Microfilament Network Is Needed for the Endocytosis of Water Channels and Not for Apical Membrane Insertion Upon Vasopressin Action (44481)

ADNAN DIBAS,*[,1] ABDUL MIA,† AND THOMAS YORIO‡

Mary Kay Ash Institute for Cancer Research, Dallas, Texas 75235; †Jarvis Christian College, Hawkins, Texas 75765; and ‡Department of Pharmacology, University of North Texas Health Science Center at Fort Worth, Fort Worth, Texas 76107

Abstract. **In the current study, a novel role for the microfilaments in vasopressin-induced water transport in toad urinary bladders, a popular model for the mammalian collecting duct, was established. Vasopressin-induced water transport was not affected by cytochalasin D (CD, 20 μ*M*) or latrunculin B (Lat B, 0.5–2 μ*M*), microfilament-disrupting reagents, suggesting that the initial trafficking of vesicles containing water channels and insertion of membranes into the apical membrane are microfilament-independent. After the removal of vasopressin, bladders treated with CD or Lat B continued to transport water at least 2–3-fold greater than those that received the vehicle. Furthermore, the enhanced water transport was inhibited by $HgCl_2$ (1 m*M*), a potent inhibitor of water channel–mediated water flow, suggesting that the enhanced water flow was through water channels. In addition, Lat B and CD inhibited vasopressin-induced endocytosis of horseradish peroxidase (HRP), a fluid endocytotic marker. These results suggested that although microfilaments are not needed for the initial trafficking of water channels to the apical side, the microfilament network is essential for the retrieval of water channels following their insertion into apical membranes.**
[P.S.E.B.M. 2000, Vol 223]

The discovery of selective water channels or aquaporins was a breakthrough in the understanding of how vasopressin activates water transport in the renal collecting duct (1). To date, there are at least nine mammalian aquaporins (2). Aquaporin-2 (AQP-2) is a unique channel that is absolutely restricted to the mammalian collecting duct (3–5), is vasopressin-sensitive, and shows translocation to the apical site upon vasopressin binding to its receptor on the basolateral membranes (6). The vasopressin receptor, or V_2 receptor, is coupled to adenylyl cyclase, and vasopressin binding activates adenylyl cyclase, produces cyclic AMP (cAMP), and activates cAMP-dependent protein kinase A (PKA) (6). The mutation of the phosphorylation site for PKA in AQP-2 prevents the translocation of AQP-2 to the apical site (7). However, water transport in isolated endosomes is not affected by the addition of purified PKA (8). Therefore, the phosphorylation of AQP-2 is needed for the translocation of the water channel rather than as part of the activation of the channel for water flow (8). However, the steps between the generation of cAMP and the shuttling of AQP-2 to the apical site are not well understood with the exception of the role of microtubules in the process (9–13).

In addition, although microfilaments have been suggested to play a role in most vesicular trafficking (14), a role in water transport has not been fully characterized. Nevertheless, a role for the microfilament involvement in vasopressin-induced water transport has been made based on the depolymerization of F-actin to G-actin by cAMP and vaso-

This work was supported by a grant from the U.S. Army (DAMD. 17–95–5086). The views and opinions and/or findings contained in this report are those of the authors and should not be construed as an official Department of Army position, policy, or decision unless so designated by other documentation.

[1] To whom requests for reprints should be addressed at Mary Kay Ash Institute for Cancer Research, 1931 Chattanooga Place, Dallas, TX 75235. E-mail: marykayinstitute@yahoo.com

Received June 11, 1999. [P.S.E.B.M. 2000, Vol 223]
Accepted September 14, 1999.

0037-9727/00/2232-0203$14.00/0

pressin in toad urinary bladders, a popular model for the mammalian collecting duct (15–19). By contrast, Franki *et al.* (18) have shown that cytochalasin D has no effect on vasopressin-activated water transport and have suggested that microfilaments are not needed for water transport in toad urinary bladders. In the present study, a role for the microfilament is suggested; however, not in the initial shuttling of water channels to the apical membranes, but rather in the retrieval or endocytosis of water channels after their insertion into the apical membranes. The disruption of microfilaments in the presence of vasopressin failed to affect water transport. However, after the removal of vasopressin, water flow was sustained at an equivalent rate to that in the absence of vasopressin.

Materials and Methods

Materials. Cytochalasin D and horseradish peroxides were purchased from Sigma Chemical Co. (St. Louis, MO). Latrunculin B was from Calbiochem (San Diego, CA). Mexican *Bufo marinus* toads were from Wards Science Establishment Inc. (Rochester, NY).

Water Transport Studies. Urinary hemibladders, excised from doubly pithed toads, *Bufo marinus*, were set up as sacs and incubated in Ringer solution (111 m*M* NaCl, 3.33 m*M* KCl, 2.7 m*M* $CaCl_2$, 4.16 m*M* $NaHCO_3$, and 5.5 m*M* glucose). A 1:10 osmotic gradient was established between the mucosal (also known as urine or apical) and serosal (also known as blood or basolateral) solutions. Cytochalasin D was added apically (not as potent as latrunculin B in disrupting microfilaments) whereas latrunculin B and vasopressin (15 mU/ml) were added to the serosal bathing solution. Water transport was measured gravimetrically according to Bentley *et al.* (20). Control hemibladders received only vehicle (DMSO, concentration was 0.1% in all experiments). The inside of the bladder sac received 1/10 Ringer during water transport. The outside of the bladder was always exposed to N-Ringer. As vasopressin was added to the serosal side, it stimulated the translocation and insertion of water channels into the mucosal side to carry out the entry of water. As water entered intracellularly, constitutive water channels at the basolateral side allowed water out of the cells. Therefore, vasopressin induced a continuous loss or movement of water from the inside of the bladder sac to its outside, and the difference in the weight of the bladder sac before and after the addition of vasopressin represented the amount of water that was transported. Water transport activity was expressed as mg/30 min.

Endocytosis of Horseradish Peroxidase. Endocytosis of horseradish peroxidase (HRP) was performed as described previously (21). Urinary bladder sacs were set up as sacs, and one sac received DMSO whereas the other received CD (20 μ*M*) or Lat B (0.5–2 μ*M*) for 30 min prior to stimulation with vasopressin. Toad urinary bladders were stimulated with vasopressin in the absence of an osmotic gradient for 15 min. The mucosal side was then replaced with 1/10 Ringer containing HRP (10 mg/ml, Sigma). This was followed by washing the serosal surface of the bladder three times to remove vasopressin. After 15–30 min incubation with HRP, the mucosal solution was washed three times in ice-cold Ringer, and tissues were fixed. For tissue fixation, the whole bladder sac was removed from the end of a glass tube, submerged into 2% glutaraldehyde, and fixed for 1 hr. Following buffer rinses, post-fixation was carried out using 1% osmium tetroxide at room temperature. Tissue processing for Transmission Electron Microscopy (TEM) was carried out as described previously (21). Bladder sacs were rinsed, minced into small pieces, and then processed for embedding in epon for ultrathin sectioning. Tissue blocks were polymerized overnight in an oven at 60°C. Ultrathin sections made with a diamond knife were collected on bare copper or nickel grids. To quantitate endocytosis, each condition was run at least 3–5 times using intact bladders. Quantitative analysis was made to compare the relative distribution of HRP-loaded endosomes in vasopressin-stimulated versus control, cytochalasin D, and latrunculin B sacs using ultrathin TEM sections. Over 25–46 pictures for each condition (vasopressin, control, cytochalasin D, and latrunculin B) were taken from several epon-embedded tissue blocks originated from at least three to five separate experiments. TEM pictures were taken at X84,000, and the results were plotted into histograms. HRP-loaded endosomes were counted, and results were expressed as number of HRP-loaded endosomes/100 μM^2 membrane.

Statistical Treatment of Data. Statistical significance was assessed by using one-way ANOVA and the Tukey multiple comparison test at $P < 0.05$.

Results

Cytochalasin D (20 μ*M*) Treatment Had No Effect on Vasopressin-Induced Water Transport from Toad Urinary Bladders. Control bladders (receiving DMSO) or experimental bladders (receiving cytochalasin D) were treated for 30 min with drugs or vehicle. Cytochalasin D had no effect on basal water transport. Water loss from control bladders in the absence of vasopressin was 13 ± 5 mg/30 min in control bladders and 18 ± 2 mg/30 min in cytochalasin D-treated bladders (data not shown). Following a stimulation with vasopressin (15 mU/ml) of both bladders, water transport was followed for 60 min. At 30 min, water loss from control bladders was 514 ± 12 mg/30 min in control bladders compared with 512 ± 53 mg/30 min in cytochalasin D-treated bladders (Fig. 1A). At 60 min, water loss from control bladders was 1017 ± 106 mg/60 min compared with 1056 ± 108 mg/60 min in cytochalasin D-treated bladders (Fig. 1A). Therefore, cytochalasin D appears to lack any inhibitory effects on water transport, suggesting that microfilaments are not needed for the initial translocation and trafficking of vesicles containing water channels to the apical side.

Enhanced Water Transport After Vasopressin-Washout in Cytochalasin D-Treated Toad Urinary Bladders. We followed water transport further after the

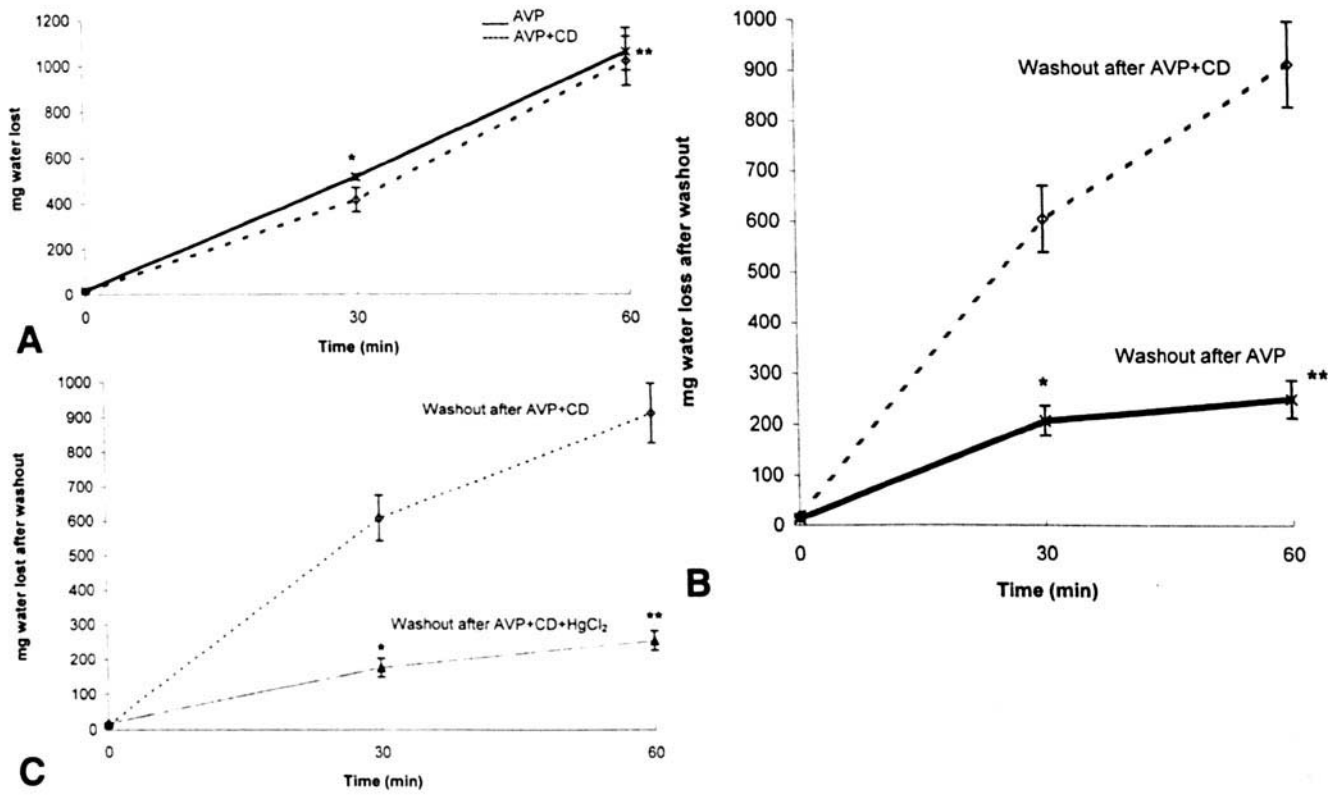

Figure 1. **(A)** Cytochalasin D (20 μ*M*) treatment had no effect on vasopressin-induced water transport from toad urinary bladders. Toad urinary bladders were treated with vehicle (DMSO, ——) or cytochalasin D (CD, 20 μ*M*, ----) for 30 min prior to the addition of vasopressin (AVP, 15 mU/ml). As shown in Figure 1A, cytochalasin D did not inhibit vasopressin-activated water transport. * and **AVP were not statistically significant from AVP/CD. **(B)** Cytochalasin D (20 μ*M*)-treated bladders continued to transport water in the absence of vasopressin at a higher rate than DMSO-treated bladders. Toad urinary bladders that received DMSO (——) or CD (----) prior to the addition of vasopressin, were washed extensively, and water transport was measured in the absence of vasopressin. As illustrated in Figure 1B, bladders receiving CD had an enhanced and sustained water transport compared with DMSO-treated bladders. Water loss in control bladders was 207 ± 29 mg/30 min whereas water loss in CD-treated bladders was 607 ± 66 mg/30 min ($P < 0.0005$ vs. control). Furthermore, at 60 min, water loss from control bladders was 250 ± 37 mg/60 min whereas water loss in CD-treated bladders was 914 ± 85 mg/60 min ($P < 0.0001$ vs. control). **(C)** $HgCl_2$ (1 m*M*) added apically, inhibited the enhanced and sustained water transport induced by cytochalasin D (20 μ*M*)-treated bladders. Toad urinary bladders were treated with CD (20 μ*M*) as described in Methods prior to the addition of vasopressin. Next, bladders were washed extensively, and water transport was measured for 60 min in CD-treated (----) and in CD-treated + $HgCl_2$ (——, added apically where aquaporins fuse to transport water). $HgCl_2$ inhibited the sustained water transport induced by CD by 71%. Water transport induced by CD (607 ± 66 mg/30 min) was attenuated by $HgCl_2$ (water loss was 176 ± 26 mg/30 min, $P < 0.008$ vs. CD alone). At 60 min, water loss in CD-treated bladders was 914 ± 85 mg/60 min compared with 256 ± 29 mg/60 min in $HgCl_2$-treated bladders ($P < 0.008$ vs CD alone).

removal of vasopressin. Both control bladders (receiving DMSO then vasopressin) and experimental bladders (receiving cytochalasin D then vasopressin) were washed extensively and allowed to sit for 60 min during which water transport was followed every 30 min. As shown in Figure 1B, water loss in control bladders was 207 ± 29 mg/30 min whereas water loss in cytochalasin D-treated bladders was much higher (607 ± 66 mg/30 min, $P < 0.0005$ vs control). Furthermore, at 60 min, water loss from control bladders was 250 ± 37 mg/60 min whereas water loss in cytochalasin D-treated bladders was 914 ± 85 mg/60 min ($P < 0.0001$ vs control). Therefore, at 30 min, although vasopressin-activated water transport was nearly the same in control (DMSO-treated) as in CD-treated bladders, water transport in CD-treated bladders after the removal of vasopressin was 293% that of the control bladders. In addition, at 60 min, no difference was observed in water transport in the presence of vasopressin. However, water transport in CD-treated bladders after the removal of vasopressin was 366% that of control bladders.

Enhanced Water Transport Induced by Cytochalasin D Was Attenuated by $HgCl_2$, a Potent Water Channel Inhibitor. To verify that the enhanced and sustained water flow was in fact due to water channels remaining at the apical site that were not retrieved, we tested the effect of $HgCl_2$ (1 m*M*, a potent inhibitor of water channel fluid transport), by adding it to the apical site, the site of water channels insertion. As shown in Figure 1C, the enhanced water transport induced by CD (607 ± 66 mg/30 min) was attenuated by $HgCl_2$ (water loss was 176 ± 26 mg/30 min, $P < 0.008$ vs CD alone). At 60 min, water loss in CD-treated bladders was 914 ± 85 mg/60 min compared with 256 ± 29 mg/60 min in $HgCl_2$-treated bladders ($P < 0.008$ vs CD alone). $HgCl_2$ induced a 71% inhibition in water flow at 30 min and 60 min. This suggested that the enhanced water transport was indeed through water channels.

Latrunculin B, a Microfilament Disrupting Reagent, Mimicked Cytochalasin D Effects on Vasopressin-Activated Water Transport. To confirm further the role of microfilaments in the retrieval of water channels, latrunculin B, another microfilament disrupting reagent, was tested on vasopressin-activated water transport in another set of animals. Similar to cytochalasin D, latrunculin B (0.5–2 μM) had no effect on vasopressin-induced water transport. Water loss in control bladders was 691 ± 42 mg/30 min compared with 654 ± 36 mg/30 min in latrunculin B-treated bladders (Fig. 2A). At 60 min, water loss in control bladders was 1300 ± 95 mg/60 min compared with 1175 ± 61 mg/60 min (Fig. 2A). After the wash of vasopressin, water transport in latrunculin B-treated bladders was sustained at a higher rate than control bladders. Water loss in control bladders was 235 ± 61 mg/30 min compared with 575 ± 51 mg/30 min in latrunculin B-treated bladders, which corresponded to 245% of control transport (Fig. 2B) ($P < 0.003$ vs control). At 60 min, water loss in control bladders was 339 ± 25 mg/60 min compared with 789 ± 94 mg/60 min, which corresponded to 233% of control transport (Fig. 2B) ($P < 0.002$ vs control).

In addition, $HgCl_2$ (1 mM) attenuated water transport in latrunculin B-treated bladders (Fig. 2C). Water loss in latrunculin B-treated bladders was 575 ± 51 mg/30 min compared with 217 ± 13 mg/30 min in $HgCl_2$-treated bladders (Fig. 2C) ($P < 0.0001$ vs latrunculin B alone). Also, water loss in control bladders was 789 ± 94 mg/60 min compared with 275 ± 17 mg/60 min in $HgCl_2$-treated bladders (Fig. 2C) ($P < 0.0001$ vs latrunculin B alone). $HgCl_2$ induced 62% and 65% inhibition at 30 and 60 min, respectively. The results are summarized in Table I.

Latrunculin B and Cytochalasin D Inhibited Vasopressin-Induced Endocytosis of HRP, a Fluid Endocytotic Marker. Data presented earlier suggested that microfilament-disrupting reagents inhibit endocytosis; therefore, the effects of latrunculin B and cytochalasin D on vasopressin-induced endocytosis of HRP were investigated. As illustrated in Figure 3B, latrunculin B attenuated vasopressin-induced endocytosis of HRP compared with vasopressin alone (Fig. 3A). A similar observation was observed with cytochalasin D (data not shown). Vasopressin-induced labeled endosomes were 14.65 ± 3.1/100 μM^2 compared with 3.5 ± 0.34/100 μM^2 in control, 3 ± 0.22/100 μM^2 in vasopressin/cytochalasin D, and 3.4 ± 0.33/100 μM^2 in vasopressin/latrunculin B (Table II). In fact, HRP appeared as dense clouds over the plasma membrane along the microvilli in latrunculin B-treated bladders as well as in cytochalasin D-treated bladders (data not shown). Vasopressin induced a 4.2-fold increase in the uptake of HRP compared with control bladders; an effect was abolished by both cytochalasin D and latrunculin B. Therefore, both microfilament-disrupting reagents inhibited vasopressin-enhanced apical endocytosis of HRP.

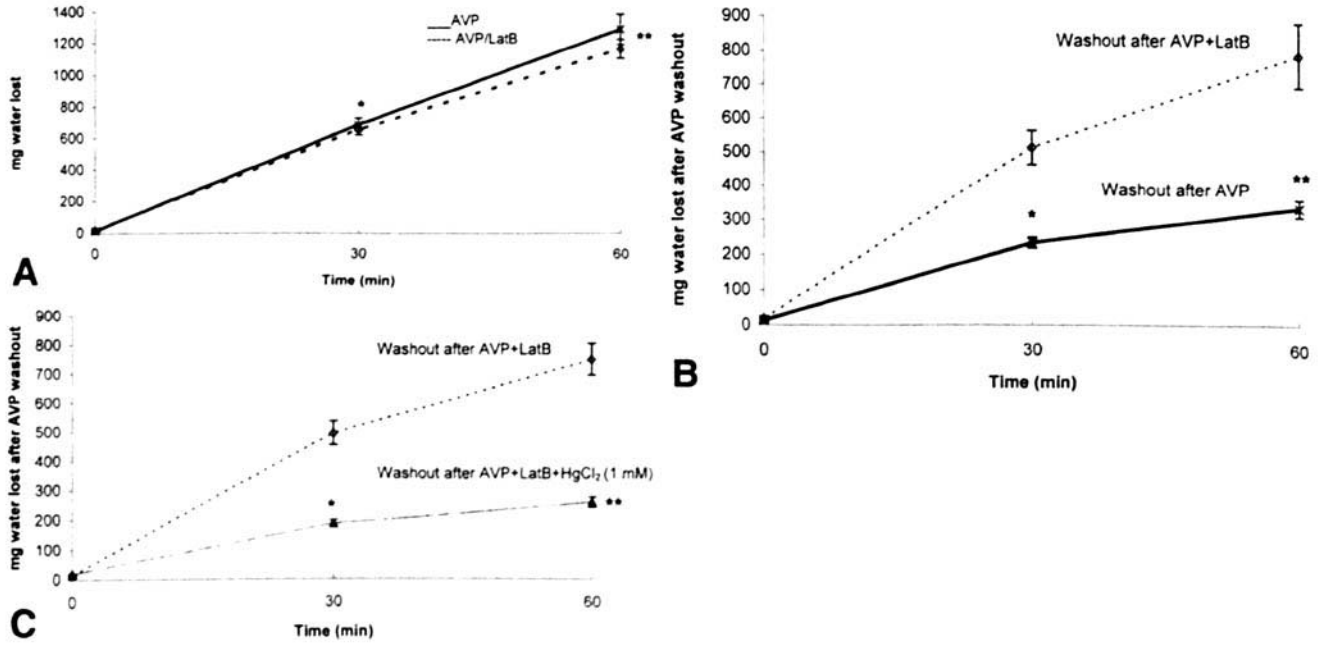

Figure 2. (A) Latrunculin B (0.5–2 μM) treatment had no effect on vasopressin-induced water transport from toad urinary bladders. Toad urinary bladders treated with vehicle (DMSO, ——) or latrunculin B (Lat B, 0.5–2 μM, ----) for 30 min prior to the addition of vasopressin (AVP, 15 mU/mL). As shown in Figure 2A, latrunculin B did not inhibit vasopressin-activated water transport. * and **AVP were not statistically significant from AVP/Lat B. **(B)** Latrunculin B (0.5–2 μM)-treated bladders continued to transport water in the absence of vasopressin at a higher rate than DMSO-treated bladders. Toad urinary bladders that received DMSO (——) or Lat B (----) prior to the addition of vasopressin, were washed extensively, and water transport was measured in the absence of vasopressin. As illustrated in Figure 2B, bladders receiving Lat B had an enhanced and sustained water transport compared with DMSO-treated bladders. **(C)** $HgCl_2$ (1 mM) added apically, inhibited the enhanced and sustained water transport induced by latrunculin B (0.5–2 μM)-treated bladders. Toad urinary bladders were treated with Lat B as described in Methods. Next, bladders were washed extensively, and water transport was measured for 60 min in Lat B-treated bladders (----) and in Lat B-treated + $HgCl_2$ (——, added apically where aquaporins fuse to transport water). $HgCl_2$ attenuated the sustained water transport induced by Lat B.

Table I. Effects of Cytochalasin D and Latrunculin B on Vasopressin-Induced Water Transport from Toad Urinary Bladders

Condition	Water loss (mg/30 min)	Water loss (mg/60 min)	Water loss (mg/30 min) after AVP removal	Water loss (mg/60 min) after AVP removal	Water loss (mg/30 min) after AVP removal/$HgCl_2$	Water loss (mg/60 min) after AVP removal/$HgCl_2$
AVP1 + DMSO	514 ± 12	1017 ± 106	207 ± 29	250 ± 37	–	–
AVP1 + CD	512 ± 53	1056 ± 108	607 ± 66	914 ± 85	176 ± 26	256 ± 29
AVP2 + DMSO	691 ± 42	1300 ± 95	235 ± 61	339 ± 25	–	–
AVP2 + Lat B	659 ± 36	1175 ± 61	575 ± 51	789 ± 94	217 ± 13	273 ± 17

Table II. Quantative Effects of Latrunculin B and Cytochlalasin D on Vasopressin-Induced Endocytosis of HRP

Treatment	No. of labeled endosomes/100 μM^2
Control	3.5 ± 0.34[a]
Vasopressin	14.65 ± 3.1
Vasopressin + latrunculin B	3.4 ± 0.33[a]
Vasopressin + cytochalasin D	3 ± 0.22[a]

[a] Indicates statistical significance versus vasopressin ($p < 0.001$)

Discussion

The current study identified a novel role for microfilaments in vasopressin-activated water transport in toad urinary bladders, a popular model for the mammalian collecting duct (22). This finding is important and will greatly improve our understanding of water transport mechanisms. The elucidation of the signal transduction pathway of vasopressin-induced water transport in toad urinary bladders and the identification of the elements involved in such a pathway may be helpful in further characterizing vasopressin-activated water transport in the mammalian collecting duct. Initial observations in toad urinary bladders were subsequently reproduced in mammalian systems (23). Franki *et al.* (18) excluded the importance of microfilaments in vasopressin-induced water transport due to the lack of inhibitory effects by CD and CB on the induction of water flow. Our current findings agree with these previous results, but although we agree that microfilaments are not important in the initial trafficking of water vesicles containing water channels, the microfilament network is needed for the recycling or endocytosis of water channels.

Our findings are also in agreement with the study of Masters *et al.* (24) who reported a lack of inhibitory effect by cytochalasin D on vasopressin-activated water transport, but observed a sustained water transport after cytochalasin D-washout. However, the latter group did not investigate this further. The current study demonstrated that the sustained water transport was likely through inserted water channels, as such an activity was greatly attenuated by $HgCl_2$. However, at least three older studies have reported an inhibitory effect by cytochalasin D and B on vasopressin-activated water transport from toad urinary bladders (25–

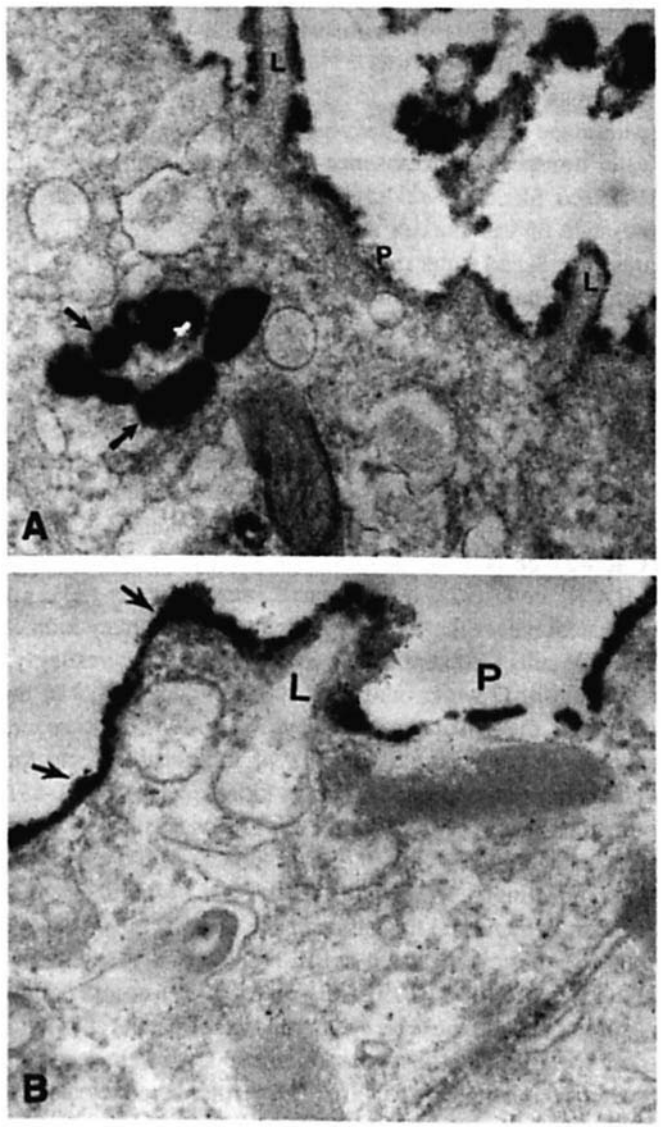

Figure 3. Vasopressin-induced endocytosis of horseradish peroxidase (HRP) was inhibited by latrunculin B. (Top) TEM of toad urinary bladder stimulated with vasopressin for 15 min and retrieved for 20 min showing the internalization of spherical to elongated endosomes (arrows) loaded with HRP through the mucosal plasma membrane (P) along the microvilli (L) in unstained section. 84,000x. (Bottom) TEM of toad urinary bladder pretreated with latrunculin B (1 μM, 15–30 min) followed by vasopressin for 15 min and retrieved for 20 min using HRP showing the inhibition of endocytosis in unstained section. HRP appears as dense clouds (arrows) over the plasma membrane (P) along the microvilli (L) at 84,000x.

27) with one study reporting only a 25% inhibition by cytochalasin D. The reason for this contradiction is not known. However, we have tested two different microfilament-disrupting reagents (cytochalasin D and latrunculin B), and both had no effect on vasopressin-induced water transport.

By contrast, marked differences in sustained water flow was observed in CD-treated vasopressin-stimulated bladders as compared with vasopressin alone. Water transport at 30 min after the removal of vasopressin was almost 40% of that in the presence of vasopressin. However, in CD-treated bladders, water transport in the absence of vasopressin was 119% of that in the presence of vasopressin. Similarly at 60 min, water transport in control bladders was 25% of that in the presence of vasopressin, whereas in CD-treated bladders, water transport was 87% of that in the presence of vasopressin. Therefore, the majority of water flow was maintained after the disruption of microfilaments. Similarly, water transport in the absence of vasopressin in latrunculin B-treated bladders was maintained at 87% of that in the presence of vasopressin at 30 min and was 67% of vasopressin-induced water transport at 60 min. Like we observed with cytochalasin D, latrunculin B maintained the majority of water transport. The disruption of the microfilament network apparently impaired the retrieval of water channels and therefore, produced a sustained water transport activity after the removal of vasopressin. Our results are also in agreement with several reports showing that cytochalasin inhibited endocytosis in bovine aortic endothelial cells (28), opossum kidney cells (OK) (29), human adenocarcinoma (Caco-2) cells (30, 31), Madin-Darby canine kidney (MDCK) cells (32), human small intestine cells (33), rat peritoneal macrophages (34), African green monkey kidney (VERO) cells (35), and Chang liver cells (36). Interestingly, cytochalasin D while inhibiting apical endocytosis, had no effect on the basolateral endocytosis (30–32). However, several studies reported the lack of inhibitory effects for cytochalasin on endocytosis in A6 kidney cells (37), A-431 human carcinoma (38), hepatocytes (39), and rat peritoneal macrophages (40, 41).

Interestingly, Verry *et al.* (37), have shown that cytochalasin D increased the basal uptake of HRP in A6 kidney cells and that cytochalasin D had no effect on vasopressin-induced uptake of HRP. The reason for such a contradiction is not known but could be due to the different cell types with some specificity in the regulation of endocytosis in each cell type. For example, PKC inhibitors attenuated caveolae-dependent endocytosis in MDCK cells suggesting a positive role for PKC in the caveolae-dependent endocytotic pathway (42). By contrast, PKC activators inhibited caveolae-mediated endocytosis in MA104 kidney cells (43) which further suggests the presence of distinct mechanisms regulating endocytosis based on cell type. In conclusion, the present study identified an important step in the signal transduction pathway of vasopressin-induced water transport from toad urinary bladders suggesting that microfilaments play an important role in water channel recycling of vasopressin-sensitive water channels.

1. Knepper MA, Wade JB, Terris J, Ecelbarger CA, Marples D, Mandon B, Chou CL, Kishore BK, Nielsen S. Renal aquaporins. Kidney Int **49**:1712–1717, 1996.
2. Dibas A, Mia AJ, Yorio T. Aquaporins (water channels): Role in vasopressin-activated water transport. Proc Soc Exp Biol Med **219**:183–199, 1998.
3. Nielsen S, Digiovanni SR, Christensen EI, Knepper MA, Harris HW. Cellular and subcellular immunolocalization of vasopressin-regulated water channel in rat kidney. Proc Natl Acad Sci U S A **90**:11663–11667, 1993.
4. Fushimi K, Uchida S, Hara Y, Hirata Y, Marumo F, Sasaki S. Cloning and expression of apical membrane water channel of rat collecting tubule. Nature **361**:549–552, 1993.
5. Fushimi K, Sasaki S, Yamamoto T, Hayashi M, Furukawa T, Uchida S, Kuwahara M, Ishibashi K, Kihara I, Marumo F. Functional characterization and cell immunolocalization of AQP-CD water channel in kidney collecting duct. Am J Physiol **267**:F573–F582, 1994.
6. Nonguchi H, Owada A, Kobayashi N, Masanobu M, Terada Y, Kioke J, Ujiie K, Marumo F, Sakai T, Tomita K. Immunohistochemical localization of V_2 vasopressin receptor along the nephron and functional role of luminal receptor in terminal inner medullary collecting ducts. J Clin Invest **96**:1768–1778, 1995.
7. Katsura T, Gustafan CE, Ausielo DA, Brown D. Protein kinase A phosphorylation is involved in regulated exocytosis of aquaporin-2 in transfected LLC-PK_1 cells. Am J Physiol **272**:F816–F822, 1997.
8. Lande MB, Jo I, Zeidel ML, Somers M, Harris WH. Phosphorylation of aquaporin-2 does not alter the membrane water permeability of rat papillary water channel–containing vesicles. J Biol Chem **271**:5552–5557, 1996.
9. Philips ME, Taylor A. Effect of colcemid on the water permeability response to vasopressin in isolated perfused rabbit collecting tubules. J Physiol **456**:591–608, 1992.
10. Reavon E, Maffly R, Taylor A. Evidence for the involvement of microtubules in the action of vasopressin in toad urinary bladder. J Membr Biol **40**:250–267, 1978.
11. Marples D, Barber B, Taylor A. Effect of dynein inhibitor on vasopressin action in toad urinary bladder. J Physiol **490**:767–774, 1996.
12. Bourguet J, Hugon JS, Svelto M. ADH-induced water permeability: What role for the microtubular network? Comp Biochem Physiol **90**:669–672, 1988.
13. Wilson L, Taylor A. Evidence for the involvement of microtubules in the action of vasopressin in toad urinary bladder. J Membr Biol **40**:237–250, 1978.
14. Harper JF. Stimulus-secretion coupling: Second messenger-regulated exocytosis. Adv Second Messenger Phosphoprotein Res **22**:193–318, 1988.
15. Hays RM, Lindberg U. Actin depolymerization in the cyclic AMP-stimulated toad bladder epithelial cells, determined by the DNAse method. FEBS Lett **280**:397–399, 1991.
16. Gao Y, Franki N, Macaluso F, Hays R. Vasopressin decreases immunogold labeling of apical actin in the toad bladder granular cell. Am J Physiol **263**:C908–C912, 1992.
17. Gao Y, Franki N, Condeelis J, Hays RM. Vasopressin depolymerizes F-actin in toad bladder epithelial cells. Am J Physiol **260**:C9–C16, 1991.
18. Franki N, Ding G, Gao Y, Hays RM. Effect of cytochalasin D on the actin cytoskeleton of toad bladder epithelial cell. Am J Physiol **263**:C995–C1000, 1992.
19. Hardy MA, DiBona DR. Microfilaments and the hydroosmotic action of vasopressin in toad urinary bladder. Am J Physiol **243**:C200–C204, 1982.
20. Bentley PJ. The effects of neurohypophysial extracts on water transfer

across the wall of isolated urinary bladder of the toad *Bufo marinus*. J Endocrinol **17**:201–209, 1958.
21. Mia AJ, Oakford LX, Yorio T. Surface remodeling following removal of vasopressin in toad urinary bladder. Tissue Cell **26**:189–201, 1994.
22. Kachadorian WA, Levine SD, Wade JD, Discala VA, Hays RM. Relationship of aggregated intramembranous particles to water permeability in vasopressin-treated urinary bladders. J Clin Invest **59**:576–581, 1977.
23. Harmanci MC, Kachadorian WA, Valtin H, DiSacala VA. Antidiuretic hormone-induced intramembranous alterations in mammalian collecting duct. Am J Physiol **235**:F440–F443, 1978.
24. Masters BR, Fanestil DD. Metabolic dependence of the offset of antidiuretic hormone-induced osmotic flow of water across toad urinary bladder. J Membr Biol **48**:237–247, 1979.
25. Pearl M, Taylor A. Actin filaments and vasopressin-stimulated water flow in toad urinary bladder. Am J Physiol **245**:C20–C39, 1983.
26. Davis WL, Goodman DBP, Jones RG, Rasmussen H. The effects of cytochalasin B on the surface morphology of the toad urinary bladder epithelium: A scanning electron microscopic study. Tissue Cell **10**:451–462, 1978.
27. Grosso A, Spinelli F, De Sousa RC. Cytochalasin B and water transport. Cell Tissue Res **188**:375–388, 1978.
28. Alexander JS, Jackson SA, Chaney E, Kevil CG, Haselton FR. The role of cadherin endocytosis in endothelial barrier regulation: Involvement of protein kinase C and actin-cadherin interactions. Inflamm **22**:419–433, 1998.
29. Gekle M, Mildenberger S, Freudinger R, Scwerdt G, Silbernagl S. Albumin endocytosis in OK cells: Dependence on actin and microtubules and regulation by protein kinases. Am J Physiol **272**:F668–F677, 1997.
30. Shurety W, Bright NA, Luzio JP. The effects of cytochalasin D and phorbol myristate on the apical endocytosis of ricin in polarized Caco-2 cells. J Cell Sci **109**:2927–2935, 1996.
31. Jackman MR, Shurety W, Ellis JA, Luzio JP. Inhibition of apical but not basolateral endocytosis of ricin and folate in Caco-2 cells by cytochalasin D. J Cell Sci **107**:2547–2556, 1994.
32. Gottlieb TA, Ivanov IE, Adesnik M, Sabatini DD. Actin microfilaments play a critical role in endocytosis at the apical but not the basolateral surface of polarized cells. J Cell Biol **120**:695–710, 1993.
33. Blok J, Scherven AA, Mulder-stapel AA, Ginsel LW, Deams WT. Endocytosis in absorptive cells of cultured human small intestine tissue: Effect of cytochalasin B and D. Cell Tissue Res **222**:113–126, 1982.
34. Pratten MK, Lloyd JB. Effects of temperature, metabolic inhibitors, and some other factors on fluid-phase adsorptive pinocytosis by rat peritoneal macrophages. Biochem J **180**:567–571, 1979.
35. Sandvig K, van Deurs B. Selective modulation of the endocytotic uptake of ricin and fluid phase markers without alteration in transferrin endocytosis. J Biol Chem **265**:6382–6388, 1990.
36. Wagner R, Rosenberg M, Estensen R. Endocytosis in Chang liver cells: Quantitation by sucrose-^{3}H uptake and inhibition by cytochalasin B. J Cell Biol **50**:804–817, 1971.
37. Verrey F, Groscurth P, Bolliger U. Cytoskeletal disruption in A6 kidney cells: Impact on endo/exocytosis and NaCl transport regulation by antidiuretic hormone. J Membr Biol **145**:193–204, 1995.
38. Haigler HT, McKanna JA, Cohen S. Rapid stimulation of pinocytosis in human carcinoma cells A-431 by epidermal growth factor. J Cell Biol **83**:80–90, 1979.
39. Klaus GG. Cytochalasin B dissociation of pinocytosis and phagocytosis by peritoneal macrophages. Exp Cell Res **79**:73–78, 1973.
40. Kyle ME, Nakae D, Sakaida I, Miccadei S, Farber JL. Endocytosis of superoxide dismutase is required for the enzyme to protect hepatocytes from the cytotoxicity of hydrogen peroxide. J Biol Chem **263**:3784–3789, 1988.
41. Piasek A, Thyberg J. Effects of colchicine on endocytosis of horseradish peroxidase by rat peritoneal macrophages. J Cell Sci **45**:59–71, 1980.
42. Patron RG, Joggerst B, Murer H. Regulated internalization of caveolae. J Cell Biol **127**:1199–1215, 1994.
43. Smart EJ, Foster DC, Ying YS, Kamen BA, Anderson RGW. Protein kinase C activators inhibit receptor-mediated potocytosis by preventing internalization of caveolae. J Cell Biol **124**:307–313, 1994.