Vasopressin regulation of the renal UT-A3 urea transporter

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Submitted 6 November 2008; accepted in final form 25 November 2008

Stewart GS, Thistlethwaite A, Lees H, Cooper GJ, Smith C. Vasopressin regulation of the renal UT-A3 urea transporter. Am J Physiol Renal Physiol 296: F642–F648, 2009. First published December 3, 2008; doi:10.1152/ajprenal.90660.2008.—Facilitative urea transporters in the mammalian kidney play a vital role in the urinary concentrating mechanism. The urea transporters located in the renal inner medullary collecting duct, namely UT-A1 and UT-A3, are acutely regulated by the antidiuretic hormone vasopressin. In this study, we investigated the vasopressin regulation of the basolateral urea transporter UT-A3 using an MDCK-mUT-A3 cell line. Within 10 min, vasopressin stimulates urea flux through UT-A3 transporters already present at the plasma membrane, via a PKA-dependent process. Within 1 h, vasopressin significantly increases UT-A3 localization at the basolateral membrane, causing a further increase in urea transport. While the basic trafficking of UT-A3 to basolateral membranes involves both protein kinase C and calmodulin, its regulation by vasopressin specifically occurs through a casein kinase II-dependent pathway. In conclusion, this study details the effects of vasopressin on UT-A3 urea transporter function and hence its role in regulating urea permeability within the renal inner medullary collecting duct.

arginine vasopressin; urea transport; membrane localization; casein kinase II


In perfused isolated IMCD, vasopressin stimulates phloro- tin-sensitive trans-epithelial urea transport (23, 28). Recent studies of rat UT-A1 (11) and mouse UT-A3 (25) expressed in Madin-Darby canine kidney (MDCK) type I cell lines showed that both isoforms can be acutely regulated by arginine vasopressin (AVP), via a cAMP-dependent pathway. Interestingly, the protein kinase A (PKA) inhibitor H89 reduced the vasopressin activation of both UT-A1 (12) and UT-A3 (25). Vasopressin is known to rapidly increase phosphorylation of UT-A1 via PKA in rat IMCDs (30) and also to increase UT-A1 membrane accumulation (16). In addition, it has recently been reported that increased cAMP levels stimulate phosphorylation of UT-A3 in rat IMCD (2) and that vasopressin increases basolateral membrane expression of UT-A3 (26). Previously, little was known concerning this vasopressin regulation of UT-A3 localization, other than that it was not through alteration of the ubiquitin-proteasome pathway (26). In this study, we now showed that vasopressin increases UT-A3 basolateral membrane expression, and hence urea transport, through a casein kinase II-dependent pathway.

METHODS

MDCK cell lines. Type I MDCK cells stably expressing mUT-A3 (termed MDCK-mUT-A3) were cultured in DMEM media plus 10% FBS (GIBCO) and 300 μg/ml hygromycin, as previously described (25). The host cell line MDCK-FLZ cells (i.e., lacking mUT-A3) were cultured in DMEM media plus 10% FBS, but lacking hygromycin. Epithelial monolayers were cultured on semipermeable Transwell supports (Corning) as previously described (19). Monolayers were fed on day 2 following seeding and then daily until use. The resistance of each monolayer was measured using an EVOM resistance meter (World Precision Instruments) and monolayers were used for experiments once trans-membrane resistance developed to >1.0 kΩ.

Flux measurements. Unidirectional basolateral uptake urea flux experiments were performed at 37 ± 0.2°C using [14C]urea (1.0 μCi per basolateral well) as the radiolabeled tracer, as previously described (25). MDCK cells were grown on semipermeable Transwell supports (Corning) and initially incubated in HBSS media (GIBCO) containing 5 mM urea, 12 mM HEPES, and the specific test compounds. After the relevant incubation time period, they were then placed in HBSS basolateral solution containing [14C]urea for 30 s. This was followed by 10 s in a basolateral solution consisting of standard 1× PBS containing 10 mM “cold” urea. Transwells were then removed from basolateral solution completely, and the apical HBSS solution was replaced with 500 μl of 5% SDS solution. Cells were allowed to dissolve for 45 min on a horizontal shaker (Stuart Scientific). SDS-cell suspensions were then transferred into scintillation vials, 3 ml of “Ecocint A” (National Diagnostics) scintillation fluid were added to each vial, and the radioactivity was counted using a 1900

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TR liquid scintillation analyzer (Packard). All test compounds were of certified grade and were made up either in sterile dH2O or anhydrous DMSO. Stock solutions were prepared from Calbiochem (unless stated): AVP (Sigma) 100 μM in dH2O, N-[2-(p-Bromo-c

agonist (e.g., 10 mM semipermeable transwells (Corning) until confluent epithelial mono-

ic.2HCl (H89) 10 mM in DMSO, hbbestatin 10 mM in DMSO, calmodulin inhibitor W-7 10 mM DMSO, protein kinase C inhibitor Calphostin-C 1 mM DMSO, and ccasein kinase II inhibitor 10 mM DMSO. Stocks were then diluted in HBSS before use. Control solutions were prepared by adding the corresponding solvent minus the test compound.

**Immunoblotting.** MDCK-mUT-A3 cells were harvested using 0.05% trypsin-EDTA (Invitrogen) and washed twice with PBS. Cell protein was homogenized with a handheld dounce homogenizer, using a standard homogenization buffer (pH 7.6) containing 12 mM HEPES, 300 mM mannitol, and peptidase inhibitors added immediately before use (1 μg/ml pepstatin, 2 μl/ml leupeptin, and 1 μg/ml phenylmethylsulfonyl fluoride, Sigma). Homogenates were initially centrifuged at 3,000 g for 5 min, the pellet was discarded, and the resulting supernatant was centrifuged at 17,000 g for 20 min. The 17,000-g pellets, containing plasma membranes (26), were retained and resuspended in homogenization buffer. All centrifugal steps were performed at 4°C. Immunoblotting experiments were then performed using the protocol previously described (24). Briefly, SDS-PAGE was performed on minigels of 12% polyacrylamide by loading 20 μg per lane of protein. After transfer to nitrocellulose membranes, immunobLOTS were probed for 16 h at 4°C with either an affinity-purified urea transporter antisemur (ML446), previously shown to detect UT-A3 (24), or anti-Na-K-ATPase antiserm (B05–369, mouse monoclonal IgG, Upstate Biotechnology). ImmunobLOTS were then washed and probed with 1:5,000 dilution of either goat anti-rabbit (for ML446) or goat anti-mouse (for α-Na-K-ATPase) horse serumid peroxidase (HRP)-linked secondary antiserm (Dako) for 1 h at room temperature. After further washing, detection of protein was performed using Western Lightning Chemiluminescence Reagent Plus (PerkinElmer) and ECL film (GE Healthcare). Images of developed film were then captured with Image Reader LAS-1000 package and the intensity of signals was quantified densitometrically using the AIDA Image Analyzer v.3.44 software package.

**Immunolocalization studies.** MDCK-mUT-A3 cells were grown on semipermeable transwells (Corning) until confluent epithelial monolayers were formed. After 15-min preincubation in experimental agonist (e.g., 10 μM casein kinase II inhibitor), cells were exposed to 10−6 M vasopressin for 1 h. These treated MDCK-mUT-A3 transwells were then fixed for 15 min using 4% paraformaldehyde (PFA) diluted in 1× PBS. Fixed transwells were then placed in a solution containing 1% BSA, 0.2% gelatin, and 0.05% saponin, diluted in 1× PBS, for three 10-min washes. The transwells were then incubated overnight in 1:100 ML446 antibody diluted in 0.1% BSA and 0.3% Triton X-100 in 1× PBS. After three 10-min washes in 0.1% BSA, 0.2% gelatin, and 0.05% saponin in 1× PBS, transwells were incubated in 1:100 dilution of FITC-conjugated AffiniPure goat anti-rabbit secondary antibody (Jackson Immunoresearch) for 1 h. After three further 10-min washes in 0.1% BSA, 0.2% gelatin, and 0.05% saponin in 1× PBS, the nitrocellulose membranes containing the MDCK-
mUT-A3 cells were removed, placed on slides, and had coverslips mounted using Eukitt mounting medium (GmbH). Slides were then viewed using an AxioPlan2 microscope (Zeiss) and images were recorded using a QICam Fast1394 camera and Q capture Pro software (Q Imaging).

**Statistical analysis.** All data values are shown as mean average ± SE, with n representing the n number. One-way ANOVA was used for statistical analysis of all urea flux experiments and immunoblot densitometry data. If the ANOVA indicated a difference, treatment comparison between groups with the Student-Newman-Keuls post hoc test was performed. Groups were deemed statistically significant if P < 0.05.

**RESULTS**

In previous studies, we showed that AVP significantly stimulates basolateral urea flux in MDCK-mUT-A3 monolayers (25, 26). Figure 1A shows a summary of the time scale of the effect of 10−6 M AVP on MDCK-mUT-A3 basolateral urea transport, with significant increases after 10-min (P < 0.01, n = 11, ANOVA) and 60-min (P < 0.001, n = 23, ANOVA) AVP exposure. No effects with AVP were observed on the basolateral urea transport in MDCK-FLZ monolayers [not significant (NS), n = 4, ANOVA]. Using the previously characterized ML446 antibody (24), we probed immunoblots of plasma membrane-enriched MDCK-mUT-A3 protein and successfully detected the expected ~45- to 50-kDa signal that represents glycosylated UT-A3 (24) (Fig. 1B). Although there was no significant effect after 10 min (NS, n = 3, ANOVA), exposure to 10−6 M AVP for 60 min before cell harvesting

![Image](http://ajprenal.physiology.org/)

**Fig. 1.** A: summary of the effect of 10−6 M arginine vasopressin (AVP) on basolateral 14C-labeled urea unidirectional flux in MDCK-FLZ and MDCK-mUT-A3 monolayers. B: immunoblots comparing the signals obtained for UT-A3 and Na-K-ATPase in 17,000-g MDCK-mUT-A3 protein samples exposed to 10−6 M AVP for different time periods. Also shown is a summary of densitometry results for 3 immunoblots, shown as a ratio of UT-A3 to Na-K-ATPase signal. *P < 0.05, **P < 0.01, ***P < 0.001 vs. basal data; numbers in brackets denote n numbers.
significantly increased the UT-A3 signal ($P < 0.05, n = 3$, ANOVA), as previously reported (26). In contrast, no such change was observed in the 100-kDa Na-K-ATPase signals that were detected in the same protein samples ($n = 3$, ANOVA; Fig. 1B), illustrating that the effect of AVP on UT-A3 localization was specific.

To further investigate AVP regulation of UT-A3, we initially studied the effect of the PKA inhibitor H89 on the AVP-stimulated increases in basolateral urea transport and membrane localization. As previously reported (25), 10 μM H89 completely inhibits the effect of 10 min of AVP exposure on basolateral urea transport (Fig. 2A). However, 10 μM H89 surprisingly failed to prevent the significant stimulation of urea transport after 60-min AVP exposure ($P < 0.05, n = 4$, ANOVA). Furthermore, H89 exposure also failed to prevent the increase in UT-A3 signal observed in 17,000-g immunoblot samples after 60-min AVP exposure ($P < 0.05, n = 3$, ANOVA; Fig. 2B). At higher doses H89 is reported to also inhibit protein kinase C (PKC; with an IC$_{50}$ of $\sim 25$ μM for PKC, compared with an IC$_{50}$ of $\sim 1$ μM for PKA); hence, we also investigated the effects of 50 μM H89. This higher dose of H89 not only prevented any significant AVP-stimulated increase in basolateral urea transport after both 10- and 60-min exposure ($n = 4$, ANOVA; Fig. 2C), it also prevented AVP increasing plasma membrane localization of UT-A3 (NS, $n = 3$, ANOVA; Fig. 2D). These results with H89 therefore strongly suggested that both PKA and PKC were involved in the vasopressin regulation of UT-A3.

We now performed experiments utilizing the specific PKC inhibitor Calphostin-C and a casein kinase II inhibitor, since it has previously been reported that urea transporters from vasopressin-treated IMCD tissue are phosphorylated at casein kinase phosphorylation sites (13). In the absence of vasopressin, neither 10 μM CKII inhibitor (NS, $n = 4$, ANOVA) nor 1 μM Calphostin-C (NS, $n = 4$, ANOVA) had any effect on basal urea transport (Fig. 3A). This was as expected since there is no UT-A3-mediated transport under these conditions (25, 26) (confirmed in Fig. 1 in this study). However, our results in Fig. 3B showed that incubation in 1 μM Calphostin-C dramatically reduced basal UT-A3 membrane localization ($P < 0.01, n = 3$, ANOVA), whereas 10 μM CKII inhibitor had no significant effect (NS, $n = 3$, ANOVA). Strong 100-kDa Na-K-ATPase

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**Fig. 2.** A: effect of 10 μM H89 on 10⁻⁶ M AVP-stimulated basolateral ¹⁴C-labeled urea unidirectional flux in MDCK-mUT-A3 monolayers. The presence of H89 prevented the stimulated increase in urea transport observed at 10 min but not the increase at 60-min AVP exposure. B: immunoblot showing the effect of 10 μM H89 on AVP-stimulated increases in 45- to 50-kDa UT-A3 17,000-g signal. Densitometry results for 3 immunoblots, shown as % of control signal, showed a significant increase in signal with 60-min AVP exposure in both the presence and absence of 10 μM H89. C: effect of 50 μM H89 on 10⁻⁶ M AVP-stimulated basolateral ¹⁴C-labeled urea unidirectional flux in MDCK-mUT-A3 monolayers. The presence of H89 prevented the stimulated increase in urea transport observed at both 10- and 60-min AVP exposure. D: immunoblot showing the effect of 50 μM H89 on AVP-stimulated increases in UT-A3 signal. Densitometry results for 3 immunoblots, shown as % of control signal, showed that the significant increase in signal with 60-min AVP exposure did not occur in the presence of 50 μM H89. *$P < 0.05$, **$P < 0.01$ vs. basal data.
positive control signals were observed in all cases, confirming the viability of each protein sample. Further investigation showed that preincubation with 1 μM Calphostin-C prevented the AVP-stimulated increase in basolateral urea flux (NS, n = 6, ANOVA; Fig. 4A). Importantly, Calphostin-C also abolished UT-A3 plasma membrane signal in the presence of AVP (Fig. 4B), so that it was significantly less than observed under basal conditions (P < 0.01, n = 3, ANOVA). In contrast, while 10 μM CKII inhibitor prevented AVP-stimulated increases in basolateral urea flux (NS, n = 4, ANOVA; Fig. 5A) and UT-A3 plasma membrane expression, the UT-A3 signal remained at a level that was not significantly different to that observed under basal conditions (NS, n = 3, ANOVA; Fig. 5B).

To further corroborate our findings, we performed immunolocalization studies on PFA-fixed monolayers of MDCK-mUT-A3 cells. As previously reported (26), 1-h vasopressin treatment caused a marked increase in UT-A3 signal in the basolateral membranes compared with cells under control conditions (Fig. 6). In contrast, cells preincubated in either Calphostin-C or CKII inhibitor before AVP exposure displayed a very different staining pattern, with the majority of UT-A3 signal being intracellular and very little being present at the basolateral membranes.

Finally, since non-muscle myosin II is a target for vasopressin in the renal collecting duct (5), we investigated the effect of the non-muscle myosin II inhibitor blebbistatin. Preincubation of MDCK-mUT-A3 cells in 10 μM blebbistatin was found to have no effect on the AVP-stimulated increases in either basolateral urea transport (P < 0.05, n = 4, ANOVA) or membrane localization (P < 0.05, n = 3, ANOVA; data not shown).

Fig. 3. A: effect of 10 μM casein kinase II (CKII) inhibitor and 1 μM Calphostin-C (protein kinase C inhibitor) on basolateral 14C-labeled urea unidirectional flux in MDCK-mUT-A3 monolayers under basal conditions (i.e., in the absence of vasopressin). B: immunoblots comparing the effects of 10 μM CKII inhibitor and 1 μM Calphostin-C on the UT-A3 signal under basal conditions. Densitometry results for 3 immunoblots, shown as ratio to Na-K-ATPase signal, showed that Calphostin-C completely abolished the UT-A3 signal, while CKII inhibitor had no effect. **P < 0.01 vs. control data.

Fig. 4. A: effect of 1 μM Calphostin-C on 10−6 M AVP-stimulated basolateral 14C-labeled urea unidirectional flux in MDCK-mUT-A3 monolayers. The presence of Calphostin-C prevented the AVP-stimulated increase in urea transport. B: immunoblot showing the effect of 1 μM Calphostin-C on AVP-stimulated increases in UT-A3 signal. Densitometry results for 3 immunoblots, shown as ratio to Na-K-ATPase signal, showed that Calphostin-C not only prevented the significant increase in UT-A3 signal normally observed with AVP, it also significantly decreased the signal compared with basal conditions. *P < 0.05 increase vs. basal data. ††P < 0.01 decrease vs. basal data.
It is also known that inhibition of calmodulin reversibly blocks vasopressin-stimulated urea permeability in the rat IMCD (13); therefore, we investigated the effects of the calmodulin inhibitor W-7. Preincubation with 25 μM W-7 completely abolished vasopressin-stimulated increases in basolateral urea flux (NS, n = 4, ANOVA; Fig. 7A). This result was further confirmed by immunoblotting results for protein from MDCK-mUT-A3 cells exposed to W-7 (Fig. 7B), where inhibition of calmodulin not only prevented vasopressin-stimulated increases in expression, but actually abolished the UT-A3 signal so that it was significantly less than that present under basal conditions (P < 0.05, n = 3, ANOVA).

DISCUSSION

Passive urea movement, through UT-A1 and UT-A3 urea transporters, across the IMCD plays an essential role in the mammalian urinary concentrating mechanism (8). As a result, acute regulation of these IMCD urea transporters by the antidiuretic hormone vasopressin is of great importance to urine production. It is already known that regulation of the UT-A3 urea transporter can occur through phosphorylation (2) and through increases in basolateral membrane expression (26). In this study, we now detailed further how vasopressin stimulates increased UT-A3 transport and basolateral membrane expression.

In the absence of vasopressin, there is no UT-A3-mediated transport even though UT-A3 transporters are present in the plasma membrane (Fig. 1). However, the response of MDCK-mUT-A3 basolateral urea transport to vasopressin exposure rapidly begins within 10 min and increases for up to an hour (Fig. 1A). This rapid response is therefore similar to that reported in MDCK-rUT-A1 cells (11, 12) and, more importantly, to that reported for urea permeability of IMCD (28). In contrast, immunoblots of MDCK-mUT-A3 protein showed that it required prolonged 60-min exposure to vasopressin to significantly increase the UT-A3 signal present in the plasma membrane-enriched samples (Fig. 1B). These results illustrate that there is not a simple linear relationship between UT-A3 basolateral membrane expression and transport function. The fact that 10 μM H89 only prevents the AVP increase in urea transport after 10 min (Fig. 2) strongly suggests that there is an initial PKA-dependent activation of the UT-A3 transporters already in the membrane. This PKA action would then presumably produce an initial phosphorylation of UT-A3, similar to that reported to take place in rat IMCD after 15 min of forskolin treatment (2).

In contrast to the initial activation of UT-A3 transporters, the effect of AVP on UT-A3 membrane localization is PKA independent (Fig. 2). Results using both a higher dose of H89, which inhibits PKC, and the specific PKC inhibitor Calphostin-C illustrated that this process actually requires PKC (Figs. 2 and 4). Detailed investigation with Calphostin-C showed that PKC is in fact required for the basic trafficking of UT-A3, even in the absence of vasopressin (Fig. 3B). As PKC inhibition completely stopped UT-A3 trafficking to the plasma membrane, it also prevented all UT-A3-mediated urea transport (Fig. 4). This was shown by the fact that urea flux in the presence of AVP plus Calphostin-C remained at the same level (Fig. 5).

![Fig. 5. A: effect of 10 μM CKII inhibitor on 10−6 M AVP-stimulated basolateral 14C-labeled urea unidirectional flux in MDCK-mUT-A3 monolayers. The presence of CKII inhibitor prevented the AVP-stimulated increase in urea transport. B: immunoblot showing the effect of 10 μM CKII inhibitor on AVP-stimulated increases in UT-A3 signal. Densitometry results for 3 immunoblots, shown as ratio to Na-K-ATPase signal, showed that CKII inhibition prevented the AVP-stimulated increase in UT-A3 signal but left an expression level that was not significantly different to that observed under basal conditions. **P < 0.01 vs. basal data.](http://ajprenal.physiology.org/)

![Fig. 6. Immunofluorescent staining of MDCK-mUT-A3 cells mounted on transwell membranes using ML446 antibody. One-hour exposure to 10−6 M AVP increases the UT-A3 signal observed at the basolateral membranes compared with basal conditions. In contrast, preincubation with either 1 μM Calphostin-C or 10 μM CKII inhibitor before AVP exposure leads to majority of UT-A3 signal remaining intracellular.](http://ajprenal.physiology.org/)
levels as under basal conditions. Overall, these findings suggest that PKC does not play a direct role in the vasopressin regulation itself, but in the basic trafficking processes that are required to transport UT-A3 to the basolateral membrane. This conclusion agrees with previous reports in rat IMCD that vasopressin has no clear effects on PKC activity per se (4). Instead, our results suggest that casein kinase II is the vital component in the vasopressin regulation pathway for UT-A3 (Fig. 5). Inhibiting casein kinase II prevented AVP-stimulated increases in both UT-A3-mediated urea flux and UT-A3 plasma membrane signal. Most crucially, however, UT-A3 plasma membrane localization remained at basal levels, indicating that casein kinase II inhibition only affects the action of vasopressin, not the basic trafficking processes. The PKC-specific nature of the CKII inhibition was confirmed by the previous lack of effect observed on UT-A3 membrane expression in basal conditions (Fig. 3B). The effect of casein kinase II inhibition on AVP-stimulated trafficking of UT-A3 to the plasma membrane was further confirmed by immunolocalization studies (Fig. 6), which also reiterated that mUT-A3 localizes to the basolateral membranes. This conclusion for the involvement of casein kinase II in the vasopressin regulatory pathway again agrees with previous reports. For example, 4-h treatment with vasopressin causes increases in protein and mRNA levels of both urea transporters and casein kinase II within the renal medulla of Brattleboro rats (3). In addition, vasopressin-treated IMCD tissue contains urea transporters (unknown whether to be UT-A1, UT-A3, or both) phosphorylated at sites S62 and S63, which are casein kinase II phosphorylation sites (14). Furthermore, there are other casein kinase II sites, including one in the COOH terminus of UT-A3 at amino acids 452–456, which may also be involved. Further studies are therefore now required to investigate the precise mechanisms and phosphorylation sites by which casein kinase II regulates vasopressin-stimulated localization of IMCD urea transporters.

This study shows that the mechanism for the change in UT-A3 localization is not the conventional IMCD membrane trafficking observed for the water channel aquaporin-2, since the non-muscle myosin II inhibitor blebbistatin has no effect on UT-A3 localization changes. Our data support the preliminary findings of Chou et al. (6) who reported that blebbistatin does not inhibit vasopressin-stimulated increases in rat IMCD urea permeability. In addition, our results suggest that calmodulin is also vital for UT-A3 to be trafficked correctly to the plasma membrane (Fig. 7). As the data for calmodulin inhibition are very similar to that obtained for PKC inhibition, we therefore can make the same conclusion that it is involved in the basic trafficking processes, rather than specifically being involved in the vasopressin regulation of these processes. It is unclear at this stage whether calmodulin involvement is simply due to its...
role in the production of cAMP (13), or whether it also has a more direct role in UT-A3 trafficking.

Overall, we are now able to propose a basic model for vasopressin regulation of UT-A3 urea transporters in the IMCD (Fig. 8). Vasopressin, via V2 receptors and adenylyl cyclase, stimulates an increase in cAMP levels. Within 5–10 min, PKA activates UT-A3 urea transporters that are already present within the basolateral membrane, presumably through a phosphorylation event, and stimulates an initial activation of urea transport. Second, within 1 h, stimulation of the trafficking process of UT-A3 occurs via a casein kinase II-dependent mechanism, so that there is a large increase in the expression level of UT-A3 in the basolateral membranes. This increased expression in turn causes a further increase in urea transport. Obviously, further investigation is now required to detail the exact phosphorylation sites, targeting proteins etc. that are involved in these processes. We can therefore see that vasopressin has a biphasic effect on UT-A3 urea transport, consisting of a rapid initial increase, followed by a slower further increase. In this manner, UT-A3 appears to be regulated by vasopressin in a similar manner to UT-A1 (16, 30). It is also interesting to note that 5 μM H89 (i.e., PKA inhibition alone) has been reported to only partially reduce UT-A1 phosphorylation (30), suggesting that UT-A1 may be similar to UT-A3 in being regulated by kinases other than PKA. More importantly, this “two-stage” process of acute vasopressin action on the urea transporters present in the IMCD probably underlies the “biphasic” increase in IMCD urea permeability observed within 1 h of vasopressin addition (28).

In conclusion, this study reports that vasopressin increases basolateral urea transport in MDCK-mUT-A3 cells in part by increasing localization of UT-A3 to the basolateral membrane. Our data suggest that vasopressin produces this effect through a casein kinase II-dependent pathway that regulates a basic trafficking process involving PKC and calmodulin. These findings have important implications to the future research of the vasopressin regulation of the urinary concentrating mechanism and how it controls water balance.

GRANTS

We thank Kidney Research U.K. for funding this work.

REFERENCES


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