Pediatr Nephrol (1993) 7: 672–679

Pediatric Nephrology

Review article

The effect of vasopressin on the cytoskeleton of the epithelial cell

Richard M. Hays[1], John Condeelis[2], Yang Gao[1], Herold Simon[1], Guohua Ding[1], and Nicholas Franki[1]

[1] Department of Medicine, Division of Nephrology, and [2] Department of Anatomy/Structural Biology, Albert Einstein College of Medicine, 1300 Morris Park Avenue, Bronx, NY 10461, USA

Received Oktober 17, 1992; received in revised form March 27, 1993; accepted May 10, 1993

Abstract. Vasopressin (AVP) promotes the fusion of vesicles containing water channels with the apical membrane of receptor cells in the amphibian bladder and mammalian kidney. Fusion is accompanied by depolymerization of the actin cytoskeleton. In this review, we present the evidence for actin depolymerization by AVP in the whole cell, and the application of confocal microscopy and immunogold electron microscopy in localizing depolymerization to the apical region of the receptor cell.

Key words: Vasopressin – Actin – Cytoskeleton – Urine concentration

Introduction

The action of vasopressin (AVP) on the collecting duct has been one of the most intensively studied processes in the kidney. The initial steps follow the pattern for a hormone whose action is mediated by cyclic AMP (Fig. 1): AVP binds to V_2 receptors on the basolateral membranes of principal cells, and, via a G protein, activates adenylate cyclase, generating cyclic AMP [1]. In parallel with the adenylate cyclase/cyclic AMP sequence, there is a V_1 receptor pathway which, via phospholipase C, activates several modulators of the hydroosmotic response to AVP; these include prostaglandins (notably prostaglandin E_2), protein kinase C, and possibly calcium. Many questions remain about the details of this dual control pathway; the reader is referred to recent reviews [2–4].

Even at the V_2 receptor step in AVP action, there are questions of interest to the pediatric nephrologist. There is good evidence, for example, that the defect in human nephrogenic diabetes insipidus lies in the V_2 receptor [5]; recent studies of the receptor are discussed by Knoers et al. at this meeting [6]. Reduced adenylate cyclase activation and cyclic AMP levels may also contribute to the reduced urinary concentrating activity in the neonatal period [7, 8].

Beyond the generation of cyclic AMP, the action of AVP is an interesting example of the cell biology of exocytosis (Fig. 1). This has been studied in most detail in the toad urinary bladder, an analog of the mammalian collecting duct. Long vesicles (aggrephores), whose walls contain aggregates of water channels, are positioned just below the granular cell apical membrane. The aggrephores maintain a strictly horizontal position in the unstimulated cell [9].

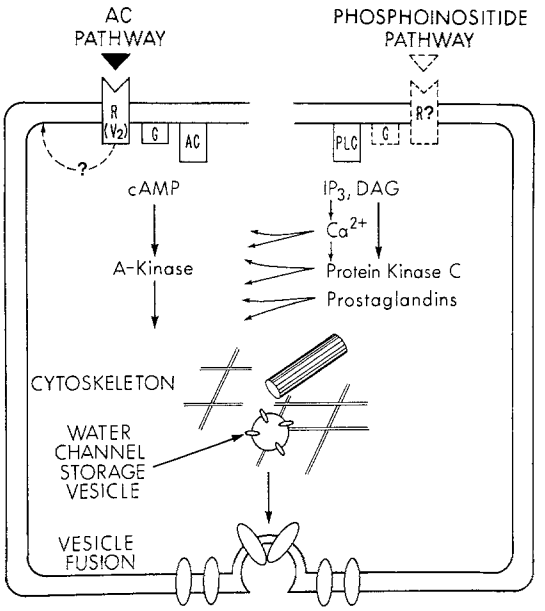


Fig. 1. Pathways of vasopressin (AVP) action in amphibian bladder and mammalian collecting duct. The V_1 receptor and G protein involved in the phosphoinositide pathway are shown by *dashed lines*, indicating some remaining questions about this pathway. From [4] with permission. *AC*, Adenylate cyclose; *PLC*, phospholipase C; *IP*$_3$; *DAG*

Correspondence to: R. M. Hays, Albert Einstein College of Medicine, 1300 Morris Park Avenue, Ullmann Building Room 617, Bronx, NY 10461, USA

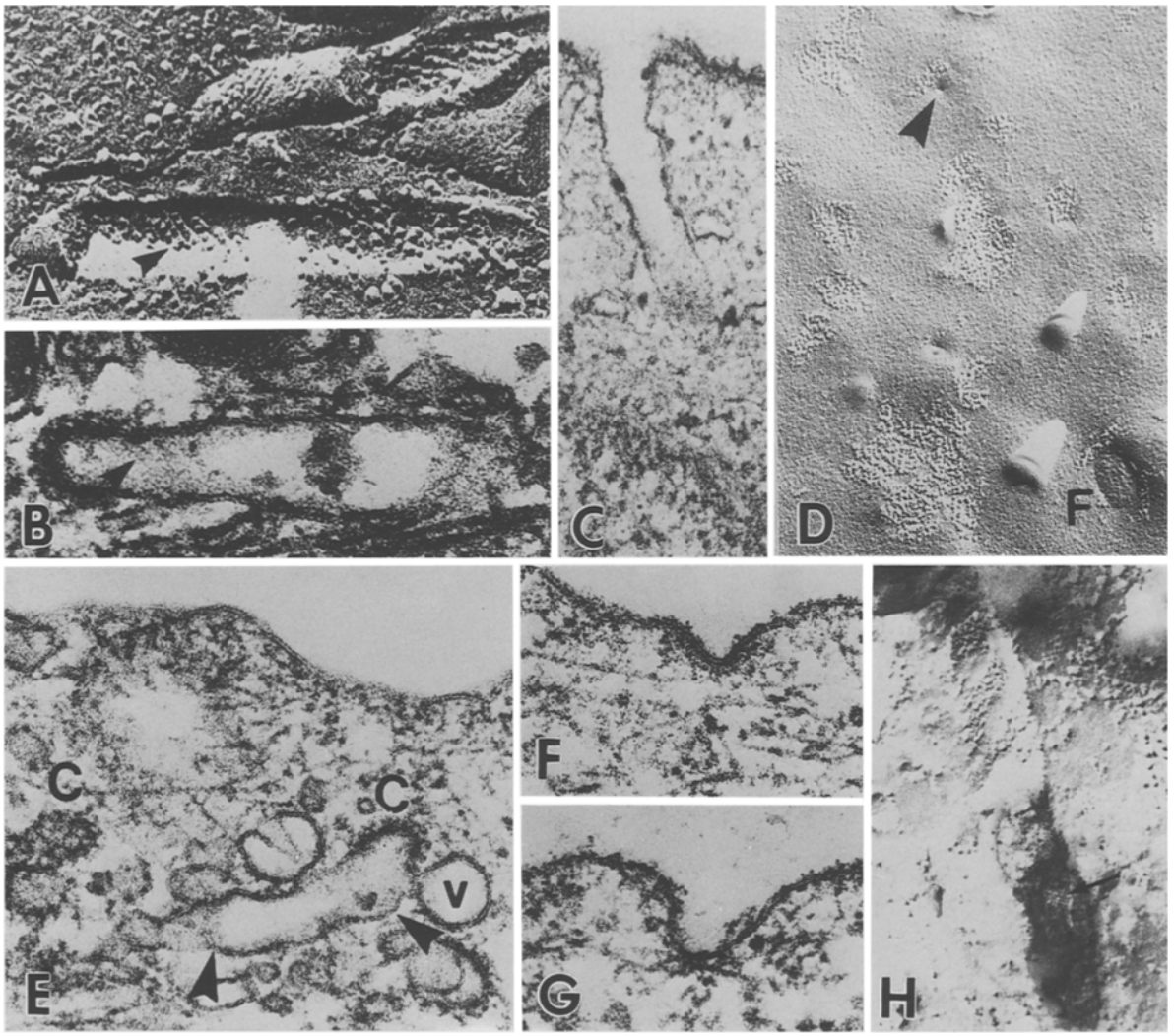


Fig. 2. Steps in the transfer of water channels in the toad and frog urinary bladder. **A** Aggrephores in the cytoplasm of a *Bufo marinus* granular cell, shown by freeze-fracture. Linear arrays of particles (*arrowhead*) are visible in both aggrephores. **B** Aggrephore (*B. marinus*) prepared by glutaraldehyde/tannic acid fixation and ultrathin sectioning. A coated vesicular head (*arrow*), as well as linear arrays (*arrowhead*) can be seen. **C** Aggrephore (*B. marinus*) fused to the apical membrane of a AVP-treated granular cell. **D** P-face of a AVP-treated granular cell (*Rana catesbeina*), showing particle arrays (*arrows*), some of which are emerging from fusion sites (*arrowheads*). The sites range from large (*bottom, F*) to small (*top*), the latter are suggestive of a small fused vesicle. **E** Aggrephore in the cytoplasm of *Rana esculenta*, showing several emerging vesicles (*V*) as well as surface coating (*C*), probably clathrin. *Arrowheads* point to short linear arrays. **F** Clathrin-coated apical membrane pit, presumably undergoing endocytosis, in the apical membrane of a AVP-treated *B. marinus* granular cell. **G** Uncoated apical membrane pit, AVP-treated *B. marinus* granular cell, undergoing exocytosis or endocytosis. **H** Aggrephore from *B. marinus* initially treated with AVP, followed by AVP withdrawal. Preparation by label-fracture method shows simultaneously P-face particles (*top*) and E-face grooves (*bottom arrow*), as well as horseradish peroxidase internalized by the aggrephore during endocytosis. **A–G**, Reproduced from [20]; **H**, reproduced from [18]. Entire figure from [2], with permission

Following stimulation by AVP, the aggrephores angulate and then fuse with the apical membrane. The water channel aggregates then move from the aggrephores to the planar membrane, and water flow begins across the cell. The sequence of events, described by investigators in a number of laboratories [10–14], is shown in Fig. 2.

The aggrephores do not remain fixed to the apical membrane in the presence of an osmotic gradient, but engage in a process of cycling between the cytoplasm and the membrane [15–18]. The cycling pattern appears to be relatively superficial, and there is no evidence at this time for acidification of the retrieved aggrephores of the kind seen in late endosomes [19].

The system of water channel delivery seen in the toad bladder is significantly modified in other species. In as closely related a species as the frog, aggrephores in the bladder epithelium rarely fuse directly with the apical membrane, but appear to act as cytoplasmic storage sites for water channels; small vesicles may carry out the actual transport of channels from aggrephore to membrane

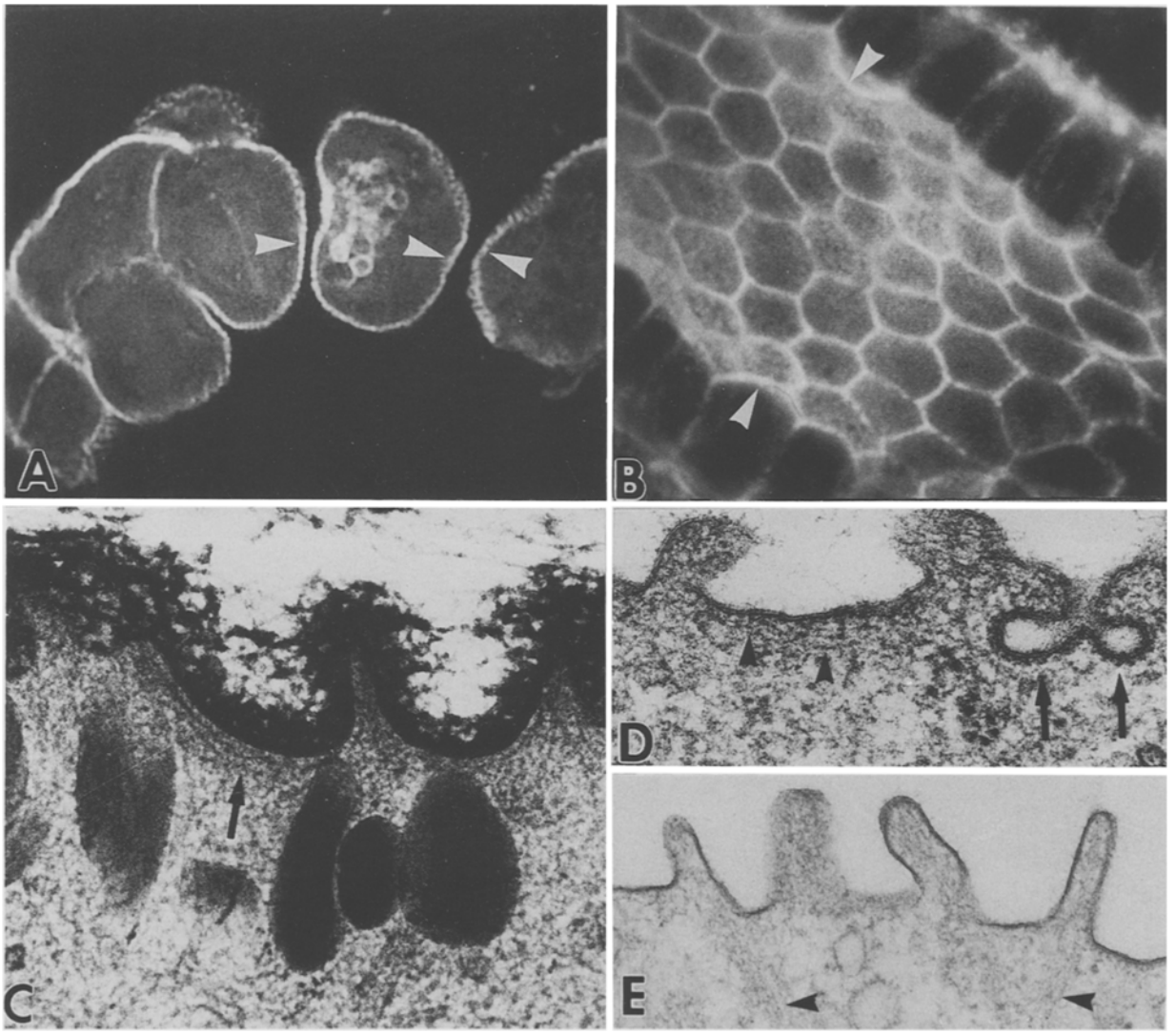


Fig. 3. Filamentous actin (F-actin) in toad bladder granular cells and the principal and intercalated cells of the inner medullary collecting duct (IMCD). **A** Rhodamine phalloidin-stained toad bladder granular cells, some of which have been separated from the epithelial sheet, showing concentration of F-actin around the cell periphery (*arrowheads*, apical region). **B** Scanning confocal microscopic view of a rhodamine phalloidin-stained section of rabbit papilla, showing an IMCD. In the cells running along the lateral border, the apical regions (*arrowheads*) are intensely stained. **C** Rapid-freeze section of the apical region of a toad bladder granular cell, showing an array of parallel filaments (*arrow*) running between adjacent microvilli (courtesy of Dr. Thomas Reese). **D** Filaments running between microvilli in IMCD principal cell (*arrowheads*); *arrows*, coated pits. **E** Course of filaments in collecting duct intercalated cell, running straight down from microvilli into terminal web (*arrowheads*). B, D, E from [32], with permission

(Fig. 2E) [20]. In the mammalian kidney, the channel delivery system is even less well understood. Channel aggregates do appear on the apical membrane of principal cells in the presence of AVP [21], and are thought to be brought to the membrane by vesicles, but neither the vesicles or their source have been clearly identified. Vesicles also appear to be involved in channel endocytosis [22].

Thus, the system for water channel delivery and recovery beyond the generation of cyclic AMP has the features of a vesicular transport system. This system, like the receptor-associated steps, appears to require time to develop during the postnatal period [23], and, like other secretory systems [24–26], the fusion of water channel-carrying vesicles in both amphibian bladder and mammalian collecting duct is accompanied by the depolymerization of F-(filamentous)actin. The following sections will summarize our evidence for the role of AVP-induced actin depolymerization in vesicle fusion.

Depolymerization of F-actin by AVP

In single cells, as well as epithelial sheets, F-actin is concentrated at the cell periphery. In epithelial cells it is particularly abundant in microvilli and the underlying terminal web. Figure 3 shows the distribution of F-actin in the toad urinary bladder and the rat inner medullary collecting duct

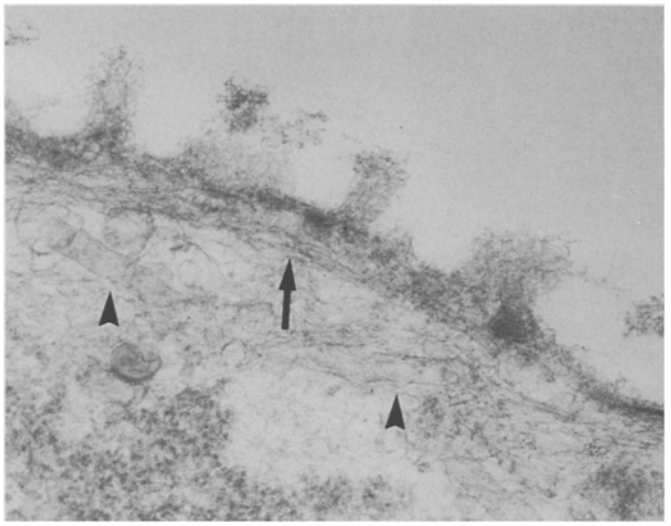


Fig. 4. Toad bladder granular cell treated with 0.1% Triton and fixed with glutaraldehyde, showing a heavy filamentous network below the apical membrane (*arrow*) and two aggrephores (*arrowheads*)

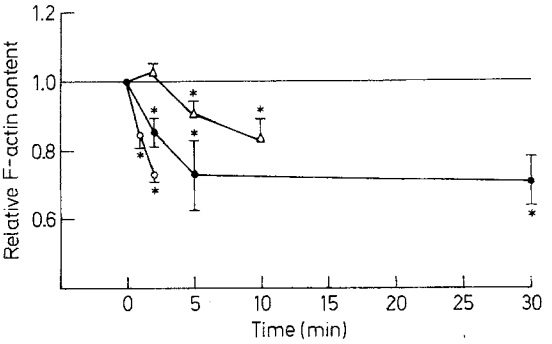


Fig. 5. Depolymerization of F-actin in toad bladder epithelial cells by AVP. ✳ indicates significant decreases in F-actin content of AVP-treated compared with paired untreated control cells. △, 3 mU AVP; ●, 10 mU AVP; ○, 100 mU AVP. From [30] with permission

(IMCD). Figure 3 C and D show more detailed views of the pattern of F-actin in the microvilli and subapical region. In both the toad bladder granular cell and the IMCD principal cell, which are the AVP-responsive cells, the F-actin follows a distinctive course, running down the microvilli and then turning to establish a dense network below the apical membrane. In contrast, the adjoining mitochondria-rich cells of the toad bladder and intercalated cells of the collecting duct (Fig. 3 E) as well as the proximal tubular cells, show the more typical course of apical F-actin: coursing straight down from the microvilli to the terminal web.

A striking example of the subapical filamentous network in the toad bladder is shown in Fig. 4. The subapical actin network in the toad bladder and collecting duct has the potential of acting as a barrier to vesicle fusion, an idea first proposed by Orci et al. [27] in studies of the pancreatic beta-cell. The idea of a barrier was extended with the demonstration in the chromaffin cell [24], the mast cell [25], and the parotid acinar cell [26] that the barrier was under agonist control, disappearing when exocytosis was stimulated. In the parotid cell, fluorescently labeled fodrin, another polymeric structure associated with cortical F-actin [28], also disappeared during exocytosis. We wondered whether AVP had a similar effect on F-actin in toad bladder and collecting duct, and began our studies by determining the concentration of F-actin in whole epithelial cells by the rhodamine phalloidin binding assay [29].

Our first studies were in the toad bladder [30], and our findings are depicted in Fig. 5, in which we show the relative F-actin content per microgram protein of isolated vasopressin-treated cells, compared with paired untreated controls. There was no osmotic gradient in these experiments. At 100 mU/ml of AVP, there was a significant decrease in F-actin content as early as 1 min after stimulation, reaching a maximum of 30% in 2 min. A significant decrease was seen at 2 min with 10 mU/ml AVP, and at 5 min with 3 mU/ml, a very low concentration. In all three cases, actin depolymerization took place before the onset of the hydroosmotic response, as determined in independent experiments on intact bladders. Similar results were obtained in the frog bladder. Depolymerization persisted as long as AVP was present in the bathing medium, in contrast to the findings in some other secretory cells, such as the chromaffin cell, in which F-actin repolymerizes within a minute of the secretory response [31]. I-Desamino [8-D-Arginine] Vasopressin, a pure V_2 agonist, and 8-bromo cyclic AMP depolymerized F-actin in a fashion comparable to AVP. When AVP was removed from the bathing medium, actin repolymerization took place as water flow returned to baseline values. When F-actin was stabilized by the introduction of 7-nitrobenz-2-oxa-1,3-diazolephallacidin (NBD-phallacidin) into the cell, the subsequent hydroosmotic effect of AVP was selectively reduced by 40%, supporting the conclusion that actin depolymerization is important in mediating the water flow response.

We have now completed a series of studies of the F-actin response to AVP in the rat IMCD [32]. We used 0.2- to 0.3-mm sections of the lower half of Sprague-Dawley rat papillae which were opened up by incubation in collagenase and hyaluronidase. The tissues were then treated with AVP or left as controls, fixed, and stained with rhodamine phalloidin as in the toad bladder experiments. Five minutes after the addition of AVP, there was significant depolymerization of F-actin over an AVP concentration range of 2.5–250 nM. Thus, in both amphibian bladder and rat IMCD, the response to AVP involves a net depolymerization of F-actin, as determined in the whole epithelial cell.

Where in the epithelial cell does actin depolymerization take place? To answer this question, we turned to confocal microscopy, which permits optical sectioning of the cell either in the horizontal ($x-y$) or vertical ($x-z$) plane [33]. We analyzed the fluorescence intensity of rhodamine phalloidin-labeled toad bladder epithelial cells comparing vertical sections of paired control and AVP-treated toad bladder epithelial cells, and found a significant reduction in the ratio of apex/side fluorescence intensity in the presence of AVP (Fig. 6) [34]. This is shown for six paired experi-

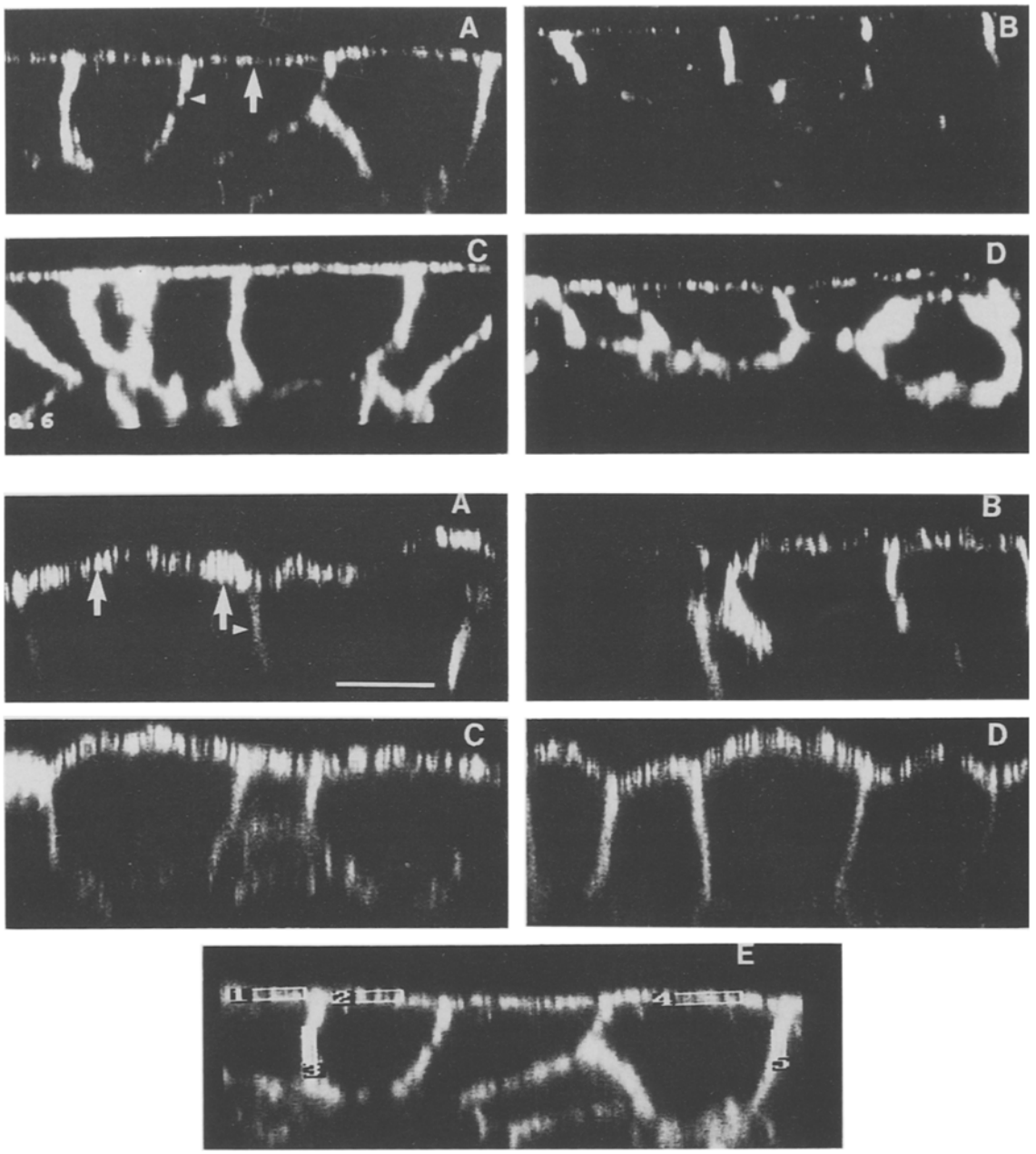


Fig. 6. Confocal views, in the vertical plane, of paired toad bladder epithelial cells stained with rhodamine phalloidin. The upper four panels (**A – D**) are from bladders mounted on plastic rings; the lower four panels (**A – D**) are from bladders mounted in a split Ussing chamber. In each set **A** and **C** were controls and **B** and **D** were treated with AVP for 15 min prior to fixation and rhodamine phalloidin staining. The apical regions are shown by *arrows*, and the lateral (side) regions by *arrowheads*. There is a reduction in the fluorescence intensity of the apical segments following AVP. From [34] with permission

ments in Fig. 7; the ratios were 3.69 ± 0.5 for controls and 2.61 ± 0.3 for AVP-treated bladders ($P < 0.05$). Thus, for the toad bladder, we were able to show that it was the apical F-actin pool that is depolymerized by AVP; this is the region of the cell in which the aggrephores are positioned and fuse.

However, the resolution of the confocal microscope is not good enough to distinguish between F-actin in the microvilli, and F-actin in the terminal web between and beneath the microvilli. It is the terminal web F-actin that is probably most critical in vesicle positioning and fusion. To make this distinction, we used immunogold electron microscopy, employing a specific antibody to actin [35]. Results in studies of the toad bladder are shown in Figs. 8–10. There was a striking decrease in immunogold labeling of actin between and below the microvilli, but no significant change in the microvillar actin. The reduction in actin labeling was greatest near the bases of the microvilli, where the majority of aggrephores fuse. Immunogold studies of the rat IMCD have also shown a significant reduction

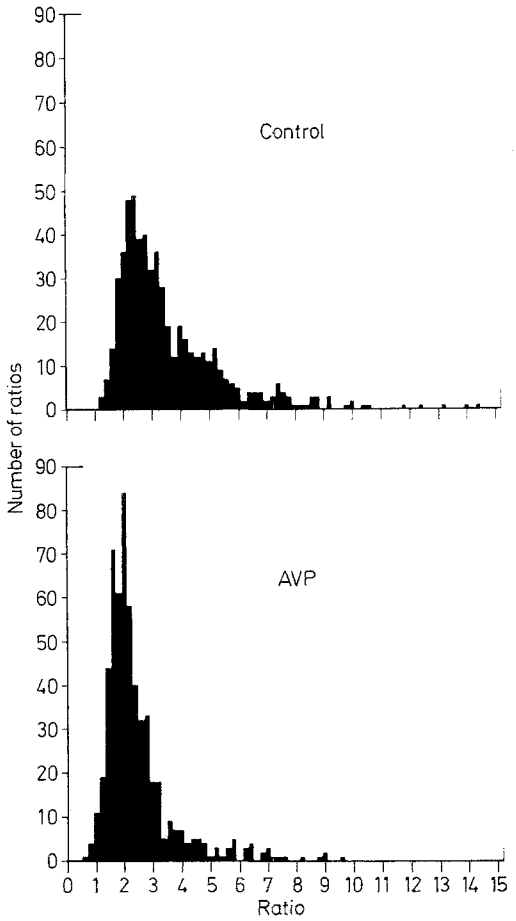
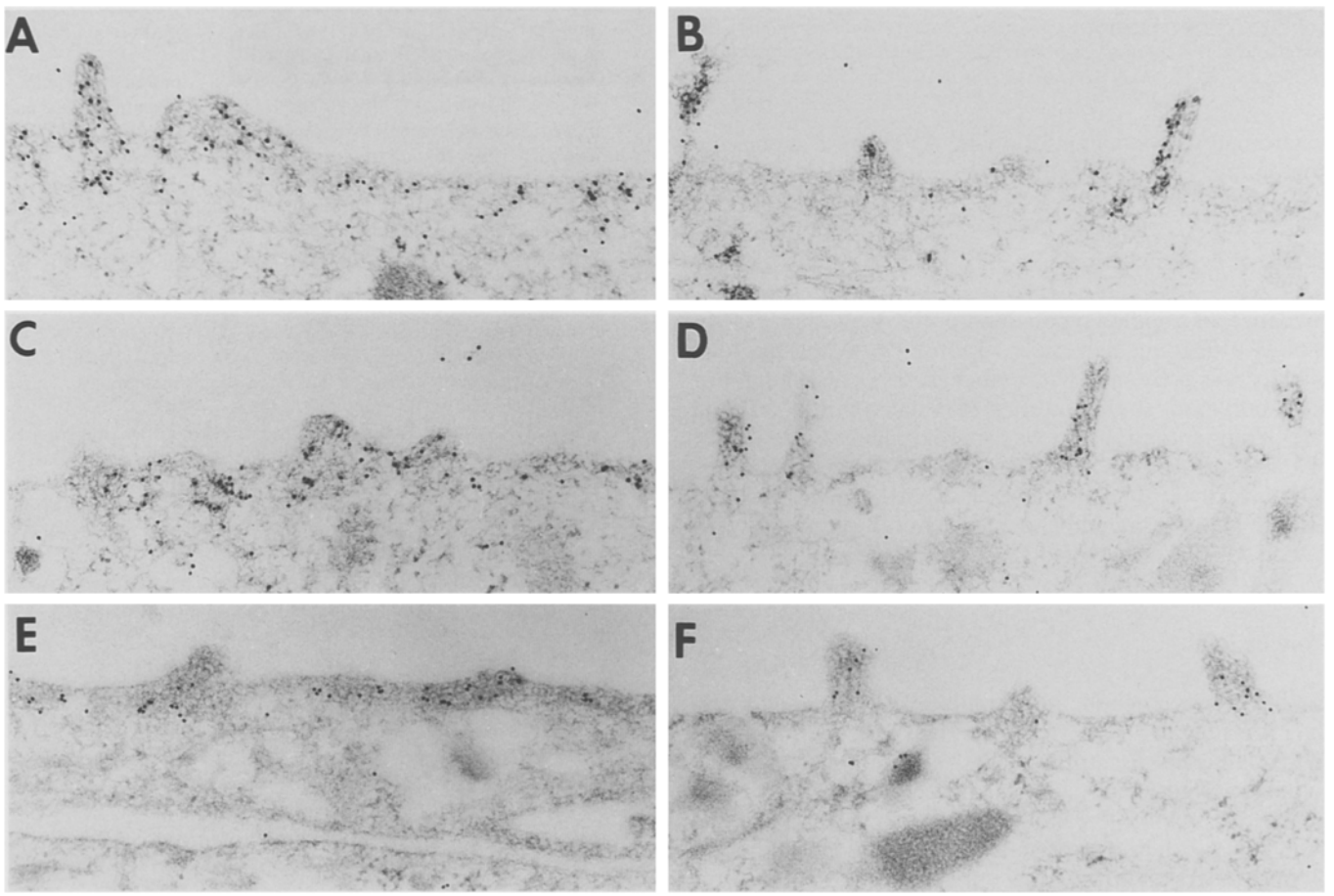


Fig. 7. Distribution of apex/side fluorescence ratios in six paired experiments carried out with the split chamber. From [34] with permission

Fig. 8. Immunogold staining of actin in the apical region of three paired experiments in the toad bladder. **A, C** and **E** are from control bladders; **B, D** and **F** are from bladders treated with AVP. There is a consistent reduction in gold particle density in the subapical cytoplasm between and beneath the microvilli following AVP. From [35] with permission



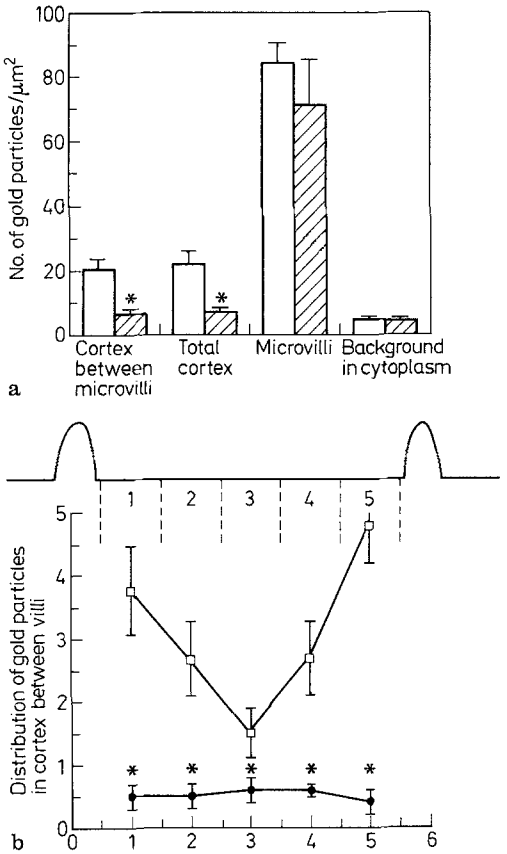


Fig. 9. Analysis of particle distribution in four paired experiments. **a** Distribution in cortex between the microvilli, the entire submicrovillar cortex, the microvilli themselves, and the more central cytoplasm. ■, Control; ▨ AVP; ✳, significant decrease. **b** Pattern of distribution of immunogold in five arbitrary segments between microvilli; □, control; ■, AVP

in terminal web labeling following AVP; these studies are continuing.

Effect of cytochalasin D

Evidence of another type supports the view that F-actin acts as a restraint to vesicle fusion [36]. When the toad bladder was pretreated with cytochalasin D, an inhibitor of actin polymerization, and then stimulated with AVP, the rate of aggrephore fusion was approximately twice that of bladders treated with AVP alone (AVP 4.9 ± 0.9, AVP plus cytochalasin D 8.5 ± 1.7 fusion events/ μm^2; $P < 0.05$). Thus, by interfering with the integrity of the actin network, cytochalasin D accelerated aggrephore fusion.

Conclusion

We have shown in both the amphibian urinary bladder and the rat IMCD that AVP depolymerizes F-actin, and that it is the subapical F-actin pool that is depolymerized. Our findings suggest that, as in other secretory cells, vesicle fusion requires the breakdown and reorganization of the actin network. These experiments mark only the beginning of our understanding of the effects of AVP on the cytoskeleton; there is good evidence for an important role of microtubules in vesicle fusion [37], and it is entirely possible that molecular "motors", such as kinesin and dynein [38], as well as other elements of the cytoskeleton, participate in the response to AVP.

References

1. Orloff J, Handler JS (1962) The similarity of effects of vasopressin, adenosine 3′, 5′-phosphate (cyclic AMP) and theophylline on the toad bladder. J Clin Invest 41: 702–709
2. Hays RM (1991) Cell biology of vasopressin. In: Brenner BM, Rector FC (eds) The kidney. Saunders, Philadelphia, pp 424–444
3. Breyer MD (1991) Regulation of water and salt transport in collecting duct through calcium dependent signaling mechanisms. Am J Physiol 260: F1–F11
4. Hays RM (1990) Water transport in epithelia. In: Kinne R, Kinne-Saffran E (eds) Comparative physiology: basic principles in transport. Karger, Basel, pp 1–30
5. Bichet DG, Razi M, Lonergan M, Arthus MF, Papukna V, Kortas C, Barjon JN (1988) Hemodynamic and coagulation responses to 1-desamino [8-D-arginine] vasopressin in patients with congenital nephrogenic diabetes insipidus. N Engl J Med 318: 881–887
6. Knoers N, Ouweland A, Fahrenholz F, Monnens L, Oost B (1993) Progress in the approach to elucidate the genetic defect in nephrogenic diabetes insipidus. Pediatr Nephrol 7: 685–688
7. Schlondorff D, Weber H (1976) Cyclic nucleotide metabolism in compensatory renal hypertrophy and neonatal kidney growth. Proc Natl Acad Sci USA 73: 524
8. Imbert-Teboul M, Chabardes D, Clique A, Montegut M, Norel F (1984) Ontogenesis of hormone-dependent adenylate cyclase in isolated rat nephron segments. Am J Physiol 247: F316–F325
9. Hays RM, Franki N, Ding G (1987) Effects of antidiuretic hormone on the collecting duct. Kidney Int 31: 530–537
10. Chevalier J, Bourguet J, Hugon JS (1974) Membrane associated particles: distribution in frog urinary bladder epithelium at rest and after oxytocin treatment. Cell Tissue Res 152: 129–140
11. Wade JB (1985) Membrane structural studies of the action of vasopressin. Fed Proc 44: 2687–2692
12. Muller J, Kachadorian WA, DiScala VA (1980) Evidence that ADH-stimulated intramembranous particle aggregates are transferred from cytoplasmic to luminal membranes in toad bladder epithelial cells. J Cell Biol 85: 83–95
13. Hays RM (1983) Alteration of luminal membrane structure by antidiuretic hormone. Am J Physiol 245: C289–C296
14. Humbert F, Montesano R, Grosso A, DeSousa RC, Orci L (1977) Particle aggregates in plasma and intracellular membranes of toad bladder (granular cell). Experientia 33: 1364–1367
15. Masur SK, Cooper S, Rubin MS (1984) Effect of an osmotic gradient on antidiuretic hormone induced endocytosis and hydroosmosis in the toad urinary bladder. Am J Physiol 247: F370–F379
16. Ding G, Franki N, Hays RM (1985) Evidence for cycling of aggregate-containing tubules in toad urinary bladder. Biol Cell 55: 213–218
17. Harris HW Jr, Wade JB, Handler JS (1986) Transepithelial water flow regulates apical membrane retrieval in ADH-stimulated toad urinary bladder. J Clin Invest 78: 703–712
18. Coleman RA, Harris HW Jr, Wade JB (1987) Visualization of endocytosed markers in freeze-fracture studies of toad urinary bladder. J Histochem Cytochem 35: 1405–1414
19. Lencer WI, Verkman AS, Arnaout MA, Ausiello DA, Brown D (1990) Endocytic vesicles from renal papilla which retrieve the vasopressin-sensitive water channels do not contain a functional H^+ ATPase. J Cell Biol 111: 379–389
20. Ding G, Franki N, Bourguet J, Hays RM (1988) The role of vesicular transport in ADH-stimulated aggregate delivery. Am J Physiol 255: C641–C652

21. Harmanci MC, Stern P, Kachadorian WA, Valtin H, DiScala VA (1980) Vasopressin and collecting duct intramembranous particle clusters: a dose-response relationship. Am J Physiol 239: F560–F564
22. Verkman AS, Lencer WI, Brown D, Ausiello DA (1988) Endosomes from kidney collecting tubule cells contain the vasopressin-sensitive water channel. Nature 333: 268–269
23. Siga E, Horster MF (1991) Regulation of osmotic water permeability during differentiation of inner medullary collecting duct. Am J Physiol 260: F710–F716
24. Burgoyne RD, Cheek TR (1987) Reorganization of peripheral actin filaments as a prelude to exocytosis. Biosci Rep 7: 281–288
25. Koffer A, Tatham PER, Gomperts BP (1990) Changes in the state of actin during the exocytotic reaction of permeabilized rat mast cells. J Cell Biol 111: 919–927
26. Perrin D, Moller K, Hanke K, Soling H-D (1992) cAMP and Ca^{2+}-mediated secretion in parotid acinar cells is associated with reversible changes in the organization of the cytoskeleton. J Cell Biol 116: 127–134
27. Orci L, Gabbay KH, Malaisse WJ (1972) Pancreatic beta-cell web: its possible role in insulin secretion. Science 175: 1128–1130
28. Goodman SR, Krebs KE, Whitfield CF, Riederer BM, Zagor IS (1988) Spectrin and related molecules. CRC Crit Rev Biochem 23: 171–234
29. Condeelis J, Hall AL (1991) Measurement of actin polymerization and cross-linking in agonist-stimulated cells. Methods Enzymol 196: 487–496
30. Ding G, Franki N, Condeelis J, Hays RM (1991) Vasopressin depolymerizes F-actin in the toad bladder epithelial cell. Am J Physiol 260: C9–C16
31. Cheek TR, Bourgoyne RD (1986) Nicotine-evoked disassembly of cortical actin filaments in adrenal chromaffin cells. FEBS Lett 207: 110–114
32. Simon H, Gao Y, Franki N, Hays RM (1992) Vasopressin (AVP) depolymerizes apical actin in rat inner medullary collecting duct (IMCD) Am J Physiol, in press
33. Inoue S (1989) Foundations in confocal scanned imaging in light microscopy. In: Pawley J (ed) Handbook of biological confocal microscopy. IMR Press, Madison, pp 1–13
34. Holmgren K, Magnusson KE, Franki N, Hays RM (1992) ADH-induced depolymerization of F-actin in the toad bladder granular cell: a confocal microscope study. Am J Physiol 262: C672–C677
35. Gao Y, Franki N, Macaluso F, Hays RM (1992) Vasopressin decreases immunogold labeling of apical actin in the toad bladder granular cell. Am J Physiol 263: C908–C912
36. Franki N, Ding G, Gao Y, Hays RM (1992) The effect of cytochalasin D on the actin cytoskeleton of the toad bladder epithelial cell. Am J Physiol 263: C995–C1000
37. Taylor A, Mamelak M, Gelbetz H, Maffly R (1978) Evidence for involvement of microtubules in the action of vasopressin in toad urinary bladder. I. Functional studies on the effects of antimitotic agents on the response to vasopressin. J Membr Biol 40: 213–235
38. Sheetz MP (1987) What are the functions of kinesin? Bioessays 7: 165–168