Urea Transporter UT3 Functions as an Efficient Water Channel

DIRECT EVIDENCE FOR A COMMON WATER/UREA PATHWAY

(Received for publication, January 30, 1998, and in revised form, February 25, 1998)

Baoxue Yang and A. S. Verkman‡
From the Departments of Medicine and Physiology, Cardiovascular Research Institute, University of California, San Francisco, California 94143-0521.

A family of molecular urea transporters (UTs) has been identified whose members appear to have an exceptionally high transport turnover rate. To test the hypothesis that urea transport involves passage through an aqueous channel, osmotic water permeability was measured in Xenopus oocytes expressing UTs. The UT3 class of urea transporters functioned as efficient water channels. Quantitative measurement of single channel water permeability (p<sub>water</sub>) using epitope-tagged rat UTs gave p<sub>water</sub> (in cm<sup>3</sup>/s × 10<sup>-14</sup>) of 0.14 ± 0.11 (UT2) and 1.4 ± 0.2 (UT3), compared with 6.0 and 2.3 for water channels AQP1 and AQP3, respectively. Relative single channel urea permeabilities (p<sub>urea</sub>) were 1.0 (UT2), 0.44 (UT3), and 0.0 (AQP1). UT3-mediated water and urea transport were weakly temperature-dependent (activation energy <4 kcal/mol), inhibited >75% by the urea transport inhibitor 1,3-dimethylthiourea, but not inhibited by the water transport inhibitor HgCl₂. To test for a common water/urea pore, the urea reflection coefficient (σ<sub>urea</sub>) was measured by independent induced osmosis and solvent drag methods. In UT3-expressing oocytes, the time course of oocyte volume in response to different urea gradients (induced osmosis) gave σ<sub>urea</sub> ~ 0.3 for the UT3 pathway, in agreement with σ<sub>urea</sub> determined by the increase in uptake of <sup>14</sup>C-urea during osmotic gradient-induced oocyte swelling (solvent drag). In oocytes of comparable water and urea permeability coexpressing AQP1 (permeable to water, not urea) and UT2 (permeable to urea, not water), σ<sub>urea</sub> = 1. These results indicate that UT3 functions as a urea/water channel utilizing a common aqueous pathway. The water transporting function and low urea reflection coefficient of UT3 in vasa recta may be important for the formation of a concentrated urine by countercurrent exchange in the kidney.

Several related urea transporters (UTs)<sup>1</sup> have been cloned recently. The UT2 transporter (rat form referred to as rUT2, Ref. 1) was first identified in rabbit by expression cloning (2). The UT2 cDNA encodes a 397-amino acid glycoprotein that is expressed in the medullary portion of descending limbs of Henle in kidney (3, 4). UT1 is larger protein (929 amino acids) that contains the UT2 sequence at its C terminus fused to a 67% identical amino acid sequence at its N terminus (5). UT1 functions a cAMP-regulated urea transporter that is expressed at the apical membrane of inner medullary collecting duct cells. UT1 and UT2 are thought to be derived from a single gene by alternative splicing. A 391-amino acid urea transporter expressed in erythrocytes was initially cloned from human bone marrow (HUT11, Ref. 6) and subsequently from rat kidney (named UT3, Ref. 7). Rat UT3 has 62% amino acid identity to UT2 and is expressed strongly in kidney in descending vasa recta in the inner stripe of outer medulla, as well as in testis and brain (7, 8). It is thought that these urea transporters have an important role in the urinary concentrating mechanism to establish a hypertonic renal medullary interstitium (9).

UT3 was initially recognized as the Kidd antigen (Jk) in erythrocytes (10–12). Humans lacking the Kidd antigen (Jk<sup>–/–</sup>) have low urea/urea urea permeability. These individuals are phenotypically grossly normal, but have a defect in their ability to produce a maximally concentrated urine (13). From the number of Kidd antigen proteins per erythrocyte and the erythrocyte urea permeability, it was estimated that the UT3 transporter rate is 2–15 × 10<sup>6</sup> urea molecules/s (9, 14). This turnover rate is substantially higher than that of usual solute carriers, suggesting a channel mechanism for urea transport. Additional functional data involving urea concentration dependence and trans-inhibition (15) support a channel-type mechanism.

The purpose of this study was to test whether urea transporters of the UT3-type (and possibly the UT2-type) transport urea by a channel mechanism involving urea passage through an aqueous pore. It was found that UT3 functions as an efficient water channel with water transport rates comparable with those of aquaporin-type water channels. Water movement through UT3 was found to be temperature-dependent and inhibited by urea transport inhibitors but not by water transport inhibitors. A key finding was that the reflection coefficient for urea was remarkably less than unity, providing strong evidence for a common water and urea pathway through UT3. These results have important implications regarding urea transporter structure and transporting mechanism and suggest a novel role for UT3 in the urinary concentrating mechanism as a facilitator of urea solvent drag in renal vasa recta.

MATERIALS AND METHODS

cDNA Constructs—Full-length cDNAs encoding rat AQP1 (GenBank™ accession number L07268), AQP3 (GenBank™ accession number D17695), UT2 (GenBank™ accession number U09957), and UT3 (GenBank™ accession number U81518) were polymerase chain reaction-amplified using rat kidney cDNA as template and primers (BanHI or BglII and XbaI engineered restriction sites underlined): AQP1 sense, 5'-CGGATCCCATGGCCACGCCAGTTAAAGAAGA-3'; AQP1-antisense, 5'-GCTCTAGATTTGGGCTTCATCTCCACCCTG-3'; AQP3 sense, 5'-CTAGAGGAGACGTCGTAGGCCTGGTAC-3'; AQP3-antisense, 5'-CTAGAGGAGACGTCGTAGGCCTGGTAC-3'; UT2 sense, 5'-GCTCTAGATTTGGGCTTCATCTCCACCCTG-3'; UT2 antisense, 5'-GCTCTAGATTTGGGCTTCATCTCCACCCTG-3'; UT3 sense, 5'-GCTCTAGATTTGGGCTTCATCTCCACCCTG-3'; UT3 antisense, 5'-GCTCTAGATTTGGGCTTCATCTCCACCCTG-3'. Amplified DNA fragments was confirmed by sequence analysis and

*This work was supported by National Institutes of Health Grants DK53124, HL49288, and DK43840. The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked "advertisement" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

‡ To whom correspondence should be addressed: 1246 Health Science Plaza, University of California, San Francisco, California 94143-0521.

1 The abbreviation used is: UT(s), urea transporter(s).
Urea Transporter UT3 Functions as an Efficient Water Channel

Fig. 1. Water and urea permeability in oocytes expressing aquaporins and urea transporters. A, time course of oocyte swelling at 10 °C in response to a 5-fold dilution of extracellular Barth’s buffer with distilled water. Oocytes were injected with 50 nl of water (as control) or cRNAs (5 ng) encoding AQP1, AQP3, UT2, or UT3. B, averaged water permeability coefficients \( P_w \) for different groups of 10 oocytes. C, percent 90-s uptake of \( \left[{^{14}}\text{C}\right] \)urea (expressed as percentage of uptake at 2 h, mean ± S.E.) at 23 °C in oocytes from the same batches as used in B. D, autoradiogram of proteins from oocyte plasma membranes that were immunoprecipitated with c-Myc antibody.

The significant intrinsic water permeability of UT3 suggests the existence of a continuous aqueous channel through the UT3 protein that passes both water and urea. Studies of temperature dependence, inhibitor specificity, and urea reflection coefficient were done to test this possibility. Fig. 2A shows a weak temperature dependence for UT3-mediated transport of both water and urea.
water and urea. The low Arrhenius activation energy (<4 kcal/mol) is consistent with an aqueous pore pathway and is in agreement with the low activation energies found for several of the aquaporin-type water channels. Fig. 2B indicates that UT3-mediated water and urea transport were each strongly inhibited by the urea analog 1,3-dimethylthiourea and by phloretin. Neither water nor urea transport were inhibited by HgCl2, a potent inhibitor of most aquaporin-type water channels. HgCl2, but not the urea transport inhibitors, strongly inhibited water permeability in oocytes expressing AQP1 (Fig. 2C). Together these results support a common aqueous route for water and urea transport through UT3.

The most direct evidence for a common water/solute pathway is the finding of a low solute reflection coefficient (19, 20). Reflection coefficient measurements have in general been challenging because of the tight coupling between volume and solute transport as described by Equations 1 and 2. Of note, different labs have reported substantially different reflection coefficients for urea transport across erythrocytes (21, 22) and NaCl transport across proximal tubule (23–25). Our strategy to determine the urea reflection coefficient ($\sigma_{\text{urea}}$) in oocytes involved independent measurements of induced osmosis and solvent drag, and quantitative comparison of experimental results with numerical solution of the Kedem-Katchalsky equations.

In the induced osmosis method, oocytes were briefly swelled in 100 mM Barth’s buffer (because oocytes do not shrink well below their normal volume, Ref. 26) and then the external solution was switched to 50 mM Barth’s buffer containing different concentrations of urea. As seen in Fig. 3A (top), oocytes expressing UT3 initially swelled for external [urea] of 200 and 400 mM and shrunk for [urea] of 600 and 800 mM, suggesting $\sigma_{\text{urea}}$ < 1. The same measurements were simulated numerically using Equations 1 and 2 for different values of $\sigma_{\text{urea}}$ (Fig. 3B). There was good agreement between the simulated and experimental data set for $\sigma_{\text{urea}}$ = 0.3 versus 1.0.

An important control study was done to validate the above approach for determination of $\sigma_{\text{urea}}$ and to show that the low $\sigma_{\text{urea}}$ is not a consequence of apparent solvent/solute coupling (“pseudo-solvent drag”) due to unstirred layers. An identical set of measurements (as in Fig. 3A, top) was done in oocytes coexpressing AQP1, which is permeable to water but not urea, and UT2, which is permeable to urea and not water. The amounts of injected cRNAs encoding AQP1 and UT2 were adjusted to give oocyte water and urea permeabilities comparable with those for UT3-expressing oocytes. Fig. 3A (bottom) shows little initial oocyte swelling or shrinking for external [urea] ~200
As an independent method to measure $\sigma_{\text{urea}}$ utilizing solvent drag, the 90-s uptake of $[14C]$urea into oocytes was measured for different external solution osmolalities. For $\sigma_{\text{urea}} < 1$, the osmotic water influx induced by a low external osmolality would result in increased $[14C]$urea uptake compared to that in hypoosmotic external solutions and depressed by a hyperosmolar solution. The model simulations for $\sigma_{\text{urea}} = 0.3$ determined above were in reasonable agreement with the $[14C]$urea uptake data.

Our results provide strong evidence that the UT3 protein is associated with an aqueous channel that transports water and urea in a coupled manner. The high UT3-mediated urea turnover and the trans-stimulation data cited in the Introduction are explainable by a common water/urea channel. Although electron crystallography data suggest the location of a putative aqueous channel, no structural information about the UT proteins to suggest the existence or location of an aqueous channel. Of note, the UT and AQP proteins share no homology. Structure and mutagenesis studies are needed to resolve the mechanism of UT3-mediated water and urea transport.

Although not directly addressed in this study, we speculate about the physiological implications of a common water/urea channel in UT3. UT3 is expressed in descending vasa recta in kidney (7, 8), where it confers high transendothelial urea permeability (29). The countercurrent exchange mechanism in the renal medulla requires the delivery of large quantities of urea and water from the vasa recta to the inner medullary interstitium (30–32). The UT3-mediated solvent drag of urea could provide a simple and elegant solution to the problem of insuring an adequate rate of urea exit from vasa recta to balance osmotically driven water exit.

Acknowledgment—We thank Dr. N. Periasamy for help in software development. The program for computation of coupled water/solute flux in oocytes is available from the authors on request.

REFERENCES

1. Smith, C. P., Lee, W. S., Martial, S., Knepper, M. A., You, G., Sands, J. M., and Hediger, M. A. (1995) J. Clin. Invest. 96, 1556–1563
2. You, G., Smith, C. P., Kanal, Y., Lee, W. S., Stelzner, M., and Hediger, M. A. (1993) Nature 365, 844–847
3. Nielsen, S., Terris, J., Smith, C. P., Hediger, M. A., Ecelbarger, C. A., and Knepper, M. A. (1996) Proc. Natl. Acad. Sci. U. S. A. 93, 5495–5500
4. Shyayakul, C., Knepper, M. A., Smith, C. P., DeGiovanni, S. R., and Hediger, M. A. (1997) Am. J. Physiol. 272, F654–F660
5. Shyayakul, C., Steel, A., and Hediger, M. A. (1996) J. Clin. Invest. 98, 2580–2587
6. Olives, B., Neau, P., Bailly, P., Hediger, M. A., Rousselet, G., Cartron, J. P., and Ripoche, P. (1994) J. Biol. Chem. 269, 31649–31652
7. Tsukaguchi, H., Shyayakul, C., Berger, U. V., Tokui, T., Brown, D., and Hediger, M. A. (1997) J. Clin. Invest. 99, 1506–1515
8. Xu, Y., Olives, B., Bailly, P., Fischer, E., Ripoche, P., Ronco, P., Cartron, J. P., and Rondeau, E. (1997) Kidney Int. 51, 138–146
9. Sands, J. M., Timmer, R. T., and Gunn, R. B. (1997) Am. J. Physiol. 273, F321–F339
10. Frohlich, O., Macey, R. I., Edwards-Moulds, J., Gargus, J. J., and Gunn, R. B. (1991) Am. J. Physiol. 260, F778–F783
11. Olives, B., Mattei, M. G., Hue, M., Neau, P., Martial, S., Cartron, J. P., and Bailly, P. (1995) J. Biol. Chem. 270, 15657–15660
12. Edwards-Moulds, J., and Kasschau, M. R. (1988) Vox. Sang. 55, 181–185
13. Sands, J. M., Gargus, J. J., Frohlich, O., Gunn, R. B., and Kokko, J. P. (1992) J. Am. Soc. Nephrol. 2, 1689–1696
14. Manouzeu, I. M., Moronne, M. M., and Nichols, M. E. (1993) J. Membr. Biol. 133, 85–97
15. Yousef, L. W., and Macey, R. I. (1989) Biochim. Biophys. Acta 954, 281–288
16. Yang, B., and Verkman, A. S. (1997) J. Biol. Chem. 272, 16140–16144
17. Zhang, R., Logee, K., and Verkman, A. S. (1990) J. Biol. Chem. 265, 15375–15378
18. Kedem, O., and Katchalsky, A. (1958) Biochim. Biophys. Acta 27, 229–246
19. Finkelestein, A. (1987) Water Movement through Lipid Bilayers, Pores, and Plasma Membranes: Theory and Reality, John Wiley & Sons, Inc., New York
20. Verkman, A. S. (1989) Am. J. Physiol. 257, C837–C850
21. Goldstein, D. A., and Solomon, A. K. (1960) J. Gen. Physiol. 44, 1–17
22. Mlekoday, H. J., Moore, R., and Levitt, D. G. (1983) J. Gen. Physiol. 81, 213–220
23. Corman, B., and DiStefano, A. (1983) Pfluegers Arch. 397, 35–41
24. Andreoli, T. E., Schafer, J. A., Troutman, L. L., and Watkins, M. L. (1979) Am. J. Physiol. 237, F455–F462
25. Shi, L. B., Fushimi, K., and Verkman, A. S. (1991) J. Gen. Physiol. 98, 379–398
26. Zhang, R., and Verkman, A. S. (1991) Am. J. Physiol. 260, C267–C34
27. Cheng, A., Van Hoek, A. N., Yaeger, M., Verkman, A. S., and Mitra, A. K. (1997) Nature 387, 627–630
28. Wals, T., Hirai, T., Murata, K., Heymann, J. B., Mitsuoka, K., Fujiyoshi, Y., Smith, B. L., Agre, P., and Engel, A. (1997) Nature 387, 624–627
29. Pallone, T. L. (1994) Am. J. Physiol. 267, R260–R267
30. Kokko, J. P., and Rector, F. C. (1972) Kidney Int. 2, 214–223
31. Stephenson, J. L. (1972) Kidney Int. 2, 85–94
32. Knepper, M. A., and Nielsen, S. (1993) Am. J. Physiol. 265, F214–F224