tubule (CCT) are responsible for the final adjustment of urine volume and composition, as well as whole animal balance of salt and water (*1*). Fluid reabsorption in the CCT is driven by large osmotic gradients with the urine being significantly hypotonic compared to the blood. The water permeability of the apical (urine-facing) membrane of the CCT and therefore the rate of water reabsorption is under the control of the posterior pituitary hormone, vasopressin, also known as antidiuretic hormone (ADH) (*2*). The effects of ADH on mammalian kidney function have been studied extensively since the early 1900s (*3*). The prevailing hypothesis is that ADH increases the water reabsorption of the renal collecting duct by controlling the insertion of water channels into the apical membranes of collecting duct cells (*1, 4, 5*). In the presence of ADH, fluid reabsorption by the CCT increases four- to sixfold (*6, 7*) and the appearance of the epithelial cells changes strikingly. A number of studies on mammalian collecting tubules (*7–9*) as well as amphibian urinary bladder [an important model of distal nephron function (*10*)] have documented dramatic distension of lateral and basal extracellular spaces, large increases (30 to 50%) in apparent cell volume with concomitant cytoplasmic dilution (*9–13*), and apparent "intracellular vacuole" formation thought to reflect the transcellular flow of dilute luminal fluid (*9*).

Epithelial thickness increases substantially in ADH-sensitive tissues during osmotic water flow induced by the hormone (*7–10*). The cross-sectional area of the CCT wall is increased significantly by bulging of the tubule cells into the lumen without significant changes in outer tubule diameter (Fig. 1 and Table 1). These large changes in area have been used, in the past, to calculate that CCT cell volume increases approximately 30% during ADH-induced transepithelial water reabsorption (*9, 14*). Apparent cell swelling of a similar magnitude has been observed in the amphibian urinary bladder and has been proposed to be a factor controlling apical membrane water permeability via a "feedback regulation" pathway (*15*). Since swelling and concomitant cytoplasmic dilution of this magnitude are thought to be lethal or very detrimental to cells, it has been proposed that cells of ADH-sensitive epithelia possess special protective mechanisms for the channelization of dilute fluid through the apical cytoplasm, which in turn prevents bulk cytoplasmic dilution (*11, 12*). Finally, by means of standard mathematical approaches for describing osmotic water flow across cells (*16*), the degree of apparent ADH-induced cell swelling has been used to estimate that osmotic water permeability (P_{osm}) of the apical membrane becomes approximately equal to that of the basolateral membrane in ADH-sensitive epithelia during hormonal stimulation (*9, 11–13, 17*).

Recently, we developed a method of video-differential interference contrast microscopy to measure directly, for the first time, P_{osm} of the individual cell membranes in cells of the rabbit CCT (*2*). Our results showed that the ratio of basolateral to apical P_{osm} was at least 7:1 in principal cells exposed to a supramaximal dose of ADH (*18*). This result is not surprising since the total basolateral surface area of principal cells is at least eight to ten times the apical surface area (*19*). These direct measurements can be used to predict that CCT cells should swell no more than 6 to 8% when stimulated with ADH and luminally perfused with a hypotonic (130 to 150 mos*M*) solution.

Because previous studies had concluded that luminal dilution during ADH stimulation caused substantial swelling of CCT cells and our P_{osm} measurements (*2*) predicted little cell volume change under these conditions, we measured cell volume directly by using optical sectioning techniques (*2*) in CCT exposed to ADH and 130 mos*M* luminal solutions. Principal and intercalated cell volume changed only (mean ± SEM) 2.3 ± 0.8% (n = 28 cells) and −0.2 ± 1.5% (n = 11 cells), respectively, under these conditions (Table 1). These changes are below the accurate detection limit of the optical sectioning method (*20*). Why then

Table 1. Effect of luminal hypotonicity and ADH on CCT. Changes in cell and total epithelial volume (mean ± SEM) in ADH-stimulated tubules luminally perfused with an isotonic (290 mos*M*) or hypotonic (130 mos*M*) solution. Change is defined as the change in the experimental. All tubules perfused with 130 mo*SM* perfusate showed dramatic changes in structure including large increases in wall thickness, basal and lateral interspace distension, and distension of basal membrane infoldings. Tubule wall area was measured and total epithelial volume was calculated as described by others (*9, 14*). Cell volumes were measured directly by K.S. using optical sectioning methods (*2*). In addition, eight randomly chosen video images of principal cells were traced by a semiautomatic method (*28*) in which control and experimental cell images were traced on separate days and the operator was unaware of the resultant control or experimental cell volume. The mean change observed was 0.43 ± 3.3% (n = 8). As a further check on the validity of these measurements, seven randomly chosen principal cell video images were traced by two additional investigators who had no knowledge of the experimental conditions or previous results of this study. The mean changes in cell volume observed by them were −2.1 ± 2.4% (n = 4) and −1.0 ± 1.1% (n = 3), respectively.

Parameter	Change (%)	Cells (n)	Tubules (n)
Tubule wall area	16.5 ± 3.1		7
Total epithelial volume	31.6 ± 5.3		7
Volume of			
Principal cells	2.3 ± 0.8	28	11
Intercalated cells	−0.2 ± 1.5	11	6

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have previous investigators concluded that transcellular water flow results in large cytoplasmic dilutions and large increases in cell volume? Measurements of epithelial thickness only have been used previously to estimate cell volume in ADH-sensitive tissues (*9, 11*). Our observations indicate that the increase in CCT wall thickness in Fig. 1 is due not to cytoplasmic dilution but instead to increases in interspace volume. Collecting tubules are surrounded by a rigid, relatively noncompliant basal lamina (*21*). Volume flow into the peritubular compartment is restricted because of the reduced basal lamina surface area compared to total basolateral membrane area, and because fluid exits basal membrane infoldings and lateral interspaces through narrow basal slits (*22, 23*). Fluid thus accumulates within the lateral interspaces and basal membrane infoldings and so generates in turn small (<1 to 2 cm H_2O) (*22*) hydrostatic pressures that drive water across the basal lamina. Because the cell membranes are at least two to three orders of magnitude more compliant than the basal lamina (*21*), these hydrostatic pressures cause cell deformation. Lateral hydrostatic pressures constrict or squeeze the cells, causing them to bulge out into the tubule lumen, whereas basal hydrostatic pressures push the cells away from the basal lamina. Thus, tubule wall thickness increases, but cell volume remains nearly constant.

In light of the above observations, we have also reexamined the ADH-stimulated formation of so-called "intracellular vacuoles" (*7, 9, 10*). In rabbit CCT these structures do not appear as a result of ADH stimulation alone or as a consequence of bilateral dilution of bath and perfusate, which causes both osmotic cell swelling and cytoplasmic dilution (*9*). We also have been unable to observe these structures in ADH-stimulated tubules bathed with a 130-mos*M* serosal solution, which causes both cell swelling and bath-to-lumen water flow. Thus, as presently described, these "vacuoles" are induced only by unidirectional water flow from lumen to bath. It has been suggested that these structures may represent an intracellular compartment, which fills slowly with water as the cytoplasm is diluted by lumen-to-bath volume flow (*9*). Figure 2 shows optical sections of a principal cell in which we observed these structures. The structures extend from the basal pole into the first 40 to 50% of the cytoplasm (mean length ± SEM = 3.7 ± 0.3 μm; $n = 9$) and then disappear. We conclude that these structures represent not "intracellular vacuoles," but basal membrane infoldings, which have become distended as fluid accumulates in the basal extracellular space. Distension of these infoldings has been described previously in electron microscopy studies (*8*).

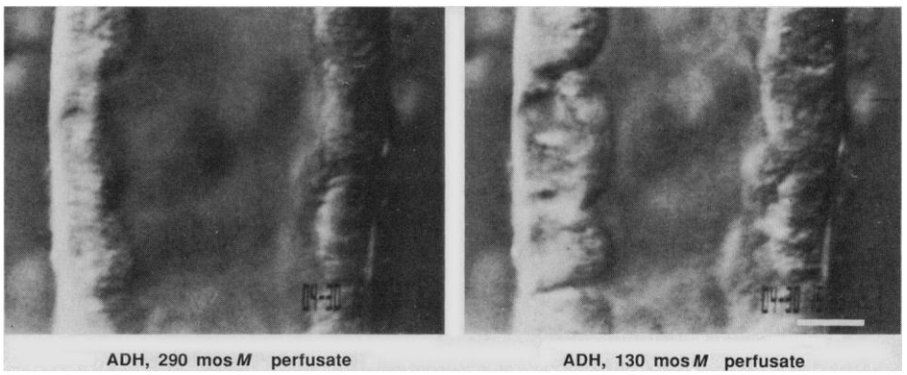

Fig. 1. Cross section of an ADH-stimulated tubule perfused with an isotonic (290 mos*M*) or hypotonic (130 mos*M*) perfusate. Total epithelial volume [see Table 1 and (*14*)] increased 33% when the tubule lumen was perfused with hypotonic saline. When the volume of two principal cells was measured directly by optical sectioning techniques in this same tubule (*2*), however, the observed changes in cell volume were −0.5 and 3.7%, respectively. Scale bar is 5 μm.

To conclude, cells of the rabbit CCT do not show significant swelling (*24*), cytoplasmic dilution, and cytoplasmic disruption (that is, "vacuole" formation) during ADH-induced transcellular water flow. Our results show that these cells are not stressed by large cytoplasmic dilutions and that there is no need to postulate protective mechanisms or complex fluid transport pathways for transcellular water movement. Furthermore, we conclude that previous indirect calculations of the relative P_{osm} of CCT apical and basolateral cell membranes are underestimates. Because the basolateral P_{osm} and membrane surface area of CCT are at least seven times those of the apical membrane (*2, 19*), water flow across the basolateral membrane into the blood is driven by small osmotic gradients, which in turn enable this tight epithelium to function in urine concentration and dilution, to withstand large changes in urine osmolality, and to support large ADH-induced variations in transcellular water flow without significant variations in cytoplasmic osmolality. The large asymmetry in membrane P_{osm} of the CCT can be compared to "leaky" epithelia where water reabsorption is driven by immeasurably small transepithelial osmotic gradients (0.1 to 3 mos*M*) (*25*) and where the ratios of basolateral to apical membrane surface areas and P_{osm} are closer to 1 to 2:1 (*26*). It is not known whether such dramatic asymmetries in membrane P_{osm} and surface area are

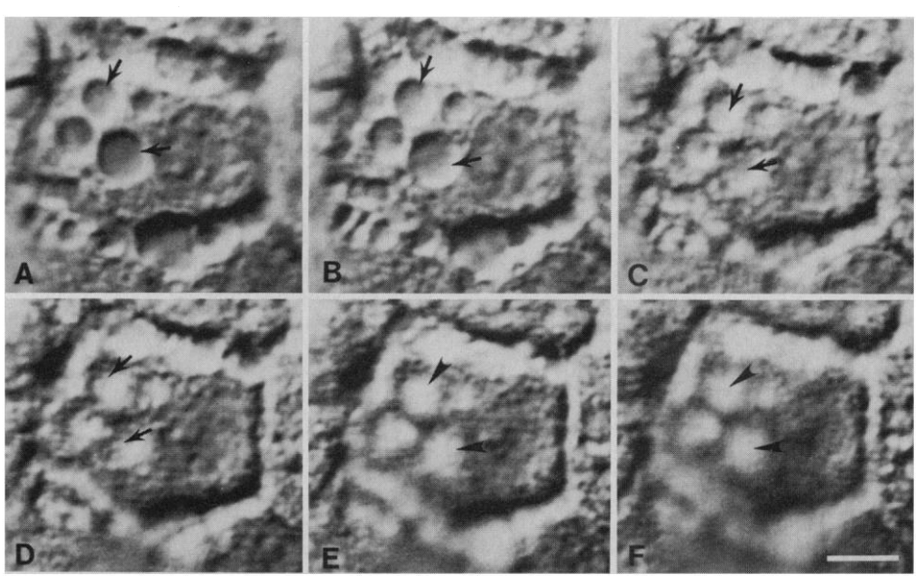

Fig. 2. Optical sections of a principal cell during ADH-stimulated transcellular water flow (**A** to **F**). Sectioning begins at the basal membrane (A) and proceeds to the apical pole (F). Basal infoldings (arrows) extend from the basal cell pole into the first 40 to 50% (C and D) of the cytoplasm. Note that the diameter of the smooth internal surface of these structures decreases until it disappears (C and D). The structures marked with arrowheads are "shadows" or out-of-focus information from the basal infoldings in the earlier optical sections. Scale bar is 10 μm.

present in all tight epithelia where water reabsorption is driven by large transepithelial osmotic gradients, but clearly the subject warrants further study (*27*).

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18. As used here P_{osm} refers to the product of the unit water permeability and total surface area for the individual membranes. The CCT epithelium is composed of principal and intercalated cells present in a ratio of approximately 3:1. Our P_{osm} measurements suggested strongly, but did not prove, that the intercalated cell was also responsive to ADH and that its ratio of basolateral to apical P_{osm} was at least 5:1 in the presence of the hormone. In the absence of ADH, the ratio of basolateral to apical P_{osm} in principal cells and intercalated cells is at least 27:1 and 17:1, respectively.
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20. As described previously (*2*), the optical sectioning technique in CCT does not allow us to detect volume changes of less than 6 to 7%. The mean coefficient of variation for tracing CCT optical sections was 5.7 ± 0.8% (n = 7). Volume changes of 10% or greater, however, can and have been easily detected in previous studies (*2*).
21. The elastic modulus for the basal lamina of rabbit CCT is 0.7×10^8 dyne/cm^2 (*22*). This value can be compared to the elastic modulus of cell membranes, which ranges between 10^{10} and 10^{11} dyne/cm^2 [E. A. Evans and R. M. Hochmuth, *Curr. Top. Membr. Transp.* **10**, 1 (1978)].
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24. The actual amount of cytoplasmic dilution observed in rabbit CCT in situ is probably even less than the 6 to 8% predicted by our P_{osm} measurements for ADH-stimulated tubules perfused with a 130-mos*M* solution. Under normal physiological conditions ADH is probably not released in chronic supramaximal doses as used in in vitro experiments. Furthermore, it seems unlikely that rabbit CCT are ever exposed to osmotic gradients as large as 160 mos*M* during antidiuresis. [See, for example, H. Wirz, *Helv. Physiol. Pharmacol. Acta* **14**, 353 (1956); C. W. Gottschalk and M. Mylle, *Am. J. Physiol.* **196**, 927 (1959)].
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27. Note that water reabsorption in the rat initial collecting duct and connecting segment is driven by extremely large transepithelial osmotic gradients (*24*) and that the ratio of basolateral to apical surface areas in principal and connecting cells of these segments ranges between 10 and 16:1 [B. A. Stanton *et al.*, *Kidney Int.* **19**, 36 (1981)].
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Arginine Vasopressin as a Thyrotropin-Releasing Hormone

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Although hypothyroidism (with concomitant increased levels of thyroid-stimulating hormone) has been associated with elevated plasma vasopressin, the role that vasopressin plays in controlling thyroid-stimulating hormone secretion from the adenohypophysis is not understood. In two in vitro pituitary cell systems, vasopressin caused a specific and dose-related release of thyroid-stimulating hormone from cells that was equal in potency to that elicited by thyrotropin-releasing hormone, the primary acknowledged regulator of thyroid-stimulating hormone release. When injected into the hypothalamus, however, vasopressin specifically inhibited the release of thyroid-stimulating hormone. Thus, vasopressin may exert differential regulatory effects on thyroid-stimulating hormone secretion in the hypothalamus and pituitary gland.

MORE THAN 20 YEARS AGO, VASOPRESSIN had been characterized as a thyrotropin-releasing hormone (TRH) by LaBella (*1*); however, this idea was soon disputed by Guillemin (*2*) and was explored essentially no further after elucidation of the structure of and synthesis of the thyroid-stimulating hormone (TSH)–releasing factor (TRH) (*3–6*). However, the conclusion was based on studies performed with lysine vasopressin that had been extracted from porcine posterior pituitary glands and by the indirect measurement of TSH by bioassay. Since the analog of vasopressin in most mammalian species, including the human and the rat, is the arginine version of the vasopressin nonapeptide (*7*), and, because TSH levels may now be measured directly by radioimmunoassay (RIA), we thus directly investigated the ability of synthetic arginine vasopressin (AVP) to release TSH from cells of the anterior pituitary with static incubation and perifusion of dispersed anterior pituitary cells and RIA for measurement of TSH; these tools were not available when this issue was considered earlier (*1, 2*). TSH functions in general metabolism and calorigenesis through stimulation of the thyroid gland (*8*). AVP is critical in fluid homeostasis, electrolyte balance (*9*), some behaviors (*10*), and the stress responses (*11*). Because AVP is involved in all of these actions and because AVP levels are elevated in conditions of experimental (*12*) and human (*13*) hypothyroidism when TSH levels are also high, we investigated whether AVP

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