PKCα regulates vasopressin-induced aquaporin-2 trafficking in mouse kidney collecting duct cells in vitro via altering microtubule assembly

Hong ZHAO1,*, Xi YAO2,*, Tao-xia WANG2, Wen-min JIN1, Qian-qian Ji1, Xiao YANG1, Qiu-hong DUAN3, Li-jun YAO2,*

1Department of Trauma Surgery, Tongji Hospital, Huazhong University of Science & Technology, Wuhan 430030, China; 2Department of Nephrology, Union Hospital, Huazhong University of Science & Technology, Wuhan 430030, China; 3Department of Biochemistry and Molecular Biology, Tongji Medical College, Huazhong University of Science & Technology, Wuhan 430022, China

Aim: Aquaporin-2 (AQP2) is a vasopressin-regulated water channel located in the collecting tubule and collecting duct cells of mammalian kidney. The aim of this study is to investigate whether PKCα plays a role in vasopressin-induced AQP2 trafficking in mouse inner medullary collecting duct 3 (mIMCD3) cells.

Methods: AQP2-mIMCD3 stable cell line was constructed by transfection of mouse inner medullary collecting duct 3 (mIMCD3) cells with AQP2-GFP construct. Then the cells were transfected with PKCα shRNA, PKCα A/25E, or PKCα scrambled shRNA. The expression levels of PKCα, AQP2, and phospho-S256-AQP2 were analyzed using Western blot. The interaction between AQP2 and PKCα was examined using immunoprecipitation. The distribution of AQP2 and microtubules was studied using immunocytochemistry. The AQP2 trafficking was examined using the biotinylation of surface membranes.

Results: Treatment of AQP2-mIMCD3 cells with 100 μmol/L of 1-desamino-8-D-arginine vasopressin (DdAVP) for 30 min stimulated the translocation of AQP2 from the cytoplasm to plasma membrane through influencing the microtubule assembly. Upregulation of active PKCα by transfection with PKCα A/25E plasmids resulted in de-polymerization of α-tubulin and redistributed AQP2 in the cytoplasm. Down-regulation of PKCα by PKCα shRNA partially inhibited DdAVP-stimulated AQP2 trafficking without altering α-tubulin distribution. Although 100 μmol/L of DdAVP increased AQP2 phosphorylation at serine 256, down-regulation of PKCα by PKCα shRNA did not influence DdAVP-induced AQP2 phosphorylation, suggesting that AQP2 phosphorylation at serine 256 was independent of PKCα. Moreover, PKCα did not physically interact with AQP2 in the presence or absence of DdAVP.

Conclusion: Our results suggested that PKCα regulates AQP2 trafficking induced by DdAVP via microtubule assembly.

Keywords: PKCα; 1-desamino-8-D-arginine vasopressin (DdAVP); aquaporin-2; microtubule; kidney; medullary collecting duct

Acta Pharmacologica Sinica (2012) 33: 230–236; doi: 10.1038/aps.2011.160; published online 2 Jan 2012

Introduction
Protein kinase C alpha (PKCα) is a member of the classic PKC family[1], which is widely expressed in the mammalian kidney[2–4] and contributes to various kidney functions, including substrate absorption and urine concentration[5, 6]. PKCα-mediated cytoskeleton remodeling results in endocytosis in epithelial cells, which increases the uptake of various substrates, such as NHE3 and albumin[7–9]. PKCα is localized in glomeruli, the intercalated cells of the cortical collecting duct, and the medullary-collecting duct in the mouse kidney[10]. Genetic knockout mice that lack PKCα exhibit decreased glomerular filtration rate, increased urinary output and lower urinary osmolality, accompanied by normal water intake and normal levels of the plasma antidiuretic hormone arginine vasopressin (AVP)[11]. These results strongly suggest that PKCα-mediated urine concentration primarily occurs within the medullary collecting duct.

Aquaporin-2 (AQP2) is a vasopressin-regulated water channel in the principal cells of the connecting tubule and the collecting duct in the kidney[10, 11]. AQP2 is stored in an intracellular compartment and plays an important role in the regulation of urine concentration[12–13]. AVP stimulates AQP2 translocation to the plasma membrane for the re-absorption of water. AQP2 is removed from the plasma membrane and returned to the intracellular compartment when the stimulation is terminated[14–18]. In addition, AVP also regulates AQP2 mRNA and protein levels, which mediates long-term regula-
Both short- and long-term regulation mechanisms are involved in the pathophysiology of AQP2-mediated urine concentration[23]. Although PKC is involved in angiotensin II-mediated AQP2 expression and trafficking[24], our previous study demonstrated that AQP2 expression is only slightly different between PKCα knockout mice and wild-type mice[25], which strongly suggests that the long-term AQP2 regulation mechanism does not contribute to PKCα-mediated urine concentration. AQP2 transportation is partially regulated by the cytoskeleton[26,27], and PKCα-mediated remodeling of the actin cytoskeleton is involved in constitutive albumin uptake in the renal proximal tubule[28]. However, the contribution of PKCα to AQP2 trafficking, the role of the cytoskeleton in PKCα-mediated AQP2 transportation and the mechanism of PKCα regulation of AQP2 translocation are less well understood.

We hypothesized that PKCα mediates AQP2 trafficking via cytoskeleton distribution because of the important role of PKCα in urine concentration and cytoskeleton remodeling. Therefore, this study revealed the crosstalk among PKCα, AVP and AQP2 in trafficking and cytoskeletal remodeling in mIMCD3 cell.

Materials and methods

Construts, antibodies, and reagents

The pEF-nero-PKCα A/25E vector was a gift from Dr Gottfried BAIER (Institute for Medical Biology and Human Genetics, University of Innsbruck, Innsbruck, Austria). The AQP2-GFP-pCMV6 construct was purchased from OriGene (Rockville, MD, USA). Lipofectamine plus and geneticin (G418) were purchased from Invitrogen (Shanghai, China). Protein A/G-agarose beads and primary antibodies against phosphorylated amino acids were purchased from Pierce (Beijing, China). Streptavidin-agarose beads were purchased from Bio-Rad (Shanghai, China). DAPI was purchased from Vector Burlingame (San Diego, CA, USA). Sulfo-Link NHS-LC-biotin and Streptavidin-agarose beads were purchased from Pierce (Beijing, China). Major apparatuses included a Mini-PROTEAN II Electrophoresis Cell (Bio-Rad, Shanghai, China) and a confocal laser-scanning microscope (Olympus FV500, Japan).

Cells and cell culture

Immortalized mouse inner medullary collecting duct 3 (mIMCD3) cells were kindly provided by Dr John M Luk (Department of Urology, University of Hong Kong, Hong Kong, China). mIMCD3 cells were maintained in Dulbecco’s modified Eagle’s medium/Ham’s F12 (1:1) (GIBCO, Invitrogen, Shanghai, China) supplemented with 10% fetal bovine serum (GIBCO, Invitrogen, Shanghai, China) and 2% penicillin-streptomycin (Amresco, Shanghai, China) in a humidified atmosphere with 5% CO₂ at 37°C.

Generation of pGCsi-U6/Neo PKCα shRNA plasmid

Three siRNAs were designed and synthesized by Invitrogen (Shanghai, China) according to the cDNA sequence of PKCα (NM_011101): #1, GTCTTCCAGTTCAAATTA; #2, GTGCATATGAAACCTCAAA; and #3, CCATCAAACACCTGGAACA. The siRNAs were cloned into the eukaryotic expression plasmid pGCsi-U6/Neo (Genechem, Shanghai, China). The constructed pGCsi-U6/Neo-PKCα-siRNA vector was transfected into mIMCD3 cells. Western blotting was used to evaluate the suppression of PKCα expression in different cell groups.

Transfection

Transfection of mIMCD3 cells with the AQP2-GFP construct was performed using Lipofectamine 2000 (Shanghai, China) according to the manufacturer’s instructions. The cells were maintained in medium containing 400 µg/mL geneticin for 24 h after transfection. Individual neomycin-resistant colonies were selected and expanded for 14 d after transfection.

AQP2-GFP was stably expressed in mIMCD3 cells (AQP2-mIMCD3 cells) that were transiently transfected with a eukaryotic expression vector encoding the constitutively activated form of PKCα A/25E (pEF-nero-PKCα A/25E)[26] using Lipofectamine 2000 according to the manufacturer’s instructions. AQP2-mIMCD3 cells were transfected with three pGCsi-U6/Neo-PKCα shRNAs and scrambled shRNA using Lipofectamine 2000 according to the manufacturer’s instructions to evaluate the inhibition efficiency of the siRNAs. The cells were harvested 48 h after transfection, and Western blot and immunofluorescence were used to evaluate the transfection efficiency.

Immunocytochemical staining

AQP2-mIMCD3 cells were divided into 3 groups: (1) Scrambled shRNA transfected; (2) PKCα A/25E vector transfected; and (3) PKCα shRNA transfected. The cells in each group were treated with 100 µmol/L DDAVP for 30 min. DDAVP was removed, and the cells were incubated with culture medium for 2 h (washout). The cells were then fixed in 4% paraformaldehyde for 10 min, rinsed twice in PBS, and blocked for 15 min in a blocking/permeabilization solution (PBS containing 0.1% BSA and 0.3% Triton X-100). The cells were washed with PBS and pre-incubated in PBS containing 1% BSA for 20 min. The cells were incubated with an α-tubulin antibody (at a final concentration of 2 µg/mL) at 4°C overnight. The cells were washed with PBS and incubated in the secondary goat anti-rabbit IgG conjugated with Cy3 antibody (at a final concentration of 2 µg/mL) for 1.5 h at RT. Finally, the cells were washed twice with PBS and mounted with DAPI for confocal laser-scanning microscopy equipped with a CoolSNAP HQ camera.

Biotinylation of surface membrane proteins

Identifiably transfected AQP2-mIMCD3 cells were cultured on polylysine-coated 60-mm dishes for 48 h and treated with...
DdAVP for 0 min, 30 min, or 30 min followed by a 2-h wash-out. The cells were washed three times with PBS/glycine (137 mmol/L NaCl, 2.7 mmol/L KCl, 10 mmol/L NaH2PO4, 1.8 mmol/L KH2PO4, pH 8.0, and 5 mmol/L glycine) and twice with PBS[27]. An ice-cold biotinylation reagent (0.5 mg/mL in PBS) was immediately added to cells. The biotinylation reagent was removed after a 10-min incubation on ice, and the cells were washed three times with PBS/glycine.

Immunoblotting and immunoprecipitation
Transfected and DdAVP-treated cells were lysed in RIPA buffer (10 mmol/L Tris-HCl, 0.15 mol/L NaCl, 1% NP-40, 1% Na-deoxycholate, 0.5% SDS, 0.02% sodium azide, and 1 mmol/L EDTA, pH 7.4). The cell lysates were clarified by centrifugation at 1000×g for 10 min at 4 °C. Protein concentrations were measured using the bicinchoninic acid protein assay reagent kit (Pierce, Thermo Fisher Scientific, Beijing, China) and adjusted to the same concentration using lysine buffer. A 0.5-mL aliquot of the cell lysates was incubated with 50 μL of streptavidin-garose beads at 4 °C overnight to capture biotinylated proteins, 50 μL of protein A/G-garose beads plus the AQP2 antibody to capture total AQP2, or 50 μL of protein A/G-garose beads plus the PKCα antibody to capture total PKCα. The beads were pelleted, washed, and boiled for 5 min in 50 μL of cracking buffer (50 mmol/L Tris-HCl [pH 7.0], 10% glycerol, 2% SDS, and 2% β-mercaptoethanol). The eluted immunoprecipitates were separated on 10% polyacrylamide gels and transferred electrophoretically to PVDF membrane. The membranes were blocked for 90 min with blocking buffer during gentle shaking and incubated overnight with antibodies against AQP2 (0.5 μg/mL), phospho-S256-AQP2 (0.5 μg/mL), PKCα (0.5 μg/mL) or GAPDH (0.5 μg/mL). The membranes were incubated with an HRP-conjugated IgG (0.1 μg/mL) for 2 h at room temperature. Finally, the immunoreactive signals were visualized using an ECL reagent (Beyotime, Shanghai, China) and quantified using the Image J program (NIH, Bethesda, MD, USA).

Statistical analyses
The values are presented as the means±SEM. The data were analyzed using SPSS software. Statistical significance of differences was assessed using unpaired t-tests or two-way ANOVAs. A value of P<0.05 was viewed as statistically significant.

Results
Inhibition of PKCα and the overexpression of AQP2 in mIMCD3 cells
All three PKCα shRNA plasmids (#1, #2, and #3) and PKCα scrambled shRNA plasmids were transfected into mIMCD3 cells to select the shRNAs with the highest inhibition efficiency to PKCα. Crude proteins were extracted from the harvested cells 48 h after transfection and subjected to Western blotting using the PKCα antibody. PKCα shRNA #1 plasmids exhibited the greatest inhibitory effect on PKCα, but all three PKCα shRNAs demonstrated visible inhibition (P<0.01) (Figure 1A).

The transfection of mIMCD3 with the AQP2-GFP-pCMV6 plasmid produced 12 geneticin-resistant clones, 10 of which were subjected to Western blot analysis (data not shown). The clone that amply expressed AQP2 was selected and named WT-7. A strong signal at 30 kDa in the selected AQP2-mIMCD3 stable cell line (WT-7) was observed, but no signal was detected in nontransfected mIMCD3 cells (Figure 1B).

![Figure 1](https://www.nature.com/aps)  
**Figure 1.** Inhibition of PKCα and the overexpression of AQP2 in mIMCD3 cells. (A) PKCα shRNA plasmid #1, #2, #3, and PKCα scramble shRNA plasmid (control) were transfected into mIMCD3 cells respectively. Forty-eight hours after transfection, crude proteins extracted from harvested cells were subjected to Western blot using antibodies against PKCα. Representative blot was shown (left) and quantitative analysis of PKCα protein levels were normalized to control group (n=3 each group) (right). bP<0.05 vs control group. (B) AQP2-GFP-mIMCD3 stable cell line was generated by transfecting to express AQP2-GFP (See Materials and methods). The expression of AQP2-GFP was detected in selected WT7 stable cell lines. (C) AQP2-GFP stably expressed WT7 cells were transfected with scramble shRNA (Con), PKCα A/25E constructs or PKCα shRNA, respectively. Forty-eight hours after transfection, crude proteins extracted from harvested cells were subjected to Western blot using antibodies against PKCα (left). Quantitative analysis of PKCα protein levels were normalized to control group (n=3 each group) (right). bP<0.05 vs control group.
These results suggest the successful preparation of AQP2-mIMCD3 stable cell lines.

The WT-7 stable cell line was used to investigate PKCα expression following different transfections of PKCα scrambled shRNA, PKCα A/25E, and PKCα shRNA (#1) using Western blot. The transfection of PKCα A/25E significantly enhanced PKCα expression, but PKCα shRNA (#1) dramatically suppressed PKCα expression ($P<0.05$) (Figure 1C). The WT-7 cell line and PKCα A/25E and PKCα shRNA (#1) plasmids were utilized in subsequent experiments.

**DdAVP enhanced the phosphorylation of AQP2 and PKCα in vitro**

The physical association of AQP2 with endogenous PKCα was examined *in vitro* in WT7 cell lines to reveal the direct role of PKCα on AQP2. Cells with or without DdAVP treatment for 30 min were lysed, and PKCα was immunoprecipitated using anti-PKCα antibodies. The immunoprecipitates were analyzed using Western blot and AQP2 antibodies. Strong signals for AQP2 and PKCα were detected in whole cell lysates and immunoprecipitates, but no AQP2 signal was detected in PKCα immunoprecipitates (Figure 2). These results suggest that PKCα did not interact with AQP2 *in vitro*. However, DdAVP treatment enhanced AQP2 phosphorylation at serine 256. Down-regulation of PKCα by shRNA did not alter DdAVP-mediated AQP2 phosphorylation at serine 256. These data indicated that PKCα did not influence DdAVP-induced AQP2 phosphorylation at serine 256 (Figure 2).

**PKCα expression influenced the distribution of α-tubulin and DdAVP-mediated AQP2 trafficking in mIMCD cells**

An increase in PKCα activity induces the re-organization of microtubules in proximal tubular cells[9]. Therefore, PKCα-induced alterations in microtubule architecture were investigated. Most cells that were transfected with scrambled shRNA plasmid or PKCα shRNA plasmids exhibited a prominent dense network of microtubules around the nucleus with a considerably lower microtubule content in the cell periphery under basal conditions. The constitutive activation of PKCα increased microtubule formation in the cell periphery (Figure 3, upper panel and middle panel). AQP2-mIMCD3 cells (WT7 cells) were incubated with DdAVP for 30 min followed by a 2-h washout to evaluate the involvement of PKCα in AVP-induced microtubule formation in the cell periphery. Cells that were transfected with the control and PKCα A/25E plasmids displayed increased microtubule formation in the cell periphery following DdAVP stimulation. In contrast, the down-regulation of PKCα expression using shRNA inhibited DdAVP-induced microtubule depolymerization (Figure 3, lower panel). Taken together, these data strongly indicated that the expression of PKCα regulated microtubule distribution in mIMCD cells.

The role of PKCα in DdAVP-induced AQP2 trafficking was investigated because PKCα influences microtubule reorganization, and the DdAVP-induced depolymerization of microtubules affects the redistribution of AQP2 to the plasma membrane[25, 28, 29]. Immunofluorescence analyses indicated that AQP2 was primarily localized around the nucleus under basal condition. Treatment with DdAVP for 30 min produced a pronounced increase in AQP2 localization in the plasma membrane. DdAVP washout restored the perinuclear localization of AQP2. The expression of constitutively activated PKCα produced a wide distribution of AQP2 throughout the cytoplasm in contrast to AQP2 localization around the nucleus under basal conditions. DdAVP also induced the translocation of AQP2 to the plasma membrane in the PKCα A/25E transfected group. The down-regulation of PKCα expression by shRNA interference inhibited DdAVP-induced AQP2 translocation to the plasma membrane (Figure 3).

**Regulation of PKCα expression influenced DdAVP-stimulated plasma location of AQP2**

mIMCD-3 surface membrane proteins were biotinylated following DdAVP treatment for 0 min, 30 min, and 30 min followed by a 2-h washout to confirm the immunofluorescence results. Biotinylated proteins were captured from whole-cell lysates using streptavidin beads, separated by SDS-PAGE and probed using an AQP2 antibody. Immunoprecipitation was performed in whole cell lysates in parallel using AQP2-specific antibodies. The DdAVP-induced increase in biotin-AQP2 was partially inhibited by PKCα down-regulation with shRNA transfection, but biotin-AQP2 was enhanced by the overexpression of PKCα A/25E (Figure 4). Moreover, different treatments only marginally affected the total amount of...
AQP2 as determined by AQP2 immunoprecipitation (Figure 4). These results were consistent with the AQP2 translocation observed by immunofluorescence detection and demonstrated that PKCα participated in DdAVP-mediated AQP2 translocation in mIMCD cells.

Discussion

PKCα exhibits different expression patterns in mouse and rat kidneys. For example, PKCα localizes in the proximal tubule of mice but not rats[3, 4]. Our previous study demonstrated that PKCα contributes to urine concentration in the mouse kidney[5]. The use of cell lines that originate from mouse IMCD cells is reasonable because AQP2 is primarily expressed in collecting duct principal cells, and the effect of PKCα on urine concentrating occurs in the inner medulla. In the present study, AQP2 trafficking was examined in an mIMCD3 cell line that was derived from mouse IMCD and exhibited the properties of IMCD cells. However, immortalized mIMCD3 cells
lack AQP2 expression[29], and AQP2 is only briefly expressed in primary cultured IMCD cells[31]. Therefore, we generated AQP2-mIMCD3 stable cell lines for the following experiments. Our results demonstrated that AQP2 was located in intracellular vesicles in non-stimulated mIMCD3 cells. DdAVP treatment induced the translocation of AQP2 from the cytoplasm to the plasma membrane, and DdAVP washout reversed AQP2 localization from the plasma membrane to the cytoplasm (Figure 4). Therefore, AQP2-mIMCD3 cells provide an optimal cell model to investigate AQP2 trafficking. AQP2 phosphorylation at serine 256 by PKA plays an important role in AQP2 trafficking[32, 33]. However, the phosphorylation of AQP2 at serine 256 is not sufficient to maintain the presence of the water channel at the plasma membrane[34], and the PKC-induced internalization of AQP2 in collecting duct cells does not depend on the phosphorylation state of AQP2[25, 34]. Our study demonstrated that DdAVP increased AQP2 phosphorylation, and the inhibition of PKCa expression did not influence DdAVP-mediated AQP2 phosphorylation at serine 256, which is consistent with previous reports. These results strongly suggest that AQP2 phosphorylation at serine 256 is PKCa independent. However, the ability of PKCa to phosphorylate AQP2 at other phosphorylation sites, such as serine 231, remains unclear. Our results indicated that PKCa did not interact with AQP2 in vitro. Therefore, the ability of PKCa to mediate the trafficking of phosphorylated AQP2 requires further investigation.

The disruption of microtubules induces AQP2 translocation to the plasma membrane[23, 29], and PKCa is involved in cytoskeletal remodeling during cell motility, phagocytosis, neurite outgrowth and the regulation of cytoskeleton-associated proteins[35-38]. Therefore, the impact of PKCa on the cytoskeleton in AQP2-mIMCD3 cells was investigated. Microtubules are part of the cytoskeleton and consist of α, β, and γ tubulins[39]. α-Tubulin is the major cytoskeletal protein related to cell trafficking. The role of PKCa in the assembly of α-tubulin was determined. PKCa overexpression or inhibition produced a constitutively active form of PKCa or down-regulated expression of PKCa, respectively, which resulted in the de- and re-polymerization of α-tubulin, respectively. Moreover, PKCa down-regulation prevented DdAVP-mediated α-tubulin depolymerization. These data indicated that PKCa is involved in the assembly of α-tubulin in AQP2-mIMCD3 cells, which is consistent with previous reports[9].

The microtubule-dependent transport of AQP2 is predominantly responsible for AQP2 trafficking and localization inside of the cell after its internalization, but it is not responsible for the exocytic transport of the water channel[25]. In the present study, the overexpression of constitutively active PKCa produced a wide distribution of AQP2 throughout the cytoplasm, which is in contrast to its translocation to the plasma membrane. This result indicates that other factors/proteins rather than α-tubulin are required for AQP2 translocation to the plasma membrane[40]. Furthermore, the consistent inhibition of α-tubulin depolymerization by the down-regulation of PKCa expression produced a loss in DdAVP-mediated AQP2 translocation. This result confirms that α-tubulin is a key element for the proper localization of AQP2 in the cytoplasmic compartment.

In summary, the use of stably expressing AQP2-mIMCD3 cells demonstrated that PKCα did not functionally associate with AQP2. The down-regulation of PKCα expression altered the distribution of α-tubulin and inhibited DdAVP-mediated AQP2 trafficking. However, constitutively activated PKCα rescued or aggravated these changes. These data directly demonstrated that PKCα mediates AQP2 trafficking by influencing the assembly of α-tubulin and underscore the complexity of the molecular events of PKCα-mediated urine concentration.

Acknowledgements
This work was supported by the National Natural Science Foundation of China (No 30871173).

Author contribution
Dr Li-jun YAO designed the research and wrote the paper; Dr Hong ZHAO performed biotinilation of cell surface protein experiment; Xi YAO performed Western blot and immunocytochemistry experiment; Tao-xia WANG prepared plasmids and analysed data; Wen-min JIN performed cell culture experiment; Qian-qian JI contributed reagents preparation; Dr Xiao YANG wrote the paper; Dr Qiu-hong DUAN performed the research.

References
1 Mellor H, Parker PJ. The extended protein kinase C superfamily. Biochem J 1998; 332: 281–92.
2 Ostlund E, Mendez CF, Jacobsson G, Fryckstedt J, Meister B, Aperia A. Expression of protein kinase C isoforms in renal tissue. Kidney Int 1995; 47: 766–73.
3 Pfaff IL, Wagner HJ, Vallon V. Immunolocalization of protein kinase C isoenzymes alpha, beta1 and beta2 in rat kidney. J Am Soc Nephrol 1999; 10: 1861–73.
4 Redling S, Pfaff IL, Leitges M, Vallon V. Immunolocalization of protein kinase C isoenzymes alpha, beta1, beta II, delta, and epsilon in mouse kidney. Am J Physiol Renal Physiol 2004; 287: F289–F298.
5 Yao L, Huang DY, Pfaff IL, Nie X, Leitges M, Vallon V. Evidence for a role of protein kinase C-alpha in urine concentration. Am J Physiol Renal Physiol 2004; 287: F299–304.
6 Yao LJ, Leitges M, Vallon V. Mice lacking protein kinase C beta present modest increases in systolic blood pressure and NH₄Cl-induced metabolic acidosis. Kidney Blood Press Res 2006; 29: 36–42.
7 Kim JH, Lee-Kwon W, Park JB, Ryu SH, Yun CH, Donowitz M. Ca²⁺-dependent inhibition of Na⁺/H⁺ exchanger 3 (NHE3) requires an NHE3-E3KARP-alpha-actinin-4 complex for oligomerization and endocytosis. J Biol Chem 2002; 277: 23714–24.
8 Quaillmann B, Kessels MM, Kelly RB. Molecular links between endocytosis and the actin cytoskeleton. J Cell Biol 2000; 150: F111–F116.
9 Hryciw DH, Pollock CA, Poronnik P. PKC-alpha-mediated remodeling of the actin cytoskeleton is involved in constitutive albumin uptake by proximal tubule cells. Am J Physiol Renal Physiol 2005; 288: F1227–F1235.
10 Fushimi K, Uchida S, Hara Y, Hirata Y, Marumo F, Sasaki S. Cloning
and expression of apical membrane water channel of rat kidney collecting tubule. Nature 1993; 361: 549–52.

11 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 1993; 90: 11663–7.

12 Takata K, Matsuzaki T, Tajika Y. Aquaporins: water channel proteins of the cell membrane. Prog Histochem Cytochem 2004; 39: 1–83.

13 Tajika Y, Matsuzaki T, Suzuki T, Ablimit A, Aoki T, Hagiwara H, et al. Differential regulation of AQP2 trafficking in endosomes by microtubules and actin filaments. Histochem Cell Biol 2005; 124: 1.

14 Rojek A, Fuchtbauer EM, Kwon TH, Frokiaer J, Nielsen S. Severe urinary concentrating defect in renal collecting duct-selective AQP2 conditional-knockout mice. Proc Natl Acad Sci U S A 2006; 103: 6037–42.

15 Takata K, Matsuzaki T, Tajika Y, Ablimit A, Hasegawa T. Localization and trafficking of aquaporin 2 in the kidney. Histochemistry Cell Biol 2008; 130: 197–209.

16 Nielsen S, Chou CL, Marples D, Christensen EI, Kishore BK, Knepper MA. Vasopressin increases water permeability of kidney collecting duct by inducing translocation of aquaporin-CD water channels to plasma membrane. Proc Natl Acad Sci U S A 1995; 92: 1013–7.

17 Yamamoto T, Sasaki S, Fushimi K, Ishibashi K, Yawata E, Kawasaki K, et al. Vasopressin increases AQP-CD water channel in apical membrane of collecting duct cells in Brattleboro rats. Am J Physiol 1995; 268: C1546–C1551.

18 Boone M, Deen PM. Physiology and pathophysiology of the vasopressin-regulated renal water reabsorption. Pflugers Arch 2008; 456: 1005–24.

19 Hozawa S, Holtzman EJ, Ausiello DA. cAMP motifs regulating transcription in the aquaporin 2 gene. Am J Physiol 1996; 270: C1695–C1702.

20 Matsumura Y, Uchida S, Rai T, Sasaki S, Marumo F. Transcriptional regulation of aquaporin-2 water channel gene by cAMP. J Am Soc Nephrol 1997; 8: 861–7.

21 Hasler U, Leroy V, Martin PY, Feraille E. Aquaporin-2 abundance in the renal collecting duct: new insights from cultured cell models. Am J Physiol Renal Physiol 2009; 297: F10–F18.

22 Li C, Wang W, Rivard CJ, Lanaspa MA, Summer S, Schrier RW. Molecular mechanisms of angiotensin II stimulation on aquaporin-2 expression and trafficking. Am J Physiol Renal Physiol 2011; 300: F1255–F1261.

23 Noda Y, Horikawa S, Kanda E, Yamashita M, Meng H, Eto K, et al. Reciprocal interaction with G-actin and tropomyosin is essential for aquaporin-2 trafficking. J Cell Biol 2008; 182: 587–601.

24 Takata K. Aquaporin-2 (AQP2): its intracellular compartment and trafficking. Cell Mol Biol 2006; 52: 34–9.

25 Vossenkamper A, Nedevtsky PI, Wiesner B, Furrkert J, Rosenthal W, Klussmann E. Microtubules are needed for the perinuclear positioning of aquaporin-2 after its endocytic retrieval in renal principal cells. Am J Physiol Cell Physiol 2007; 293: C1129–C1138.

26 Baier-Bitterlich G, Ueralli F, Bauer B, Fresser F, Wachter H, Grunicke H, et al. Protein kinase C-theta isoform selective stimulation of the transcription factor complex AP-1 in T lymphocytes. Mol Cell Biol 1996; 16: 1842–50.

27 Goel M, Zuo CD, Schilling WP. Role of cAMP/PKA signaling cascade in vasopressin-induced trafficking of TRPC3 channels in principal cells of the collecting duct. Am J Physiol Renal Physiol 2010; 298: F988–F996.

28 Hays RM, Condeelis J, Gao Y, Simon H, Ding G, Franki N. The effect of vasopressin on the cytoskeleton of the epithelial cell. Pediatr Nephrol 1993; 7: 672–9.

29 Nedevtsky PI, Tamma G, Beulushhausen S, Valenti G, Rosenthal W, Klussmann E. Regulation of aquaporin-2 trafficking. Handb Exp Pharmacol 2009, 190: 133–57.

30 Umenishi F, Nariyoi T, Schrier RW. Effect on stability, degradation, expression, and targeting of aquaporin-2 water channel by hyperosmolality in renal epithelial cells. Biochem Biophys Res Commun 2005; 338: 1593–9.

31 Maric K, Okseh A, Rosenthal W. Aquaporin-2 expression in primary cultured rat inner medullary collecting duct cells. Am J Physiol 1998; 275: F796–801.

32 Katsura T, Gustafson CE, Ausiello DA, Brown D. Protein kinase A phosphorylation is involved in regulated exocytosis of aquaporin-2 in transfected LLC-PK1 cells. Am J Physiol 1997; 272: F817–F822.

33 Christensen BM, Zelenina M, Aperia A, Nielsen S. Localization and regulation of PKA-phosphorylated AQP2 in response to V(2)-receptor agonist/antagonist treatment. Am J Physiol Renal Physiol 2000; 278: F29–F42.

34 Nejsum LN, Zelenina M, Aperia A, Frokiaer J, Nielsen S. Bidirectional regulation of AQP2 trafficking and recycling: involvement of AQP2–S256 phosphorylation. Am J Physiol Renal Physiol 2005; 288: F930–F938.

35 Van Balkom BW, Saveikou PJ, Markovich D, Hofman E, Nielsen S, van der Sluijs P, et al. The role of putative phosphorylation sites in the targeting and shuffling of the aquaporin-2 water channel. J Biol Chem 2002; 277: 41473–9.

36 Kamsteeg EJ, Hendriks G, Boone M, Konings IB, Oorschot V, van der Sluijs P, et al. Short-chain ubiquitination mediates the regulated endocytosis of the aquaporin-2 water channel. Proc Natl Acad Sci U S A 2006; 103: 18344–9.

37 Holm A, Teje K, Gunnarsson T, Magnusson KE, Descoteaux A, Rasmusson B. Role of protein kinase C alpha for uptake of unpoisoned prey and phagosomal maturation in macrophages. Biochem Biophys Res Commun 2003; 302: 653–8.

38 Beaudry H, Gendron L, Guimond MO, Payet MD, Gallo-Payet N. Involvement of protein kinase C alpha (PKC alpha) in the early action of angiotensin II type 2 (AT2) effects on neurite outgrowth in NG108-15 cells: AT2-receptor inhibits PKC alpha and p21ras activity. Endocrinology 2006; 147: 4263–72.

39 Belin RJ, Sumandea MP, Allen EJ, Schoenfelt K, Wang H, Solaro RJ, et al. Augmented protein kinase C-alpha-induced myofilament protein phosphorylation contributes to myofilament dysfunction in experimental congestive heart failure. Circ Res 2007; 101: 195–204.

40 Abeyweera TP, Chen X, Rotenberg SA. Phosphorylation of alpha6-tubulin by protein kinase Calpha activates motility of human breast cells. J Biol Chem 2009; 284: 17648–56.

41 Nogales E, Wolf SG, Downing KH. Structure of the alpha beta tubulin dimer by electron crystallography. Nature 1998; 391: 199–203.