Biochemical and Biophysical Research Communications **256,** 240–248 (1999)
Article ID bbrc.1999.0306, available online at http://www.idealibrary.com on IDEAL®

Aquaporin-1 Expression in Proximal Tubule Epithelial Cells of Human Kidney Is Regulated by Hyperosmolarity and Contrast Agents

Wheamei Jenq,*,†,[1] Denise R. Cooper,†,‡ Polly Bittle,*,† and German Ramirez*,†

Divisions of Nephrology and Hypertension, James A. Haley VA Medical Center, †Department of Internal Medicine, and †Department of Biochemistry and Molecular Biology, University of South Florida, Tampa, Florida 33612

Received January 25, 1999

Primary cells of renal proximal tubule epithelium (S1 segment) of human kidney (HRPTE cells) up-regulate aquaporin-1 (AQP-1) expression in response to hyperosmolarity. NaCl and D(+)-raffinose increased (2-2.5 fold) AQP-1 expression when medium osmolarity was 400 and 500 mOsm/kg.H_2O. Urea did not have this effect. Unlike our previous findings with mIMCD-3 cells, vasopressin (10^{-8}M) did not affect AQP-1 expression in HRPTE cells in isosmolar or NaCl-enriched hyperosmolar conditions. Furthermore, HRPTE cells increased (3-4 fold) AQP-1 expression when exposed to hyperosmolar Reno-60 and Hypaque-76 (diatrizoates, ionic) contrast agents at 400 and 500 mOsm/kg.H_2O. Isosmolar (290 mOsm/kg H_2O) Visipaque (iodixanol, non-ionic) at 10% (v/v) concentrations also increased AQP-1 expression, and 25% v/v of Visipaque rendered morphological alterations of HRPTE cells and a 3-fold increase in AQP-1 expression after 24h exposure. Finally, semi-quantitative RT-PCR of HRPTE cells subjected to various isosmolar or hyperosmolar conditions demonstrated up-regulation of AQP-1 mRNA and protein levels. Our results suggest AQP-1 up-regulation in HRPTE cells exposed to environmental stresses such as hyperosmolarity and high doses of isosmolar contrast agents. © 1999 Academic Press

Aquaporin-1 (AQP-1) was first discovered in the plasma membrane of erythrocytes as a water channel for high osmotic water permeability (1). AQP-1 (CHIP-28) is expressed in various epithelial and capillary endothelial cells, and is abundantly present in kidney proximal tubules and descending thin limbs (1, 2, 3, 4). AQP-1 has been suggested to possibly play a role in "constitutive" water reabsorption, especially in the proximal tubule and the descending limbs of Henle (5, 6).

The vasopressin (AVP) hormone acts primarily in mammalian kidney collecting duct by binding to V2 receptors in the basolateral membranes of principal cells to stimulate adenylate cyclase. This enzyme activation initiates the intracellular trafficking and the insertion of the aquaporin-2 (AQP-2) to the apical membrane for increasing water permeability (7, 8, 9, 10). AVP was also found to augment AQP-1 expression in cultured mIMCD-3 cells under hyperosmolarity condition with NaCl osmolyte, though the mechanism was unknown (11).

A hyperosmolar environment stresses cells due to the osmotic efflux of water that concentrates intracellular salts and as a consequence, affects the structure and activity of the intracellular macromolecules, including proteins and DNA (12). The adaptation of renal medullary cells to hyperosmolarity involves the altered expression of a number of genes including aldose reductase, the transporters for betaine, taurine, inositol, heat shock proteins, and MAP kinases (13). Hyperosmolarity produced by increasing the concentration of NaCl or cell-impermeable D(+)-raffinose stimulates aldose reductase gene transcription, due to the elevated intracellular Na^+ and K^+ concentrations following the initial cell shrinkage (14, 15). However, the hyperosmolal addition of urea cannot increase aldose reductase, implying that hyperosmolarity per se is not sufficient to up-regulate specific gene expressions (15).

It has been documented that a significant amount of AQP-1 is present in the renal proximal tubules (2, 3, 16, 17). This nephron segment, in conjunction with the descending thin limbs, is responsible for an 80-90% fluid reabsorption of glomerular filtrate (18). Our previous results of NaCl (400 or 500 mOsm/kg.H_2O) induced AQP-1 expression in cultured mIMCD cells (11), prompted us to further study the effects of hyperosmolarity on AQP-1 expression in human renal proximal

[1] To whom correspondence should be addressed at Division of Nephrology and Hypertension, James A. Haley V.A. Medical Center, Bldg. #2, Research Service 151, 13000 Bruce B. Downs Blvd., Tampa, FL 33612. Fax: (813) 972-7623. E-mail: wjenq@com1.med.usf.edu.

0006-291X/99 $30.00

Copyright © 1999 by Academic Press
All rights of reproduction in any form reserved.

tubule epithelial (HRPTE) cells. In our experiments, we increased the osmolarity of culture media for HRPTE cells with NaCl, urea, and the impermeant D(+)-raffinose in order to test their osmotic effects on AQP-1 inducibility. In addition, radiopaque contrast agents (CA) have been reported to induce alterations in renal hemodynamics and perhaps have a direct effect of proximal tubular toxicity, especially patients with pre-existing renal insufficiency (19, 20, 21, 22). Previous studies with Madin Darby canine kidney (MDCK) cells by Hizoh *et al.* (23), demonstrated DNA fragmentation, a hallmark feature of apoptosis, in cells exposed to diatrizoates (hyperosmolar CA) and, to a lesser extent, NaCl or mannitol—but not urea, as evidence of renal cell toxicity. In addition, Haller *et al.* (24) indicated that polarized MDCK cells redistribute their surface marker proteins in the presence of CA, and that the ionic diatrizoate is more toxic than the non-ionic iopamidol, partly due to its high osmolarity. The fact that AQP-1 is a major water channel protein in proximal tubules encouraged us to investigate the effect of CA on cellular expression of AQP-1.

MATERIALS AND METHODS

Proximal tubule epithelial cells of human kidney (HRPTE) and antibodies. Normal proximal tubule epithelial cells were originally isolated from segment 1 of the proximal convoluted tubule of normal human kidneys. The primary HRPTE cells are commercially available from Clonetics (San Diego, CA). The cells were maintained in REGM medium (also from Clonetics) and only the cells of the 1st and 2nd passages were used in experiments. The polyclonal rabbit anti-AQP-1 antibody, graciously given to us by Dr. Mark A. Knepper of National Institutes of Health, was used for this study. HRP-conjugated donkey anti-rabbit IgG as the secondary antibody was obtained from Amersham (Arlington, IL).

Isosmolar and hyperosmolar treatments of HRPTE cells. HRPTE cells (3000-3500 cells/CA^2) were seeded to 6-well or 100-mm culture dishes in REGM and were grown to sub-confluency (~80% confluent) prior to various osmolar treatments for up to 24 h. The isosmolar (~290mOsm/kg.H_2O) media include REGM, REGM + PBS (25% v/v), REGM + AVP (10^{-8}M), REGM + Visipaque (iodixanol at 10%, 20%, and 25% v/v), and the 20% v/v of each pre-diluted in sterile water to 290 mOsm/kg.H_2O, Omnipaque (iohexol), Reno-60 (diatrizoate meglumine), and Hypaque-76 (diatrizoate meglumine sodium) in REGM. The hyperosmolar media (400 and 500 mOsm/kg.H_2O) tested in this study were REGM supplemented with NaCl, D(+)-raffinose, urea, Reno-60, and Hypaque-76. Both Visipaque (isosmolar) and Omnipaque (low-osmolar) are non-ionic, whereas the high-osmolar Reno-60 and Hypaque-76 are ionic.

Electrophoresis and immunoblotting. The sub-confluent HRPTE cells (in 6-well dishes) grown in REGM were treated with or without NaCl ± AVP for 6h or 24h. Similarly, the sub-confluent cells were exposed for 24h to the normosmotic media or hyperosmotic NaCl, D(+)-raffinose, urea, or the contrast agents as described above. Total cell lysates were made by adding a 250 μl per well of boiling sample buffer at 2X concentration (1X = 125mM Tris-HCl, pH 6.8, 2% SDS, 5% glycerol, 0.003% bromophenol blue, and 1% β-mercaptoethanol). Rabbit medullary extract (RME) was obtained by homogenization and extraction of the normal rabbit kidney medulla, and was used as the positive control for the presence of AQP-1 water channel protein. Samples of cell lysate were diluted to < 0.05% SDS for protein measurement by BCA method (Pierce, Rockford, IL) or Bradford dye-binding assay (Bio-Rad, Richmond, CA). Total proteins of 5 μg from each sample were electrophoresed in 12% SDS-PAGE alongside with RME and pre-stained protein markers. The proteins on the gels were then blotted electrophoretically onto 0.45 μm nitrocellulose membranes, and the AQP-1 protein bands were probed with anti-AQP-1 antibody, followed by imaging with enhanced chemiluminescence method (Amersham, Arlington, IL) and density analysis by video scanning densitometry.

Total RNA isolation and Reverse Transcription/PCR (RT-PCR). Two million HRPTE cells with or without hyperosmolar treatments (400 mOsm/kg.H_2O) for 24h were each lysed in 1 ml of RNA STAT-60 (TEL-TEST"B," Inc., Friendswood, TX). Each cell lysate was then mixed with 0.2 ml of $CHCl_3$, vigorously shaken for 15 sec, and incubated for 5 min at room temperature before centrifugation at 12,000 xg for 15 min at 4 ºC. The total RNA in the upper aqueous phase was precipitated with isopropanol. The RNA pellets thus obtained were washed once with 75% ethanol, air-dried, and dissolved in diethylpyrocarbonate (DEPC) treated RNase-free water. One μg each of total RNA together with oligo-dT primer and RNaseH$^-$ reverse transcriptase (Life Technologies, NY) were used to generate the first strand cDNA mix. Each cDNA mix was equally divided into two parts, in which each served as a template for an independent polymerase chain reaction (PCR). Sequence-specific primers for human AQP-1 (5′-CTTGGACACCTCCTGGCTATTGAC-3′ and 5′-AGC-AGGTGGGTC-CCTTTCTTTCAC-3′) and for human β-actin (5′-GCA-TCCTCACCCTGAAGTACC-3′ and 5′-GCTGGAAGAGTGCCTCAGG-3′) were employed in PCR, using GeneAmp PCR reagents (Perkin Elmer, Branchburg, NJ). Both sets of primer sequences were derived from the published cDNA sequences of AQP-1 (accession no. M77829) and β-actin (accession no. X00351). The PCR products along with low molecular weight DNA markers (Research Genetics, Huntsville, AL) were analyzed by electrophresis on 1.2% agarose gels. Video scanning densitometry was employed to analyze band density of cDNA fragments.

The effects of osmotic agents and the contrast agents on the morphology of HRPTE cells. Sub-confluent HRPTE cells were exposed for 24h in normosmotic Visipaque (290 mOsm/kg.H_2O, 25% v/v) or hyperosmotic REGM (400 and 500 mOsm/kg.H_2O) which contained NaCl, D(+)-raffinose, urea, Reno-60, or Hypaque-76. The sub-confluent HRPTE cells incubated in the regular REGM growth medium for 24h served as the control. Micrographs of HRPTE cells were taken 24h post treatments, using a Nikon phase-contrast microscope with a 10x objective lens.

RESULTS

AQP-1 protein expression in normal HRPTE cells exposed to AVP and/or NaCl supplemented REGM. As shown in Fig. 1 (upper and lower panels) of Western blotting analyses, AQP-1 up-regulation occurred at least 6h after the cells were exposed to hyperosmotic media with the addition of NaCl (400 or 500 mOsm/kg.H_2O) ± AVP (10^{-8}M). Although a similar level of AQP-1 was present in the normosmotic control medium (Ctl or Ctl + AVP, 290 mOsm/kg.H_2O) after 6h or 24h incubation, a 24h hyperosmolar exposure enabled the cells to increase AQP-1 levels to 2-2.5 fold higher than those of normosmotic Ctl or Ctl + AVP (Fig. 1, upper and lower panels). According to data shown in Fig. 1, under our experimental conditions, AVP had no effect on AQP-1 expression in HRPTE cells in either normosmotic or hyperosmotic condition.

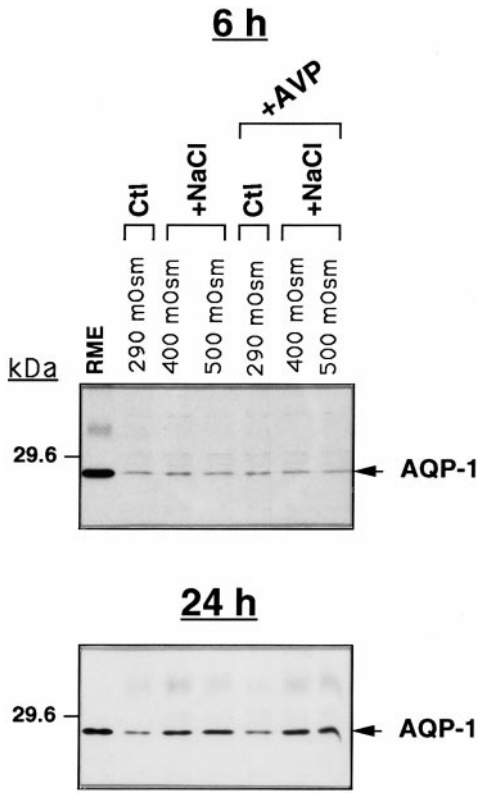

FIG. 1. Western blot analyses of AQP-1 of HRPTE cells under isosmolar (Ctl and Ctl + AVP) or hyperosmolar (NaCl ± AVP, 400 and 500 mOsm/Kg.H_2O) conditions. Despite various treatments, the level of AQP-1 in cells remained rather low and constant after 6h of incubation. However after 24h of challenge with NaCl ± AVP, AQP-1 levels increased to 2-2.5 fold as compared to those of isosmotic controls (Ctl and Ctl + AVP). AVP (10^{-8}M) supplement showed no or insignificant effect on the expression of AQP-1 channel protein, either in isosmolar or hyperosmolar media. The positive controls, AQP-1 in rabbit medullary extract (RME) and a 29.6 kDa protein marker, are shown on the far left. Four separate experiments yielded the representative results as shown.

The AQP-1 expression in HRPTE cells treated for 24h with hyperosmotic agents or impermeant osmolytes. The cell exposure to impermeant D(+)-raffinose spiked media (400 and 500 mOsm/kg.H_2O) triggered the up-regulation (2-2.5 fold increase) of AQP-1, is demonstrated in Fig. 2. Similarly, NaCl supplement in medium to the same hyperosmolarity conferred a similar increase of AQP-1. However, the same hyperosmolarity achieved by the addition of urea, did not affect the AQP-1 expression.

The effects of contrast agents on cellular expression of AQP-1. Figure 3 revealed the AQP-1 up-regulation (3-4 fold increase) in HRPTE cells upon a 24h exposure to various contrast agents (CA) supplemented REGM, under our experimental conditions. Both hyperosmolar Reno-60 and Hypaque-76 (diluted to 400 and 500 mOsm/kg.H_2O) as well as the isosmolar Visipaque (290 mOsm/kg.H_2O, 25% v/v) enabled the cells to increase AQP-1 expression (Fig. 3). The effects of Visipaque at 20% and 25% v/v concentration in REGM as assessed by immunoblotting analyses, indicated up-regulation of AQP-1 channel protein in HRPTE cells (Fig. 3), but this up-regulation occurred to a lesser degree when a lower concentration (10% v/v) was used (data not shown). When Reno-60, Hypaque-76, and Visipaque, in 20% v/v concentration, were included in the media (Reno-60 and Hypaque-76 were pre-diluted to an osmolarity of 290 mOsm/Kg.H_2O), the up-regulation of AQP-1 was only present with Visipaque (Fig. 4). This experiment indicated that Visipaque had a direct stimulatory effect on AQP-1 expression at isosmolar concentrations, while Reno-60 and Hypaque-76 only affected AQP-1 expression by increasing medium osmolarity.

Up-regulation of AQP-1 mRNA levels of osmotically challenged or contrast agents- treated HRPTE cells by semi-quantitative RT-PCR. Our semi-quantitative RT-PCR results of HRPTE cells (24h post-treatments) revealed the discrete cDNA fragments of 557 bp and 603 bp for human AQP-1 and β-actin, the housekeeping control, respectively (Fig. 5, upper and lower panels). There is an approximate 2-fold increase in AQP-1 mRNA in cells stressed with 290 mOsm/kg.H_2O of 10% v/v Visipaque or 400 mOsm/kg.H_2O each of NaCl and NaCl + AVP as opposed to the normosmotic control cells (Ctl-290 mOsm). A very faint band corresponding to AQP-1 cDNA fragment in normosmotic control was present in the agarose gel and the original print (not

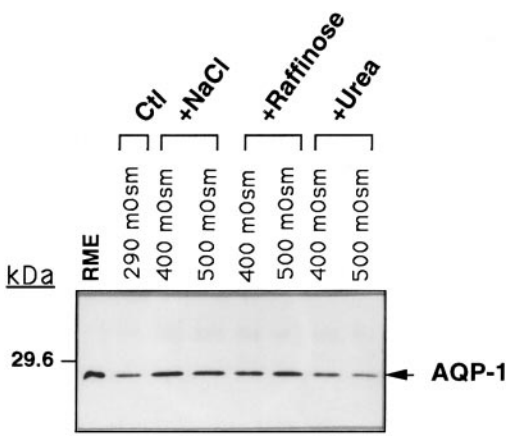

FIG. 2. The effect of osmotic agents on the expression of AQP-1 water channel in HRPTE cells. The addition of either NaCl or impermeant raffinose to REGM growth media, to achieve an osmolarity of 400 or 500 mOsm/kg.H_2O, rendered a 2-2.5 fold increase of AQP-1 expression in cells after a 24h incubation. On the contrary, the hyperosmotic challenge of cells with urea to 400 or 500 mOsm/kg.H_2O showed a similar level of AQP-1 as compared with that of normosmotic control (Ctl) and did not affect the cellular expression of AQP-1. The 28 kDa AQP-1 of rabbit medullary extract (RME) and a protein marker of 29.6 kDa are indicated in the far left. The data shown from video scanning densitometry analyses are representative of four independent experiments.

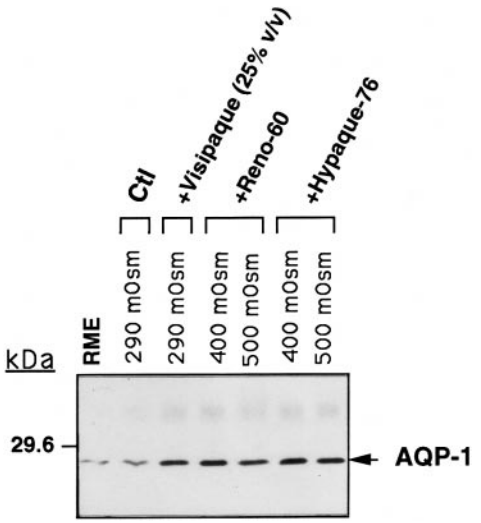

FIG. 3. The impact of ionic hyperosmolar (Reno-60 and Hypaque-76) and non-ionic isosmolar (Visipaque) contrast agents (CA) on the expression of AQP-1 in HRPTE cells. Except Reno-60 supplemented medium of 500 mOsm/kg.H_2O, which conferred cells with a 3-fold increase in AQP-1, supplements of Visipaque (25% v/v), Renal-60 (to 400 mOsm/kg.H_2O), or Hypaque-76 (to 400 and 500 mOsm/kg.H_2O, respectively) resulted in a 3.5-4 fold cellular increase of AQP-1 as compared to that of control (Ctl, 290 mOsm/kg.H_2O). The 28 kDa AQP-1 in RME (as a positive control) and a marker of 29.6 kDa are shown in the far left. According to triplicate experiments and video scanning densitometry, the representative AQP-1 protein bands and data are shown.

shown), though the resolution seemed to be diminished in its photo-reprints. While urea (400 mOsm/kg.H_2O) treatment did not significantly affect the level of AQP-1

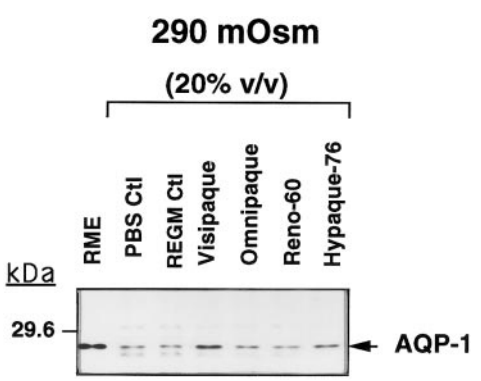

FIG. 4. AQP-1 expression of HRPTE cells in REGM supplemented with isosmolar PBS, Visipaque, or pre-diluted (to 290 mOsm/kg.H_2O in sterile water) Omnipaque, Reno-60 and Hypaque-76, all were 20% v/v of supplemental concentration. The non-ionic Visipaque (64mg I/ml of medium) invoked a 2-2.5 fold AQP-1 increase in cells as opposed to PBS Ctl or REGM Ctl. Whereas, none of the diluted Omnipaque (non-ionic, 26.1mg I/ml of medium), Reno-60 (ionic, 12.1mg I/ml of medium), or Hypaque (ionic, 12.4 mg I/ml of medium) significantly altered the cellular expression of AQP-1 as compared to those of PBS Ctl and RECA Ctl. The AQP-1 of RME and a 29.6kDa protein marker are indicated. Six immunoblots from triplicate experiments yield the representative results as shown.

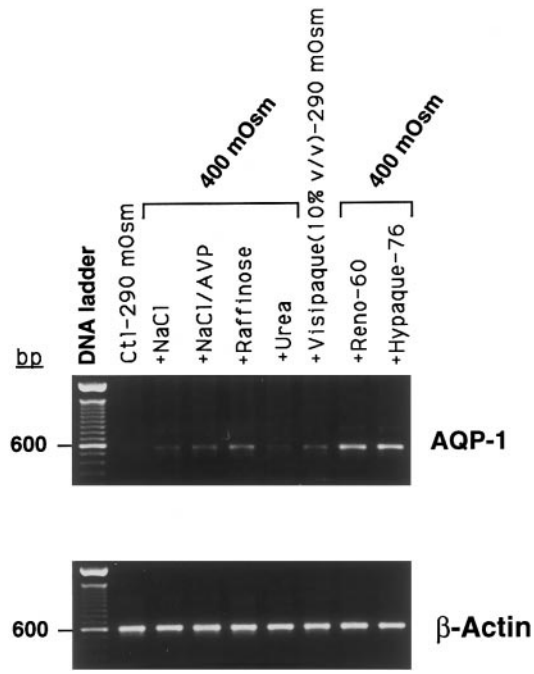

FIG. 5. The semi-quantitative RT-PCR measurements of AQP-1 expression in HRPTE cells in the presence or absence of various osmotic agents and contrast agents for 24h. RT-PCR using AQP-1 specific primers showed, as compared to the normosmotic control cells, an approximate 2-fold increase in AQP-1 mRNA in cells stressed by 10% v/v Visipaque of 290 mOsm/kg.H_2O or 400 mOsm/kg.H_2O each of NaCl and NaCl+AVP. While urea (400 mOsm/kg.H_2O) treatment did not significantly increase the AQP-1 mRNA, D(+)-raffinose rendered a 2.5-fold increase in cellular mRNA level of AQP-1. Furthermore, 24h exposure of cells to either Reno-60 or Hypaque-76 contrast agents of 400 mOsm/kg.H_2O resulted in at least a 4-fold increase in the mRNA level of AQP-1. Nevertheless, the housekeeping β-actin in HRPTE cells remains rather constant despite the treatments. Four independent RT-PCR experiments involving cells of passages 1 and 2, followed by the video scanning densitometry, yield the representative data as shown. DNA markers of 600 bp are indicated in the far left.

mRNA, D(+)-raffinose supplement to 400 mOsm/kg.H_2O hyperosmolarity rendered a 2.5-fold increase in cellular mRNA level of AQP-1. Moreover, a 24h exposure of cells to either Reno-60 or Hypaque-76 CA of 400 mOsm/kg.H_2O revealed at least a 4-fold increase in the mRNA level of AQP-1 (Fig. 5, upper panel). Nevertheless, the level of β-actin in HRPTE cells remained rather constant and is independent of osmolarity changes in the media (Fig. 5, lower panel). The up-regulation of mRNA levels suggests that either transcriptional or post-transcriptional mechanisms increase mRNA abundance.

Morphological alterations of HRPTE cells upon 24h exposure to osmotic agents and contrast agents. Micrographs of HRPTE cells showed similar cell morphology and size (110 $\pm$ 10 cells/ mm^2) when comparing Ctl cells (290 mOsm/kg.H_2O) with cells subjected to hyperosmolarity with NaCl (400 mOsm/kg.H_2O), D(+)-

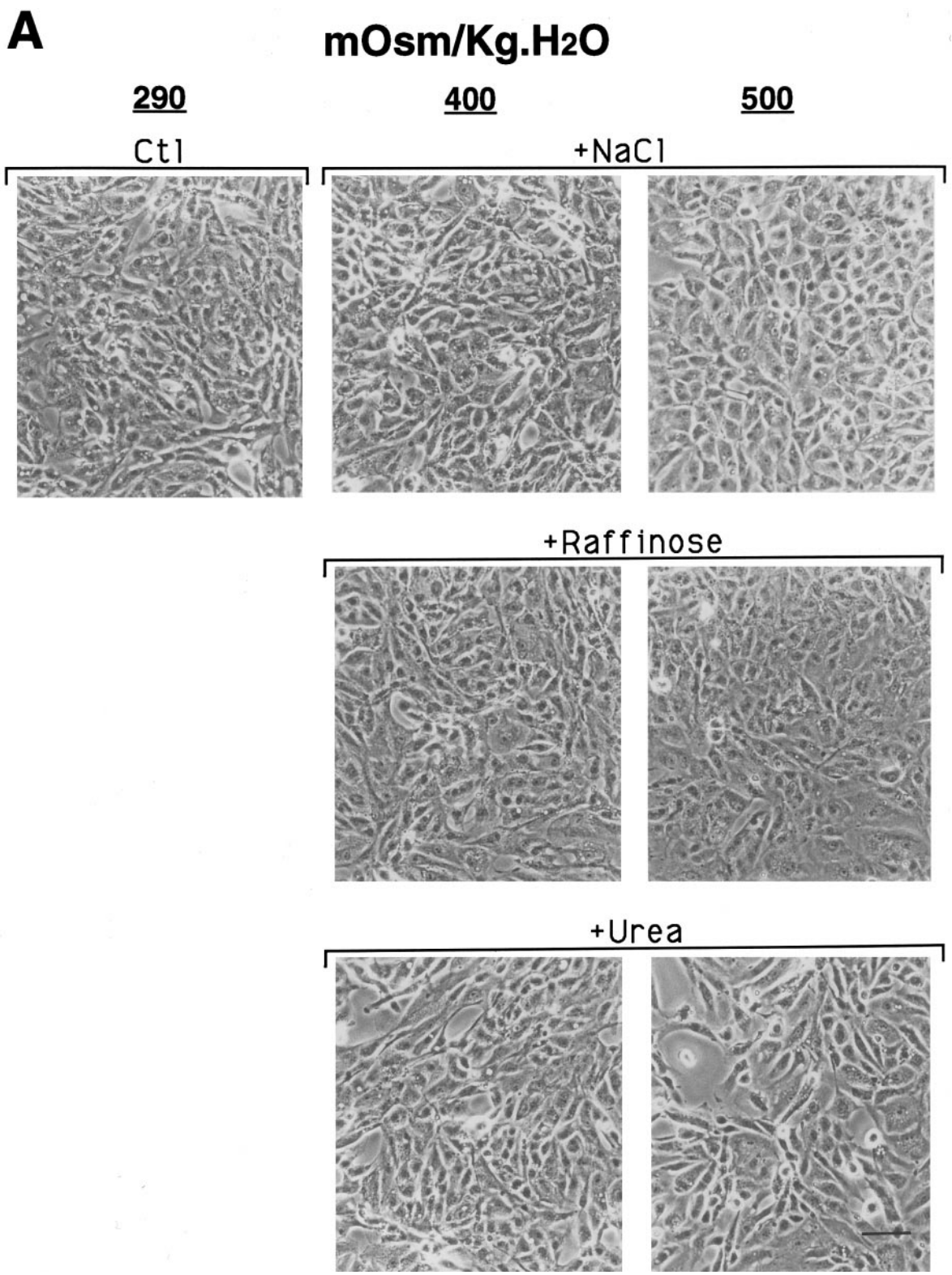

FIG. 6. Micrographs showing HRPTE cells after a 24h exposure in REGM growth media alone and in REGM supplemented with various osmotic agents or contrast agents. Panel A demonstrated the similar cell morphology and size (110 ± 10 cells/mm^2) when comparing Ctl cells (290 mOsm/kg.H_2O) with cells treated with NaCl (400 mOsm/kg.H_2O), D(+)-raffinose (400 and 500 mOsm/kg.H_2O), or urea (400 and 500 mOsm/kg.H_2O). Panel A also resulted in a significant cell swelling phenomenon upon cellular exposure for 24h in NaCl supplemented REGM (500 mOsm/kg.H_2O, 85 ± 5 cells/mm^2). In addition, cells exposed to the isosmolar Visipaque (25% v/v in REGM) for 24h, as shown in panel B, exerted an alteration in cell morphology without affecting cell size (108 ± 5 cells/mm^2). Also shown in panel B, while cells adapted to

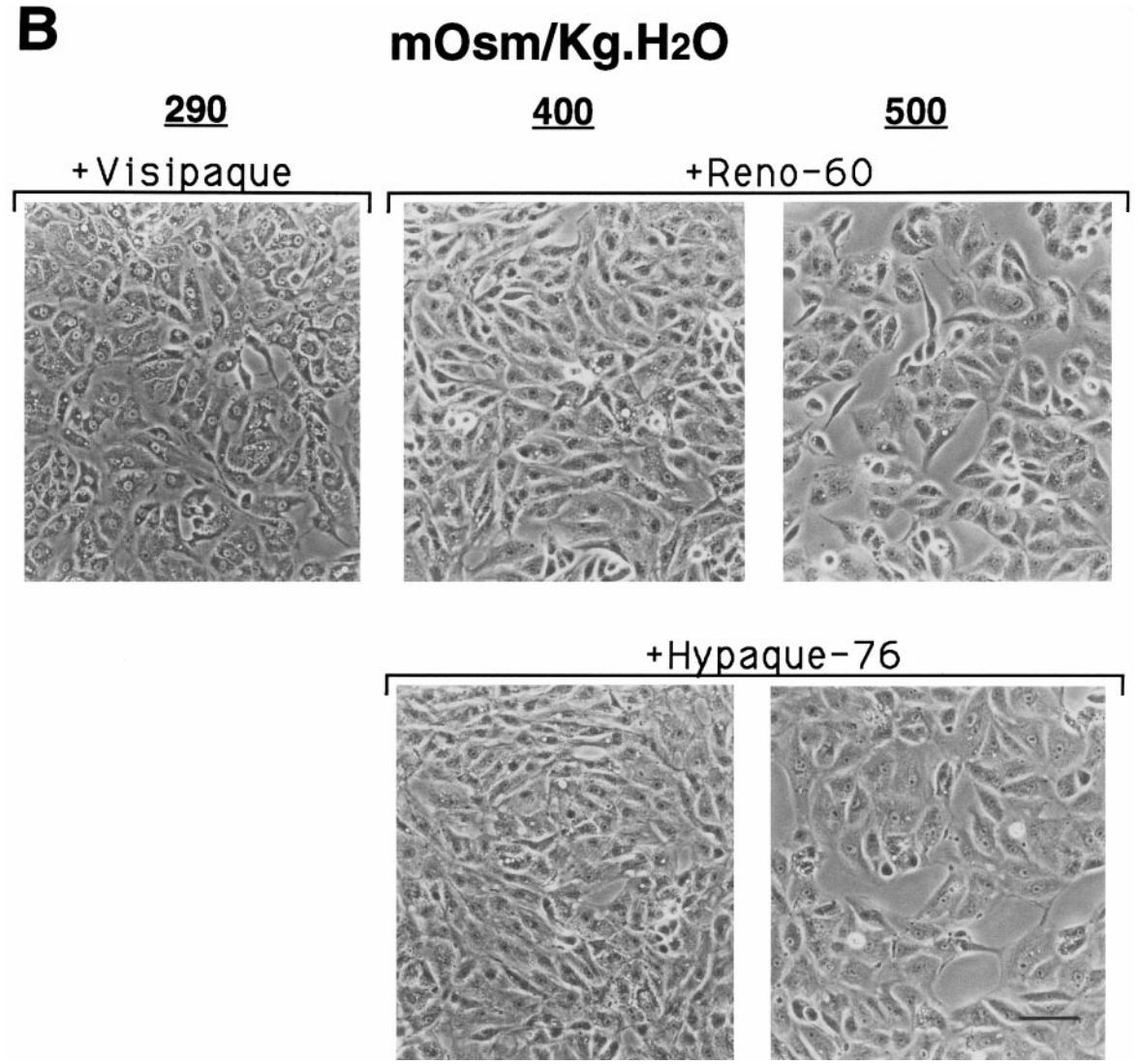

FIG. 6—*Continued*

raffinose (400 and 500 mOsm/kg.H_2O), or urea (400 and 500 mOsm/kg.H_2O). However, a significant cell swelling phenomenon occurred with increased cell size (85 ± 5 cells/mm^2) when cells were exposed to 500 mOsm/kg.H_2O of NaCl supplemented REGM for 24h (Fig. 6, panel A). Furthermore, cells exposed to isosmolar Visipaque (25% v/v in REGM) for 24h, as shown in panel B of Fig. 6, showed alterations in cell morphology consistent in better definition of the cell nuclei without changing the cell size (108 ± 5 cells/mm^2), as compared to the Ctl cells shown in panel A. Also shown in panel B, HRPTE cells incubated with Reno-60 or Hypaque-76 supplemented REGM (both 400 mOsm/kg.H_2O in osmolarity) did not show appreciable changes in cell morphology or size (105 ± 5 cells/mm^2). However, as with the cells treated with NaCl, when the osmolarity of the media was enhanced to 500 mOsm/kg.H_2O, cell swelling with reduced cell number per area equivalent (77 ± 5 cells/mm^2) was observed after a 24h incubation.

DISCUSSION

Our previous data using cultured mIMCD-3 cells had indicated that AQP-1 is a gene that is up-regulated

Reno-60 and Hypaque-76 supplemented REGM (both 400 mOsm/kg.H_2O) without any appreciable change in cell size (105 ± 5 cells/mm^2) and morphology. However, the enhanced supplements of both CA in REGM to 500 mOsm/kg.H_2O encouraged cells to swell (77 ± 5 cells/mm^2) upon a 24h treatment. Cell numbers per unit area as Mean ± SE were obtained from at least five measurements. Bar represents 25 μm.

by hyperosmolarity with NaCl, and further augmented by AVP (11). In this study we showed that HRPTE primary cells express AQP-1 under isosmotic conditions, in concert with the understanding of the proximal tubule as being a major site of nephron for the presence of AQP-1 and water reabsorption (5, 16). The response of HRPTE cells to hyperosmolarity by up-regulating the AQP-1 expression over normosmotic control cells seems to agree with our former findings with mIMCD-3 cells, except that the AQP-1 channel protein expression in the latter was not up-regulated under isosmotic conditions. Although with hyperosmotic exposure there was at least a 6h delay for HRPTE cells to up-regulate AQP-1 protein expression (as was the case for mIMCD-3 cells), the addition of AVP to either isosmotic or hyperosmotic media did not affect the AQP-1 expression in HRPTE cells (Fig. 1). This suggests differential regulatory mechanisms of AQP-1 expression in different segments of the nephron. We found that NaCl and D(+)-raffinose osmolytes (400 and 500 mOsm/kg.H_2O) stimulated AQP-1 up-regulation to a similar extent, whereas urea did not (Fig. 2). This may relate to the finding of Okazaki *et al.* (25) which demonstrated that human embryonic kidney cells exposed to hypertonic NaCl, but not urea, elicited phosphorylation of certain nuclear proteins to weaken their binding activity to oligo B, a negative calcium responsive element that mediates hyperosmolarity-induced gene stimulation. Furthermore, cell exposure to hyperosmolarity activates the osmotic response elements (ORE) that govern the up-regulation of aldose reductase, organic osmolyte transporters, and other related genes (13, 15, 26).

Urea transporters (UT1, UT2 and UT3) found in rat kidney, and human urea transporter HUT11 (rat UT3 homologue) found in bone marrow and red cell membranes also can function as water channels (27, 28, 29). There is also an AVP-inducible urea-specific HUT2 found in human renal collecting duct (28). Interestingly, the newly discovered AQP-9 from human leukocytes also facilitates urea transport (30). The presence of urea transporters and the possible presence of AQP water channels other than AQP-1 might explain, at least in part, our results showing no significant effect of AQP-1 expression upon cellular exposure to hypertonic urea. Although D(+)-raffinose is an impermeant, while NaCl can be transported in and out of the cells through various ion channels, these cannot preclude the water transport through aqueous channels, and perhaps AQP-1. Our data with HRPTE cells indicated a similar up-regulation of hyperosmotic NaCl and D(+)-raffinose in raising AQP-1 expression at both mRNA and protein levels (Fig. 2 and 5). This suggested that AQP-1 gene expression is regulated by specific ORE, though the actual signal transduction pathways might vary between NaCl and raffinose osmolytes.

In general, renal and cardiovascular failure in human are recognized to be risk factors for contrast agents (CA)-induced renal impairment or renal failure. Contrast agents may cause renal vasoconstriction that can lead to the pathogenesis of CA-induced nephrotoxicity. Both high-osmolar CA diatrizoate and low-osmolar CA iopamidol were found to induce osmotic diuresis, and tubular dysfunction of the proximal nephron in the chronic renal failure patient (21). Although the cellular mechanisms of CA-induced renal dysfunction are not clear, it prompted us to examine their impact on AQP-1 expression in HRPTE cells, since AQP-1 is responsible for major water reabsorption in the proximal nephron. We found that both ionic hyper-osmolar Reno-60 and Hypaque-76 (diluted to 400 and 500 mOsm/kg.H_2O) stimulated a 3-4 fold increase of cellular AQP-1 (Fig. 3). The non-ionic isosmolar Visipaque (290 mOsm/kg.H_2O, 25% v/v) also raised AQP-1 levels 3.5-fold (Fig. 3). However, when Omnipaque (non-ionic), Reno-60, and Hypaque-76 were diluted to 290 mOsm/kg.H_2O prior to cell incubation (20% v/v in REGM), the levels of AQP-1 expression remained rather constant as compared to those of normosmotic REGM Ctl and PBS Ctl (Fig. 4). In addition, isosmolar Visipaque supplemented to REGM at 20% v/v elevated the level of AQP-1 to 2.5-fold as opposed to the normosmotic controls (Fig. 4). The results shown in Figs. 3 and 4 reveal that both osmolarity and dose of CA affect cellular expression of AQP-1. In any event, the doses of isosmolar Visipaque (20% and 25% v/v in REGM) employed in this study were higher than the diluted low- or high-osmolar CA. A dose response relationship between Visipaque doses (10%, 20% and 25% v/v) and the levels of AQP-1 expression was also observed (data not shown), which confirms that normosmotic (290 mOsm/kg.H_2O) CA doses alone contributed to AQP-1 up-regulation in HRPTE cells. According to Dobrota *et al.* (31), rats receiving a high dose of dimeric isotonic visipaque (iodixanol at 3 g I/kg) showed numerous large protein-containing vacuoles or droplets in the cells of proximal convoluted tubule. We speculate that the higher final concentration of organically bound iodine (reflecting higher iodine doses) in Visipaque than those of diluted high-osmolar CA used in this study may, in part, account for the cellular up-regulation of AQP-1.

In our semi-quantitative RT-PCR experiments, the level of AQP-1 mRNA (Fig. 5) reflected immunoblotting results of AQP-1 channel protein up-regulation (Figs. 1–4), suggesting the transcriptional up-regulation or post-transcriptional stabilization of AQP-1 in HRPTE cells exposed to various hyperosmotic agents or contrast agents. As expected, the mRNA levels of housekeeping β-actin remained rather constant in cells with or without hyperosmotic or CA treatments. Nevertheless, the elevated cellular AQP-1 mRNA levels, under hyperosmolarity or high concentration of normosmotic

contrast agents, might be due to either the increased transcription of the gene, the increased stability of its mRNA, or both. In addition, the putative osmotic response elements (OREs) reside in promoter sequence of AQP-1 gene is speculated.

Finally, panels A and B of Fig. 6 are cell morphology overviews of HRPTE cells impacted by hyperosmotic agents and contrast agents. Cells treated with 25% v/v Visipaque (80 mg I/ml, 290 mOsm/kg.H_2O) manifested a distinctive morphological change in which the nuclei and nucleoli stand out without altering the cell size (panel B). Since the degree of contrast enhancement is directly related to iodine content in an administered dose of CA, the high iodine content in Visipaque (25% v/v) media may have contributed to the enhanced imaging of cellular nuclei and nucleoli we observed in HRPTE cells. However, cell-swelling phenomena were discerned in cells after a 24h treatment with 500 mOsm/kg.H_2O each of NaCl (panel A), Reno-60 and Hypaque-76 (panel B). According to Wener and Tinel (32), the regulatory volume increase of rat hepatocytes upon hypertonic exposure (300 to 400 mOsm/l) can be attributed to elevated Na^+ conductance, Na^+-H^+ exchange, and Na^+-K^+-2Cl^-symport. Moreover, the overall osmotic balance of hyperosmotic stressed cells depends on balancing the sum of Na^+ influx via Na^+ conductance, Na^+-H^+ exchange and Na^+-K^+-2Cl^-symport, to the amount of Na^+ extrusion via Na^+-K^+-ATPase plus the actual increase of intracellular Na^+. Since the activity of Na^+-K^+-ATPase is volume-induced along with the increased Na^+ conductance in hyperosmotic condition (33), the Na^+-K^+-ATPase activity might have reached maximum levels at certain high osmolarity conditions. Thus, we postulate that at 500 mOsm/kg.H_2O, HRPTE cells tend to retain Na^+, which in combination with an increase in the AQP-1 water channel might, in part, explain the cell swelling phenomenon. In addition, upon cellular challenge with hyperosmolarity, the transported plus synthesized organic osmolytes and undetermined solutes (idiogenic osmoles) (34), along with up-regulated AQP-1, might also contribute to the observed cell size increase.

To date, this is the first report demonstrating that primary cells of human proximal tubules can respond to hyperosmolarity by increasing AQP-1 expression. It is also a novel discovery since the hyperosmolar Reno-60 and Hypaque-76, together with the isosmolar contrast medium Visipaque (≥10% v/v) are capable of triggering AQP-1 up-regulation. Our data indicate that AQP-1 is osmoinducible in primary cells of human proximal tubules. Thus, it is essential to further investigate the mechanisms of AQP-1 up-regulation at the molecular level. Such information will allow us to gain more insight in the area of physiology and water homeostasis in kidney tubules.

ACKNOWLEDGMENTS

This research was supported by the Division of Nephrology and Hypertension, James A. Haley VA Medical Center in Tampa, Florida. The authors thank Dr. M. A. Knepper of NIH for providing us AQP-1 antibodies and Dr. M. S. Farber of James A. Haley VA Medical Center for giving us the contrast agents for this study. The authors also gratefully acknowledge the valuable discussion of the study with Dr. S. J. Wu.

REFERENCES

1. Denker, B. M., Smith, B. L., Kuhajda, F. P., and Agre, P. (1988) *J. Biol. Chem.* **263,** 15634–15642.
2. Sabolic, I., Valenti, G., Verbavatz, J. M., Van Hoek, A. N., Verkman, A. S., Ausiello, D. A., and Brown, D. (1992) *Am. J. Physiol.* **263,** C1225–1233.
3. Nielsen, S., Smith, B. L., Christensen, E. I., Knepper, M., and Agre, P. (1993) *J. Cell Biol.* **120,** 371–383.
4. Zhang, R., Skach, W., Hasegawa, H., van Hoek, A. N., and Verkman, A. S. (1993) *J. Cell Biol.* **120,** 359–369.
5. Knepper, M. A., Wade, J. B., Terris, J., Ecelbarger, C. A., Marples, D., Mandon, B., Chou, C. L., Kishore, B. K., and Nielsen, S. (1996) *Kidney Int.* **49,** 1712–1717.
6. Yamamoto, T., Sasaki, S., Fushimi, K., Ishibashi, K., Yaoita, E., Kawasaki, K., Fujinaka, H., Marumo, F., and Kihara, I. (1997) *Am. J. Physiol.* **272,** F198–F204.
7. Harris, H. W., Strange, K., and Zeidel, M. (1991) *J. Clin. Invest.* **91,** 1–8.
8. Verkman, A. S. (1992) *Annu. Rev. Physiol.* **54,** 97–108.
9. Nielsen, S., Chou, C. L., Marples, D., Chritensen, E. I., Kishore, B. K., and Knepper, M. A. (1995) *Proc. Natl. Acad. Sci. USA* **92,** 1013–1017.
10. Marples, D., Knepper, M. A., Christensen, E. I., and Nielsen, S. (1995) *Am. J. Physiol.* **269,** C655–C664.
11. Jenq, W., Mathieson, I. M., Ihara, W., and Ramirez, G. (1998) *Biochem. Biophys. Res. Commun.* **245,** 804–809.
12. Gardner, M. M. and Burg, M. B. (1994) *Am. J. Physiol.* **266,** C877–892.
13. Burg, M. B., Kwon, E. D., and Kültz, D. (1997) *Annu. Rev. Physiol.* **59,** 437–455.
14. Uchida, S., Garcia Perez, A., Murphy, H., and Burg, M. B. (1989) *Am. J. Physiol.* **256,** C614–C620.
15. Burg, M. B., Kwon, E. D., and Kültz, D. (1996) *FASEB J.* **10,** 1598–1606.
16. Maunsbach, A. B., Marples, D., Chin, E., Ning, G., Bondy, C. P. Agre, and Nielsen, S. (1997) *J. Am. Soc. Nephrol.* **8,** 1–14.
17. Maeda, Y., Smith, B. L., Agre, P., and Knepper, M. A. (1995) *J. Clin. Invest.* **95,** 422–428.
18. Knepper, M. A. and P. C. Rector, Jr. (1991) *In* The Kidney (Brenner, B. M. and Rector, F. C., Eds.), pp. 445–482, W. B. Saunders Co., Philadelphia, PA.
19. Donadia C., Tromonti, G., Lucchesi, A., Giodani, R., Lucchetti, A., and Bianchi, C. (1996) *Renal Failure* **18,** 647–656.
20. Donadia C., Lucchesi, A., Tromonti, G., Calderazzi, A., Gibilisco, G., Paolicchi, A., Giodani, R., and Bianchi, C. (1996) *Renal Failure* **18,** 657–666.
21. Russo, D., Minutolo, R., Cianciaruso, B., Memolo, B., Conte, G., and De Nicola, L. (1995) *J. Am. Soc. Nephrol.* **6,** 1451–1458.
22. Donadia C., Tromonti, G., Lucchesi, A., Giodani, R., Lucchetti, A., and Bianchi, C. (1998) *Renal Failure* **20,** 319–324.
23. Hizoh I., Strater, J., Schick, C. S., Kubler, W., and Haller, C. (1998) *Nephrology, Dialysis, Transplantation* **13,** 911–918.

24. Haller, C., Schick, C. S., Zorn, M., and Kubler, W. (1997) *Cardiovascular Res.* **33,** 655–665.
25. Okazaki, T., Ishikawa, T., Nishimori, S., Igarashi, T., Hata, K., and Fujita, T. (1997) *J. Biol. Chem.* **272,** 32274–32279.
26. Ruepp, B., Bohren, K. M., and Gabbay,K. H. (1996) *Proc. Natl. Acad. Sci. USA* **93,** 8624–8629.
27. Tsukaguchi, H., Shayakul, C., Berger, U. V., Tokui, T., Brown, D., and Hediger, M. A. (1997) *J. Clin. Invest.* **99,** 1506–1515.
28. Wintour, E. M. (1997) *Clin. & Exp. Pharmocol. & Physiol.* **24,** 1–9.
29. Yang, B. and Verkman, A. S. (1998) *J. Biol. Chem.* **273,** 9369–9372.
30. Ishibashi K., Kuwahara, M., Gu, Y., Tanaka, Y., Marumo, F., and Sasaki, S. (1998) *Biochem. Biophys. Res. Commun.* **244,** 268–274.
31. Debrota, M., Powell, C. J., Holtz, E., Willin, A., and Vik, H. (1995) *Acta Radiol.-Suppl.* **399,** 196–203.
32. Wehner, F. and Tinel, H. (1998) *J. Physiol.* **506,** 127–142.
33. Wehner, F., Sauer, H., and Kinne, R. K. H. (1995) *J. Gen. Physiol.* **105,** 507–535.
34. Ayus, A. C., Amstrong, D. L., and Arieff, A. I. (1996) *J. Physiol.* **492,** 243–255.