Vasopressin: Effect on Deformability of Urinary Surface of Collecting Duct Cells

Abstract. *In isolated renal collecting ducts vasopressin decreased the negative pressure required to suck a hemispherical bulge of the epithelial cells' urinary surface into the tip of a micropipette. Increased surface deformability may be related to the increased permeability to water which also occurs in the apex of the cells in response to the hormone.*

In mammals antidiuretic hormone facilitates maximum concentration of urine by increasing the permeability of renal cortical (*1*) and papillary collecting tubules to water (*2*). The change in permeability must occur in or near the lumenal surface of the tubules, since osmometric swelling of the cells is enhanced by the hormone only when the lumenal fluid is hypotonic with respect to that bathing the blood side of the tissue (*3*). A barrier located in the apex of epithelial cells has been implicated as the site of permeability changes induced by antidiuretic hormone in other tissues as well (*4*). In none of these epithelia, however, have alterations in apical ultrastructure attributable to vasopressin been detected by electron microscopy. Hormone-induced submicroscopic alterations in structure may affect the mechanical as well as the chemical properties of the permeability barrier. I have studied whether vasopressin alters the deformability of the apical region of isolated collecting duct cells.

The entire papilla was removed from the kidney of an adult female New Zealand White rabbit; the surface epithelium was removed by scraping with a scalpel blade. The papilla was cut into approximately 1 to 2 mm pieces and transferred into 30 ml of incubating medium in a 40-ml silicon-coated conical glass centrifuge tube. The medium contained 150 m*M* NaCl in final concentration, 25 m*M* $NaHCO_3$, 5 m*M* KCl, 10 m*M* $NaC_2H_5O_3$, 1.2 m*M* NaH_2PO_4, 1.0 m*M* CaCl, 1.2 m*M* $MgSO_4$, 300 m*M* urea, 0.1 percent dextrose, and 5 percent by volume of calf serum. Penicillin (100 unit/ml) and streptomycin (0.1 mg/ml) were also added. Experiments were performed at room temperature (22°–24°C). The solution was agitated gently by O_2 (95 percent) and CO_2 (5 percent) bubbled through it. The pieces of tissue were rinsed twice in incubating medium; then approximately 10 ml of the incubating solution containing collagenase (Worthington Biochemical, 100 mg/100 ml) was added. Every 30 minutes the tube was agitated for 10 seconds with a vortex mixer. The tissue was kept in the collagenase solution for 120 minutes. Enzymatic action was interrupted by centrifuging the suspension at 800 rev/min for 3 minutes and then rinsing the sediment twice with 30 ml of fresh incubating medium. Collagenase caused the individual tubular elements in the papilla to separate from one another and to lie relatively free in suspension. Both ends of a collecting duct fragment everted, exposing the urinary surface of the epithelial cells to the incubating medium. The cells remained attached along the lateral borders and only the urinary surface was accessible to direct study. Portions of the suspension were transferred to a chamber mounted on the stage of an inverted microscope and illuminated as described previously (*3*). The cells were observed directly at a magnification of × 1000.

Rand and Burton (*5*) devised a method for quantifying surface stiffness of intact cells, based on earlier studies by Mitchison and Swann (*6*). A portion of a cell was drawn into the tip of a glass capillary tube of constant bore with a known negative pressure. Surface stiffness was determined from the relation between the pressure applied and the distance a bulge of tissue was drawn into the capillary. I used a similar method for measuring the surface deformability. Microcapillary borosilicate glass pipettes of constant bore were made with a Stoelting microforge. The inner diameter of the pipette tips ranged between 5.0 and 7.0 μm. Each micropipette was filled with incubating solution and connected to a reservoir which could be raised and lowered. Zero hydrostatic pressure drop across the tip of the pipette was determined by observing the movement of debris into and out of the pipette. The zero pressure point could be determined with an accuracy of ± 0.2 cm-H_2O. The deformability of the urinary surface and underlying cytoplasm was determined by sucking the apical portion of the cell into the pipette. Firm attachment could usually be made with a negative pressure of less than 5 cm-H_2O. The distance the bulge moved into the pipette was recorded exactly 5 seconds after the pressure was changed. Collecting duct cells are relatively stiff, and reliable measurements of the distance the bulge was pulled into the pipette could not be made at pressures lower than 5 cm-H_2O. Occasionally an irregular tongue of tissue was pulled into the pipette, usually when the pipette had been inadvertently positioned over a junction between adjacent cells. Measurements were accepted only when the pipette was clearly attached to the rounded surface of a single collecting duct cell and a smooth bulge was sucked into the pipette. Apical surfaces deformed in this manner exhibited hysteresis; cytolysis was rarely observed after performance of the deformability test at high negative pressure. Measurements were accepted only if the surface returned to its original configuration within 30 seconds after the pressure was reduced to zero and if no cytolysis occurred.

I define deforming pressure as the negative pressure required to suck a hemispherical bulge of lumenal surface and cytoplasm into the micropipette. Consequently, increased surface deformability is reflected by decreased deforming pressure and vice versa. For study of the effect of antidiuretic hormone on deformability, the tissue sus-

Table 1. Effects of 8 m*M* dibutyryl C-AMP and of 5 m*M* theophylline with 4m*M* dibutyryl C-AMP on deforming pressure. Numbers in columns A through C are means and S.E.M. of ten measurements of deforming pressure; those in the remaining columns represent differences. The mean difference of columns A and B is 9.6 ± 2.1 ($P < .025$); that of columns A and C is 13.6 ± 1.4 $P < .01$).

Experiment	Deforming pressure (cm-H_2O ± S.E.M.)				
	Control (A)	Dibutyryl C-AMP (B)	Dibutyryl C-AMP theophylline (C)	A–B	A–C
1	37.0 ± 1.7	28.8 ± 1.9	24.3 ± 0.8	8.2	12.7
2	40.3 ± 1.3	25.7 ± 1.5	23.3 ± 1.9	14.6	17.0
3	39.8 ± 1.1	33.9 ± 1.6	28.7 ± 2.5	5.9	11.1

pension was divided into two equal portions; Pitressin (Parke-Davis) was added to one of these to a final concentration of 250 μU/ml. This dosage is greater than that which causes maximum effects for collecting duct cells (*2, 3*). Deforming pressure was measured randomly on both control and experimental cells, and the same pipette was used without the operator's knowing which suspension contained the hormone. Measurements in the control and experimental suspensions were started 20 minutes after addition of hormone.

The deforming pressure of at least six cells was measured before a different sample of suspended tubules was studied. On the average, 20 control cells and 18 experimental cells from each papilla were deformed over a period ranging from 21 to 210 minutes. In suspensions of nine papillae, the deforming pressure of control cells averaged 30.4 ± 2.0 cm-H_2O (S.E.M.). The deforming pressure of the paired cells treated with vasopressin was less—mean change 7.4 ± 0.8 cm-H_2O (S.E.M.), an indication that the hormone increased surface deformability. In some of the studies more than one comparison between control and experimental cells was technically possible, and the data have been grouped according to the approximate times the measurements were made (Fig. 1). The effect on deforming pressure was detected 20 minutes after addition of hormone and persisted for more than 3 hours.

The viscous nature of the cells was apparent when high negative pressures were applied for longer than 5 seconds. The apical surface rapidly bulged into the pipette several microns and then slowly crept up the pipette. The effect of vasopressin on creep was tested. In control and experimental cells a negative pressure of 50 cm-H_2O was rapidly applied and the distance the bulge traveled after 5 and 15 seconds was recorded (Fig. 2). The distance the bulge traveled was always greater in the treated group; furthermore, the rate of creep between 5 and 15 seconds in these paired studies was increased by the hormone (1.5 μm/10 sec ± 0.5 S.E.M., $P < .05$). Rupture of the cell bulge [viscoelastic breakdown (*7*)] was occasionally seen but only in those cells treated with vasopressin.

It is generally accepted that cyclic adenosine 3′,5′-monophosphate (C-AMP) is the intracellular mediator of the hydrosmotic effect of vasopressin in several receptor tissues, including

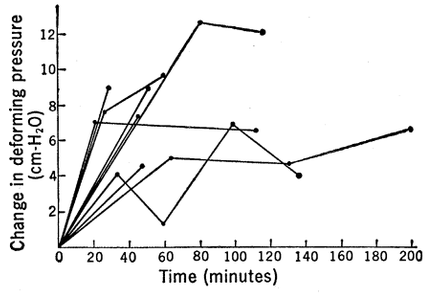

Fig. 1. Time course of effect of vasopressin on deforming pressure of collecting duct cells in nine papillae. Vasopressin (250 μU/ml) was added at zero time. Each point is the difference in mean deforming pressure (control minus vasopressin).

isolated collecting tubules (*1, 8, 9*). The dibutyryl derivative of C-AMP (Calbiochem) was added to suspensions of collecting duct cells and the effect on deforming pressure was evaluated in the same manner as for vasopressin. At a concentration of 8 mmole/liter the nucleotide significantly decreased the deforming pressure, an effect similar to that of vasopressin (Table 1). Neither 4 m*M* C-AMP nor 5 m*M* theophylline (Calbiochem) had a detectable effect; however, combining the agents at these concentrations caused a decrease in deforming pressure that was greater than that caused by 8 m*M* C-AMP alone (Table 1). Apparently, theophylline enhanced the effectiveness of the lower concentration of C-AMP by interfering with its intracellular degradation.

Changes in volume may affect the surface deformability of the cells (*5, 6*); therefore, the effect of vasopressin on cell volume was determined. The length as well as the diameter of collecting

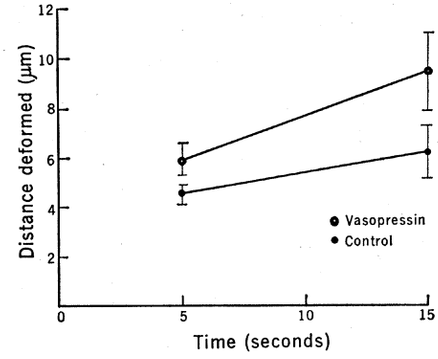

Fig. 2. Effect of vasopressin on creep. A constant negative pressure (50 cm-H_2O) was applied at zero time, and the distance the bulge advanced was recorded at 5 and 15 seconds. Vasopressin was added to the experimental medium at least 30 minutes before determination of creep. Results are from six studies. Vertical bars ± 1 S.E.M.

tubules changes when placed in anisosmotic media (*3*). Thus, I assumed that changes in cell volume would be reflected by a change in the width of the cells. The distance between the apical tight junctions of individual cells at the lumenal surface was measured by means of a calibrated reticle in the microscope eyepiece. In control cells the average distance was 14.7 ± 0.9 μm and in the hormone-treated cells 14.4 ± 0.9. The paired differences were not statistically significant. There was no apparent effect of the hormone on the configuration of the lumenal surface.

As an additional test of specificity, it would be desirable to measure the effect of vasopressin on the deformability of the lateral and basilar surfaces of collecting duct cells. Unfortunately, these surfaces are inaccessible to direct study. Since fluid absorption in the proximal tubule is not affected by vasopressin (*10*), the effect of the hormone on deforming pressure of the urinary surface of proximal tubule cells was tested as a control. A suspension of cortical tubules was prepared as described by Burg and Orloff (*11*). At the everted ends of the tubule fragments the brush border was easily recognized along the apical surfaces. In seven experiments the deforming pressure of the proximal tubular cells was much less than in collecting duct cells (5.5 ± 0.5 cm-H_2O, S.E.M.); in paired studies vasopressin had no effect on deformability (mean change 0.1 ± 0.5 cm-H_2O).

Apparently hormone-induced changes in permeability involve some reorganization or conformational change in membrane structure. Measurement of deformability is a direct method for detecting changes in the physical characteristics of membrane interfaces. Vasopressin increases the deformability of the urinary surface of collecting duct cells. Several observations are consistent with the view that the physical alteration may be associated with the permeability changes known to occur in this tissue. First, increased permeability and deformability both occur at the lumenal side of the cell. Second, the time course of the hormone effect on deformability parallels that of increased hydrosmotic flow in perfused tubules (*1, 9*). Third, C-AMP, the intracellular mediator of vasopresssin, mimics the effect of the hormone on surface deformability. The changes in surface deformability caused by vasopressin cannot yet be definitely localized to the plasma membrane. It is generally assumed that the viscosity of cytoplasm does not contribute signifi-

cantly to membrane deformability, an assumption that is probably warranted in tissues in which cytoplasmic viscosity has been measured and found to be low (*6, 12*). However, on the basis of other micrurgy experiments I have the impression that the cytoplasm of collecting duct cells is considerably more viscous than water. Consequently, until proven otherwise the possible role of the subapical cytoplasm in altering surface deformability in response to vasopressin cannot be dismissed. Once the exact location of the hormone-induced mechanical alteration in the cell apex is identified, it may be possible to characterize further the physicochemical nature of the permeability barrier.

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Cross-Reactions between Streptococcal M Proteins and Human Transplantation Antigens

Abstract. *Allogeneic antiserums against human lymphocytes were specifically inhibited by M protein from beta hemolytic group A* Streptococcus pyogenes, *type 1. Analogous M proteins from streptococcus types 3, 4, 5, 6, 12, and 14 had little or no inhibitory activity. The specific inhibition by M protein is not a result of anticomplementary activity or of coating of the lymphocyte surface. Streptococcal polysaccharide and 73 other polysaccharides were inactive. Because all seven HL-A specificities tested were inhibited, it is inferred that M1 protein has a structure common to human histocompatibility antigens.*

Streptococci are known to be associated with many suppurative diseases, such as pharyngitis, mastoiditis, and otitis media. They have also been implicated in triggering nonsuppurative diseases (glomerulonephritis, rheumatic fever) and acceleration of skin allograft rejection in the guinea pig (*1*). Similarities between animal and bacterial antigens have been suspected of being the basis for these phenomena (*2, 3*). Human erythrocyte antigens A, B, O, and Le are closely related in chemical structure to pneumococcal polysaccharide, and substances with blood group activity are found among many bacteria (*4*). Molecular "evolution" may have taken place among human antigens from ancestral substances common to bacteria and man. In addition, pathogenic bacteria may have developed antigens similar to human antigens by convergent evolution (*5*).

Patients with chronic glomerulonephritis have the HL-A2 antigen in a higher proportion than normal persons have (*6*). The HL-A antigens function as transplantation antigens (*7*). With this clue as to the possible association of transplantation and streptococcal antigens, we investigated their cross-reactivities, using the ability of a variety of substances to inhibit antiserums to HL-A, which are cytotoxic to lymphocytes. We used well-characterized human antiserums defining seven HL-A specificities; these were from our laboratory and from the serum bank of the National Institute of Allergy and Infectious Diseases. These antiserums were either from multiparous women or immunized persons. Lymphocytes were typed for HL-A specificities, and antiserums were titrated before use. One microliter of several dilutions of allogeneic antiserums was used above and below the dilution at which 50 percent of the cells are killed. Substances used as inhibitors were dissolved in phosphate-buffered saline generally at a concentration of 5 to 10 μg/μl. One microliter each of antiserum and inhibitor was incubated at room temperature for 1 hour; 1 μl of a lymphocyte suspension (1000 cell/μl) was added, incubated first for another 30 minutes, and then incubated for 1 hour with 5 μl of rabbit complement. For staining the nonviable cells, 2 μl of 5 percent eosin was added; within 1 minute 3 μl of formaldehyde was added (*7*). Each dilution of antiserum, used in control and in experimental tests, was run in triplicate, and the percentage of dead cells was averaged. Each of the two antiserums for each HL-A specificity was tested on lymphocytes from three different persons.

The M proteins were isolated from group A streptococci essentially according to the method of Fox, Wittner, and Dorfman (*8*). Preparations of bacterial cell walls were obtained from group A streptococci, type 12 (*9*). The extraction with hot formamide described by Fuller (*10*) and modified by Zittle and Harris (*11*) was used to obtain the group-specific polysaccharide (APS).

In initial trials, 84 substances (*12*) were screened for their cross-reactivities with transplantation antigens. Among the substances tested were proteins from streptococci, polysaccharides from *Streptococcus pyogenes, Escherichia coli, Diplococcus pneumoniae, Shigella flexneri, Paracolon ballerup, Serratia marcescens, Pasteurella pseudotuberculosis, Salmonella poona,* and human glomerular membrane extract. Most of the substances did not cross-react with HL-A antigens.

Results of a typical inhibition experiment are shown in Fig. 1. Antiserum to HL-A2 was strongly inhibited by M1, but it was not affected noticeably by the six other M proteins or by APS. Inhibition of antiserum to HL-A of five other specificities, HL-A1, -3, -7, -8, and -9, was similar.

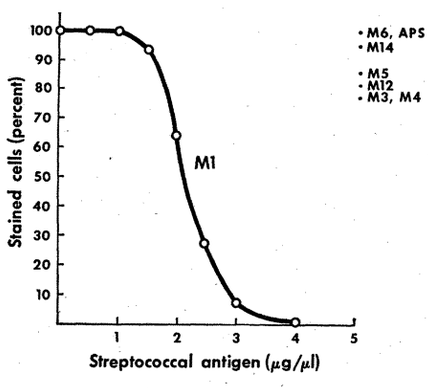

Fig. 1. Inhibition of an antiserum to HL-A2 by streptococcal antigens. The M1 protein was tested at varying concentrations, whereas M6, APS, M14, M5, M12, M3, and M4 were tested only at 5 μg/μl.