Expression and Functional Analysis of Water Channels in a Stably AQP2-transfected Human Collecting Duct Cell Line*

Giovanna Valenti‡§, Antonio Frigeri‡, Pierre M. Ronco*, Cinzia D'Ettorre‡, and Maria Svelto‡

From the ‡Istituto di Fisiologia Generale, Università degli Studi di Bari, Via Amendola 165/A, 70126 Bari, Italy, *Hôpital Tenon, Inserm Unité 64, Paris, France, and †Research Center, Dompé S.p.a., L’Aquila, Italy

In this study, we describe the establishment of a stably transfected epithelial cell line with the cDNA for the rat aquaporin 2 (AQP2). To this end, we used a human cell line (HCD) derived from the cortical collecting duct and having characteristics of principal cells (Prie, D., Friedlander, G., Coureau, C., Vandewalle, A., Cassigena, R., and Ronco, P. M. (1995) Kidney Int. 47, 1310–1318). The HCD cells were first screened for the constitutive expression of AQPs. By Western blot analysis, we found a low expression of immunoreactive AQP2 and AQP4 proteins. In contrast, transfected cells (clone CD8) probed with AQP2 antiserum expressed an intense 29-kDa protein on immunoblot in addition to a broad band between 35–45 kDa corresponding to the glycosylated form of the protein, indicating that full maturity of the protein is attained in transfected cells. Immunofluorescence demonstrated that AQP2 was located in intracellular vesicles. After vasopressin stimulation, the staining redistributed from an intracellular site to the apical pole of the cells, an effect similar to that described on collecting duct principal cells in vivo (Sabolic, I., Katsura, T., Verbavatz, J. M., and Brown, D. (1995) J. Membr. Biol. 143, 165–175) and in perfused tubules (Nielson, S., Chou, C. L., Marples, D., Christensen, E. L., Kishore, B. K., and Knepper, M. A. (1995) Proc. Natl. Acad. Sci. U. S. A. 92, 1012–1017).

The redistribution of AQP2 in CD8 cells was accompanied by an approximately 6-fold increase in osmotic water permeability coefficient ($P_w$), which was inhibited by 0.3 mM HgCl$_2$. These data indicate that functional vasopressin-sensitive water channels are expressed in transfected cells. The stably transfected cells represent a suitable model to unravel by direct experimental approach the intracellular signals involved in the translocation of AQP2 to the apical plasma membrane in the presence of vasopressin.

The mammalian kidney plays the principal role in the regulation of water balance. The coordinate function of different portions of the renal tubule allows the nephron to accomplish this task. Insight into mechanisms underlying kidney water transport physiology is principally based on experiments performed on a microperfused portion of the nephron (4). In humans, indirect data can be provided by clearance studies (5), but it is difficult to evaluate the individual role of a specific nephron segment. During the last 3 years, it became clear that different segments of the nephron express homologous proteins, the aquaporin (AQP) water channels, endowed with the apparently similar function of water transport (6), which makes it difficult to extrapolate the contribution of a particular water channel in the overall function of the tubule.

Heavy expression of AQP1 in both apical and basolateral membranes of proximal tubule and descending limb was found (7, 8). Those segments of the nephron possess a high constitutive water permeability that accounts for the nearly isotonic water reabsorption of up to 70% of glomerular filtrate. Three aquaporins are expressed in collecting duct principal cells: AQP2, AQP3, and AQP4. AQP2 is predominantly localized to the apical plasma membrane and subapical vesicles of the collecting duct principal cells (9, 10). Several lines of evidence indicate that AQP2 is the vasopressin-regulated water channel. Studies on isolated perfused tubules have demonstrated that regulation of collecting duct water permeability by vasopressin occurs through the translocation of AQP2 water channels from intracellular vesicles to the apical membrane (3). AQP3 and AQP4 are found in the basolateral membrane of collecting duct principal cells (11–17). The physiological relevance of the expression of homologous proteins having the same apparent function to transport water is still unexplained. Because of the complexity of the overall organization of the water transport regulation in the renal tubules, the availability of a cell culture system in which a pathway for water channels is retained “in vitro” would provide a powerful cellular tool for physiological studies.

Recently, the establishment of the first human cell line deriving from cortical collecting duct (HCD) has been described (1). HCD is a polarized epithelial cell line immortalized by SV40 virus and expressing principal cell characteristics. Cells are sensitive to vasopressin which strongly stimulates intracellular cAMP production. In addition, HCD cells express both A1 and A2 receptors for adenosine which have been demonstrated to modulate water reabsorption in the rabbit cortical collecting duct (18). In an attempt to search for a valuable cell culture system to study the cell biology of water channels, we tested the expression of members of the aquaporin family in HCD cells. Our findings demonstrate that HCD is the first cell line in which the AQP2 and AQP4 are constitutively expressed, although in a very low amount. In order to make this cell line suitable for cell biology studies of vasopressin-regulated water channel, HCD cells were stably transfected to overexpress AQP2. Transfected cells probed with AQP2 antiserum, intensely expressed a 29-kDa protein on immunoblot in addition to a broad band between 35 and 45 kDa which corresponds to the glycosylated form of the AQP2 protein. In addition, our

* §This work was supported by Grant 95.1726.CT04 from Consiglio Nazionale delle Ricerche-Italy. Part of this work was presented at the Aquaporins and Epithelial Water Transport International Symposium, Manchester, United Kingdom, September 13, 1995. 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.

1 The abbreviations used are: AQP, aquaporin; hAQP, human AQP; rAQP, rat AQP; PBS, phosphate-buffered saline; TIR, total internal reflection microfluorimetry.
results show that AQP2 is redistributed from an intracellular site to the apical pole of the cells after vasopressin stimulation and this effect is accompanied by a dramatic increase of the osmotic water permeability coefficient. These data suggest that transfected cells represent an interesting cell culture model for studying water channel biology.

**EXPERIMENTAL PROCEDURES**

**Cell Culture**

The human collecting duct HCD cell line was established from the normal part of a kidney removed for a localized adenocarcinoma as described previously (1). HCD were cultured at 37°C in a hormonally defined medium (DNEM-Ham’s F-12 1:1 (v/v), 5 μg/ml transferrin, 50 nm sodium selenate, 2 mM glutamine, 5 × 10^-5 M dexamethasone, 5 μg/ml insulin, and 20 mM Hepes, pH 7.4) containing 2% of newborn calf serum.

**Immunofluorescence**

Rat kidney cryostat sections (4 μm) were prepared as described previously (19). Sections were placed on Superfrost/Plus Microscope Slides (Fisher Scientific, Pittsburgh, PA), kept in PBS for 10 min, preincubated for 15 min with 1% BSA in PBS, and then incubated at room temperature for 2 h with either preimmune or immune serum at the dilutions indicated in the figure legends. Sections were washed twice for 5 min in PBS containing 2.7% NaCl (high salt PBS) and twice in regular PBS. The sections were then incubated for 60 min with fluorescein-conjugated goat anti-rabbit IgG (10 μg/ml in PBS, Sigma), followed by washing twice for 5 min in high-salt PBS and twice in normal PBS.

Cells grown on glass coverslips were fixed in a mixture containing 2% paraformaldehyde, 10 mM sodium periodate, and 75 mM lysisine for 20 min. Cells were rinsed 3 × for 5 min in PBS and permeabilized with 0.1% Triton X-100 in PBS for 4 min. After blocking in 0.1% gelatin in PBS for 15 min, cells were incubated at room temperature for 2 h with AQP2 affinity-purified antibodies. Cells were rinsed 3 × 5 min with 0.1% gelatin in PBS and incubated for 60 min with fluorescein-conjugated goat anti-rabbit IgG (10 μg/ml in PBS, Sigma), followed by washing twice for 1 min in high salt PBS and twice in regular PBS. The coverslips or the slides were mounted in 50% glycerol in 0.2 mM Tris-HCl, pH 8.0, containing 25% n-propyl gallate to retard quenching of the fluorescence. The samples were examined with a Nikon FX photomicroscope equipped for epifluorescence and photographed using Kodak T-Max 400 film.

**Video Confocal Microscopy**

Video confocal microscopy was used to determine the targeting of AQP2 in transfected cells after vasopressin treatment. In the latter instrumentation, a novel imaging technique (20, 21) was used with arc lamps in place of lasers. Cells grown on coverslips were fixed and processed for immunocytochemistry as described above and examined by video confocal microscopy. Multi-point illumination and fluorescence detection permit joint capabilities, such as improved resolution confocal performance and limited specimen invasiveness. UV excitation of fluorophores and multi-color imaging are eventually possible. Images were taken in the xy plane at steps of 320 nm, using an oil-immersion objective (40 × 1.40 normal aperture); z sections were extracted from the set of planar images, with the overall depth corresponding to 6 μm approximately.

**Production of Polyclonal Antibodies**

Rabbit antisera were raised against the synthetic peptides corresponding respectively to the 15 COOH-terminal amino acids of rat AQP2 (CELHSPQSPLRPSGKA), rat AQP3 (EAEVKLAMHKHKKQ), and rat AQP4 (CDEKKKGDSVGSLSV). The Multiple Antigen Peptide system was used for the preparation of anti-peptide antibodies. This approach uses a small polypeptidic core matrix bearing radially branching synthetic peptides as dendritic arms, as described previously (22). Anti-AQP1 antibodies were raised against AQP1 purified from human red blood cells according to Zeidel et al. (23). Anti-human AQP2 antibodies, raised against a synthetic peptide corresponding to the 15 COOH-terminal amino acids of human AQP2, were gently provided by Dr. H. Knorr (Gießen, Germany).

New Zealand White rabbits were immunized subcutaneously with the conjugated peptides (200 μg) in complete Freund’s adjuvant (1:1) and subsequently in incomplete adjuvant for booster immunizations. Serum was collected from after three booster injections and checked for the presence of specific antibodies by dot-blot analysis.

The antiserum was affinity-purified by a passage over a SulfoLink coupling gel column (Pierce) to which AQP2 peptide had been attached covalently. The purified anti-AQP2 antibodies were eluted at pH 2.5, followed by rapid titration to pH 7.5.

**Plasmid Construction and Transfection**

The cDNA coding for rat AQP2 was amplified by PCR and ligated into expression vectors pcDNA3 containing the cytomegalovirus promoter and the gene for resistance to Geneticin. Transfection was performed by use of Lipofectin (24). Cells were plated in 100-mm dishes for 12 h before transfection. Twenty μg of Lipofectin was diluted into 1 ml of serum-free medium and with 1 ml of medium containing the recombinant plasmid. The mixture was added to cells and incubated for 12 h at 37°C. The cells were grown for 2 days and then trypsized and transferred to three-10-mm dishes. A selection of cells containing transfected DNA was obtained with a medium containing 500 μg/ml Life Technologies, Inc.) for 10–15 days. Resistant clones were isolated and transferred for expansion and analysis. One of these clones, referred to as CD8, was used in subsequent studies.

**Northern Blot Analysis**

Total RNA was extracted from 107-108 HCD or CD8 cells using a Qiagen kit. Thirty μg of total RNA were electrophoresed in formaldehyde-agarose gels and blotted onto a nylon membrane. Hybridization was performed at high stringency for 18 h with the full-length AQP2 cDNA probe labeled with [α-32P]dCTP (Amersham Corp.) and prepared by random priming (Amersham Corp.). Hybridization was visualized by autoradiography.

**SDS-Polyacrylamide Gel Electrophoresis and Western Blotting**

**Preparation of Cell Homogenates**—Cells were subcultured for serial passages and used from confluent monolayer grown in 25-cm² culture flasks. For homogenate preparations, cells were washed three times in PBS and resuspended in ice-cold lysis buffer containing 50 mM Tris, 110 mM NaCl, 0.5% Triton X-100, 0.5% Nonidet P-40, and 2 mM phenylmethylsulfonyl fluoride, pH 8. Cell suspension was incubated on ice for 1 h and vortexed several times. Insoluble material was pelleted at 11,000 × g, and protein content was determined by the method of Lowry et al. (25). Cell homogenates were stored at −20°C until used for immunoblotting studies.

**Preparation of Membranes**—For crude membrane preparation, the rat kidney papilla or the rat brain were removed, cut into small slices, and homogenized in ice-cold buffer containing 300 mM mannitol and 12 mM HEPES-Tris, pH 7.4. All subsequent steps were performed at 4°C. The suspension was centrifuged at 2500 × g for 15 min, and the pellet containing nuclei and unbroken cells was discarded. The supernatant was spun down at 47,000 × g for 45 min, and the pellet was resuspended in PBS/0.1 mM phenylmethylsulfonyl fluoride. For the detection of AQP3 and AQP4, papain homogenate was spun at low speed (1000 × g) for 10 min to remove nuclei and incompletely homogenized fragments; then the supernatant was centrifuged at 17,000 × g, and the pellet was resuspended in PBS/0.1 mM phenylmethylsulfonyl fluoride.

Membranes or cell homogenates were dissolved in SDS loading buffer, heated to 60°C for 10 min, and separated by electrophoresis on 13% SDS-polyacrylamide gel electrophoresis slab gels and transferred to Immobilon-P membrane. To check the efficiency of the transfer, membrane was briefly stained with Coomassie Blue, destained, blocked in blotting buffer containing 5% nonfat dry milk, 150 mM NaCl, 1% Triton X-100, and 20 mM Tris-HCl, pH 7.4, for 60 min and incubated for 2 h or overnight with either preimmune or immune serum (1:500 diluted in blotting buffer). Membranes were then washed in several changes of blotting buffer and incubated for 60 min with alkaline phosphatase conjugated goat anti-rabbit antibody (Sigma) diluted 1:5000 in blotting buffer, washed again, and revealed for alkaline phosphatase using 0.56 mM 5-bromo-4-chloro-3-indolyl phosphate, 0.48 mM nitro blue tetrazolium in 10 mM Tris-HCl, pH 9.5 (Jansen Pharmaceutica).

**Osmotic Water Permeability Measurement by Total Internal Reflection Microfluorimetry (TIR)**

The osmotically induced cell volume changes were measured by total internal reflection microfluorimetry. This method allows the measurement of small volume changes in adherent cells, as described previously (26). Cells were grown to confluence on 20-mm circular glass coverslips and washed in PBS; then the cytosolic compartment was labeled by incubation of a cytosolic fluorescent marker 5-(and-6)-chloromethyl-benzoyl-amino-tetramethylrhodamine at a concentration of 10 μM for 20 min at 37°C. The coverslip was mounted in a chamber, and fluorescence was excited by a laser beam at 546 nm. The fluorescence
signal was collected by a ×20 objective, filtered, and detected by a photomultiplier.

The osmotic water permeability coefficient \( P_f \) was calculated from the exponential time constant, \( \tau \), by the relation \( P_f = \left( \frac{A/V_0}{V_w} \right) \tau \left( \frac{V_w}{V_0} \right)^{-1} \), where \( A/V_0 \) is the cell surface:volume ratio measured by serial confocal images, \( V_w \) is the partial molar volume of water (18 cm\(^3\)/mol), and \( V_0 \) is the initial perfusate osmolality.

**Materials**

Synthetic peptides were synthesized by Research Center, Dompe’ S.p.a. (L’Aquila, Italy). The tetramethylrhodamine derivative, 5-(and-6)-4-chloromethyl-benzoyl-amino-tetramethylrhodamine, was purchased from Molecular Probe.

**RESULTS**

Expression of Water Channels in HCD Cells—Since vasopressin strongly stimulates cAMP production in HCD cells, we investigated whether this cell line could represent a cell culture system in which a regulated pathway for water channels is retained.

Because water transport is a highly polarized process, we first verified that HCD is a polarized epithelial cell line. Fig. 1 shows an electron micrograph of HCD cells grown on plastic dishes. The cells appear to be polarized, as illustrated by apical microvilli and lateral intercellular digitations.

We then began to examine whether these cells express the AQP2 water channel. To this end, we raised polyclonal antibodies against a synthetic peptide corresponding to the 15 COOH-terminal amino acids of rat AQP2 (rAQP2). The specificity of the antibodies was assessed by Western blot and by immunocytochemistry on rat kidney frozen sections. The fluorescent staining of rat kidney section was localized to collecting duct only. The staining was confined to the apical region of a subpopulation of collecting duct epithelial cells, which most likely correspond to principal cells (Fig. 2A). When these antibodies were probed on human kidney, a similar fluorescence pattern was observed, although the staining was much weaker (data not shown). Western blot analysis of rat kidney papilla membrane preparation revealed that these antibodies detected a band at 29 kDa and a diffuse band at 35–45 kDa, which was shown to represent a glycosylated form of the 29-kDa protein (27). No bands were detected in the same preparation by preimmune serum (Fig. 2B).

Fig. 3A shows the Western blot analysis of HCD cell homogenate probed with rAQP2. A band at 29 kDa was detected by anti-rAQP2 antibodies that comigrated with that obtained from rat kidney papilla membrane preparations. Similar results were obtained when HCD cell homogenate was probed with hAQP2 antiserum (Fig. 3B). This band comigrated with the 29-kDa band recognized by hAQP2 antiserum in human papilla cell membrane preparation (Fig. 3B). In contrast, AQP1 antibodies failed to detect any bands in HCD cell homogenate (Fig. 3B), indicating that, as in parental cells, AQP1 protein is not expressed in HCD cells. The same figure shows that AQP1 antiserum recognizes both the glycosylated and not glycosylated form of the human AQP1 in the membrane preparation from human kidney papilla.

Immunofluorescence on HCD cells, using rAQP2 affinity-purified antibodies, showed that AQP2 was localized intracellularly, often close to the nucleus. No staining of the plasma membrane was detected (Fig. 4). Control experiments with peptide adsorbed serum were negative (Fig. 4). Identical results were obtained using hAQP2 antibody (data not shown).

We also examined HCD cells for the presence of AQP3 and AQP4. Immunoblot analysis using AQP4 antiserum raised against specific peptides corresponding to the respectively cloned rat proteins showed that a weak AQP4-like protein at 30 kDa is recognized in HCD cell homogenate (Fig. 5B). This protein had a similar migration as in the rat brain and in rat kidney papilla, where AQP4 is highly expressed (16, 28). No bands were recognized in the sample from HCD cell homogenate.
Expression of AQP2 in Transfected Cells—To make this cell line suitable for studying the regulated intracellular trafficking of AQP2, HCD cells were stably transfected with the coding region of AQP2. Clonal cell lines derived from stably transfected HCD cells were expanded and examined for AQP2 mRNA and protein. There was variable levels of expression in cells transfected with the coding region of AQP2. Fig. 6A shows a Northern blot of RNA isolated from one of the clonal cell lines, referred to as clone CD8, probed with $^{32}$P-labeled probe specific for rat AQP2 cDNA. A major band at 1.5 kilobases, corresponding to the predicted size of the rAQP2, was observed. No signal was observed in the HCD cells, indicating that AQP2 mRNA in HCD cells may be present at a concentration below the limit of Northern blot detection.

When whole homogenate from CD8 cells was analyzed by immunoblotting with rAQP2 antisemur, a sharp band at 29 kDa was detected in the lane containing CD8 cell homogenate (Fig. 6B) corresponding to the non-glycosylated AQP2, together with a broad band at 35–45 kDa, corresponding to glycosylated AQP2. These bands comigrated with those obtained from rat kidney papilla membrane preparation, the positive control. The expression of AQP2 was much higher in CD8 cells than in wild-type HCD cells. No bands were detected in the blots of the same samples probed with peptide adsorbed antisemur. These studies demonstrated the expression of rat AQP2 mRNA and immunoreactive protein in CD8 cells.

The localization of AQP2 in CD8 cells was examined by indirect immunofluorescence using affinity-purified antibodies.

In control cells, the staining was almost exclusively localized to intracellular vesicles without any significant staining of plasma membranes (Fig. 7A). After exposure to 10 nM vasopressin for 15 min at 37°C, the staining for AQP2 appeared to redistribute to the apical region of the cells (Fig. 7B). Most of the intracellular staining disappeared, and occasionally, patches of punctate staining were visible. This observation is consistent with a translocation of AQP2 to the apical pole of the cells, similar to that described in collecting duct principal cells in perfused tubules (3). Vasopressin stimulation did not affect the distribution of ZO-1, a tight junction marker, localized to cell periphery of CD8 cells (Fig. 7D versus Fig. 7C).

The apical redistribution of staining for the AQP2 protein after vasopressin stimulation was clearly confirmed by xz reconstruction of planar images obtained with video confocal microscopy (Fig. 8, +VP). In stimulated cells, the labeling for AQP2 is localized to the apical pole of the cells, whereas it appears diffuse in the cytoplasm under control conditions (Fig. 8, Control).

Osmotic Water Permeability Measurements—To examine the water permeability properties of HCD cells versus CD8 cells, the time course of cell swelling in response to changes in perfusate osmolality was measured by TIR fluorescence of 5-(and-6)-4-chloromethyl-benzoyl-amino-tetramethylrhodamine-labeled cells. Fig. 9A shows the time course of TIR fluorescence in HCD cells in response to a 200-mosm outwardly directed NaCl gradient. The experiments were performed at 10°C to reduce the contribution of the diffusional water permeability. The signal produced by TIR illumination was dependent upon perfusate osmolality. The signal decreased upon perfusion with hypoosmotic solution as a consequence of osmotic water influx, cell swelling, and decrease of fluorophore concentration. The rate of the swelling response was slightly but, on average, not significantly increased after vasopressin treatment (+VP).

In contrast, the rate of cell swelling was dramatically increased after vasopressin treatment in CD8 cells. Fig. 9B reports a representative TIR fluorescence time course of CD8 cells in response to a 200-mosm NaCl gradient. In presence of vasopressin (+VP), the rate of cell swelling was remarkably increased. This effect was completely abolished by 0.3 mM HgCl2.

Fig. 9C shows averaged $P_f$ values of CD8 cells. $P_f$ in the CD8 cells was about six times increased by vasopressin treatment, and the increase in $P_f$ was inhibited by HgCl2. In wild-type HCD cells, the calculated $P_f$ value under basal conditions was
comparable to that found in CD8 cells (data not shown).

Taken together, the 6-fold increase in Pf under vasopressin stimulation and the inhibition by HgCl2 indicate that the expressed AQP2 in CD8 cells is a functional vasopressin-inducible water channel and that CD8 cells seem to possess the cellular machinery involved in the correct sorting of the AQP2 water channel to the apical pole.

DISCUSSION

Vasopressin is the principal hormone that regulates the water balance through a modulation of water reabsorption in the kidney. Much effort is now devoted to clarify the complex cellular machinery involved in the targeting of AQP2 from an intracellular site to the apical plasma membrane after vasopressin stimulation. Cell cultures are useful tools to unravel the mechanism of epithelial transport and hormonal regulation at cellular and molecular levels. The homogeneity of cell cultures is a definite advantage for studies of hormone interaction with receptors coupling to triggering steps that transduce the hormonal signal to an intracellular signal. An interesting cell culture model of vasopressin-stimulated water permeability has been reported recently (29). LLC-PK1 cells were stably transfected with cDNA encoding AQP2 tagged with a COOH-terminal c-Myc epitope. Interestingly, in those cells, AQP2 recycling between intracellular vesicles and the plasma membrane was observed. However, the authors reported a mis-sorting of the expressed AQP2 to the basolateral membrane. They postulated that the c-Myc-tagged AQP2 could be responsible for this behavior.

The aim of this study was to establish an epithelial cell line that would stably express large quantities of functional AQP2 in the setting of a principal cell phenotype to be useful for identification of intracellular signals involved in the targeting of the AQP2 water channel to the plasma membrane. To this end, we selected HCD cells, a polarized epithelial cell line that was derived from human cortical collecting duct and has retained characteristics of principal cells (1), such as its hormonal responsiveness to vasopressin which stimulates cAMP synthesis.

We first analyzed the constitutive expression of water channels in HCD cells. A Western blot of HCD cell homogenate demonstrated the expression of AQP2 at the protein level. The staining of this band is weak but disappears using the peptide adsorbed antiserum, indicating that the staining is due to the presence of specific AQP2 antibodies. In addition, we confirmed that a band of identical size and intensity was detected by anti-hAQP2 antibody, suggesting that the low intensity of the recognized band was not due to a poor cross-reactivity of anti-rAQP2 antibody with HCD cells. In agreement with these findings, a functional study showed that vasopressin treatment results only in a slight increase in cell swelling in the wild-type HCD cells.

Immunoblot studies indicated also the presence of an AQP4-like protein in HCD cells. The AQP3 protein, which is also expressed in the basolateral membrane of collecting duct principal cells, does not seem to be expressed in HCD cells, based on Western blot experiments, although the possibility that an anti-rat AQP3 antibody does not cross-react with the human AQP3 cannot be ruled out.

The constitutive expression of AQP2 and AQP4 proteins in HCD cells appears, however, very low. To make this cell line suitable for cell biology study of vasopressin-sensitive water
channels, HCD epithelial cells were, therefore, stably transfected with cDNA encoding rat AQP2. High levels of AQP2 mRNA and protein were expressed in HCD cells that were transfected with AQP2 cDNA in plasmids harboring cytomegalovirus promoter. The transfected cells were referred to as CD8. This clone displays several features that make it unique and particularly useful for studies on the function and trafficking of the water channel AQP2. (a) It expresses the glycosylated and nonglycosylated forms of the AQP2, both recognized by specific antibodies raised against the COOH-terminal end of the protein, which indicates that full maturity of the protein is attained in transfected cells. (b) Light and video confocal microscopy indicated that AQP2 was translocated from an intracellular site to the apical pole of the cells after vasopressin stimulation, an effect similar to that described in collecting duct principal cells in vivo (2) and in perfused tubules (3). (c) The redistribution of AQP2 in CD8 cells was accompanied by a dramatic increase in osmotic water permeability, as demonstrated by TIR experiments, consistent with a high level of functional AQP2 expression in transfected cells. The osmotic water permeability coefficient $P_f$ in vasopressin-stimulated CD8 cells was more that six times greater than that calculated under basal conditions. This effect of vasopressin was abolished by HgCl$_2$, which should not affect the functionality of AQP4, the mercurial-insensitive aquaporin, which appears to be also expressed in HCD cells. In contrast, in the corresponding native cell line HCD, vasopressin treatment resulted in a slight but, on average, not significant increase of osmotic water permeability coefficient.

In conclusion, the stably transfected cell line developed here exhibited the major features described in the parental collecting duct principal cells: 1) sensitivity to vasopressin; 2) expression of AQP2 in intracellular vesicles, which are shuttled to the apical pole after hormonal stimulation; and 3) dramatic, vasopressin-dependent increase in osmotic water permeability coefficient. To our knowledge, it represents the first cell culture model expressing functional AQP2 that is targeted to the apical membrane of the epithelial cell upon vasopressin exposure. The stably transfected cells can provide important direct experimental evidence for the intracellular signals involved in the translocation of AQP2 to the apical plasma membrane in presence of vasopressin.

Acknowledgments—We thank Dr. Pio Benedetti for video confocal experiments. In addition, we thank Drs. Giuseppe Procino, Paola Nicchia, and Barbara Landolfi for their valuable contribution to this work.

REFERENCES
1. Prié, D., Friedlander, G., Courbe, C., Vandewalle, A., Cassigena, R., and Ranc, P. M. (1995) Kidney Int. 47, 1310–1318
2. Sabolic, I., Katsura, T., Verbal, J. M., and Brown, D. (1995) J. Membr. Biol. 143, 165–175
3. Nielsen, S., Chou, C. L., Marples, D., Christensen, E. I., Kishore, B. K., and Knepper, M. A. (1995) Proc. Natl. Acad. Sci. U.S.A. 92, 1013–1017
4. Verkman, A. S. (1989) Am. J. Physiol. 257, C837–C850
5. Chabardes, D., Gagnan-Brunette, M., Imbert-Teboul, M., Gentcharevskiaia, O., Monseigut, M., Clique, A., and Moref, F. (1980) J. Clin. Invest. 65, 439–448
6. Ager, P., Brown, D., and Nielsen, S. (1995) Curr. Opin. Cell Biol. 7, 472–483
7. Sabolic, I., Valenti, G., Verbalat, J. M., Van Hock, A. N., Verkman, A. S., Ausiello, D. A., and Brown, D. (1992) Am. J. Physiol. 263, C1225–C1233
8. Nielsen, S., Smith, B. L., Christensen, E. I., Knepper, M. A., and Ager, P. (1993) J. Cell Biol. 120, 371–383
9. Fushimi, K., Uchida, S., Hara, Y., Hirata, Y., Marumo, F., and Sasaki, S. (1995) Nature 361, 549–552
10. Nielsen, S., DiGiovanni, S. R., Christensen, E. I., Knepper, M. A., and Harris, H. W. (1993) Proc. Natl. Acad. Sci. U.S.A. 90, 11663–11667
11. Ishibashi, K., Sasaki, F., Fushimi, K., Uchida, S., Kuwahara, M., Saito, H., Parakawa, T., Nakajima, K., Yamaguchi, Y., Gojo, T., and Murumo, F. (1994) Proc. Natl. Acad. Sci. U.S.A. 91, 6269–6273
12. Eshevarria, M., Windhager, E. E., Tate, S. S., and Frindt, G. (1994) Proc. Natl. Acad. Sci. U.S.A. 91, 10949–10953
13. Ecelbarger, C., Terris, J., Frindt, G., Eshevarria, M., Marples, D., Nielsen, S., and Brown, M. A. (1995) Am. J. Physiol. 269, F663–F671
14. Frigeri, A., Gropper, M. A., Umenishi, F., Kawashima, M., Brown, D., and Verkman, A. S. (1995) J. Cell Sci. 108, 2993–3002
15. Frigeri, A., Gropper, M. A., Turk, C. W., and Verkman, A. S. (1995) Proc. Natl. Acad. Sci. U.S.A. 92, 4328–4331
16. Jung, J. S., Bhat, R. V., Preston, G. M., Guggino, W. B., Baraban, J. M., and Agre, P. (1994) Proc. Natl. Acad. Sci. U.S.A. 91, 13052–13056
17. Terris, J., Ecelbarger, C. A., Marples, D., Knepper, M. A., and Nielsen, S. (1995) Am. J. Physiol. 267, F775–F785
18. Dillingham, M. A., and Anderson, R. J. (1995) J. Membr. Biol. 88, 277–281
19. Valenti, G., Verbalat, J. M., Sabolic, I., Ausiello, D. A., Verkman, A. S., and Brown, D. (1991) Am. J. Physiol. 267, C812–C820
20. Benedetti, P. A., Evangelista, V., Guidarini, D., and Vestri, S. (1995) SPINE Proc. 2413, 56–62
21. Benedetti, P. A., Evangelista, V., Guidarini, D., and Vestri, S. (1995) Zool. Stud. 34, Suppl. 1, 186–188
22. Tam, J. P. (1988) Proc. Natl. Acad. Sci. U.S.A. 85, 5497–5413
23. Zeidel, M. L., Ambudkar, S. V., Smith, B. L., and Agre, P. (1992) Biochemistry 31, 7435–7440
24. Fedner, P. L., and Ringold, G. M. (1989) Nature 337, 387–388
25. Lowry, O. H., Rosebrough, N. J., Farr, A. L., and Randall, R. J. (1951) J. Biol. Chem. 193, 265–275
26. Farinas, J., Simaneke, V., and Verkman, A. S. (1995) Biochim. Biophys. Acta. 387, 1613–1620
27. Fushimi, K., Yamamoto, T., Hayashi, M., Furukawa, T., Uchida, S., Kuwahara, M., Ishibashi, K., Kawasaki, M., Kihara, I., and Murumo, F. (1994) Am. J. Physiol. 267, F573–F582
28. Hasegawa, H., Ma, T., Skach, W., Matthy, M. A., and Verkman, A. S. (1994) J. Biol. Chem. 269, 5497–5500
29. Katsura, T., Verbalat, J. M., Farinas, J., Ma, T., Ausiello, D., Verkman, A. S., and Brown, D. (1995) Proc. Natl. Acad. Sci. U.S.A. 92, 7212–7216