Regulation of Aquaporin mRNA Expression in Rat Kidney by Water Intake

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Abstract. Three aquaporins (AQP) are present in the membrane of the principal collecting duct cells. On the apical side, the levels of AQP2 protein are increased in response to both arginine vasopressin and water deprivation. However, whether this change parallels changes in the abundance of AQP3 and AQP4 in the basolateral membrane is less well known. This study evaluates the effect of either dehydration or water loading on the rat kidney mRNA expression of AQP2, AQP3, and AQP4. Poly(A⁺)RNA was prepared from renal cortex and medulla of control, water-deprived, well hydrated, and water-deprived rats treated with OPC31260, a V2 receptor antagonist. Northern blots were done and mRNA levels were quantified using a PhosphorImager system. Relative to control, water deprivation increased the expression of cortical AQP2, -3, and -4, whereas water loading decreased the cortical and medullar expression of AQP2, -3, and -4. Therefore, in addition to AQP2 and -3, AQP4 expression is also regulated by water intake. Treatment with OPC31260 (40 mg/kg of weight per d) inhibited up to 20 to 30% the upregulation of AQP-mRNA induced by water deprivation. Blood values of arginine vasopressin and aldosterone were significantly increased by water deprivation, whereas they were unchanged by water overloading. Taken together, these results indicate that renal AQP2, -3, and -4 expression is regulated in a coordinated manner. Simultaneous up- or downregulation of the three transcripts occurred upon either water deprivation or water loading of animals, respectively. However, the signaling mechanism for the two long-term adaptive processes may be different, and, in addition to arginine vasopressin, other factors may be involved in the transcriptional regulatory processes.

Water permeability of the collecting duct (CD) depends strictly on the presence of arginine vasopressin (AVP). The principal and inner medullary cells of the collecting duct, but not the intercalated cells, are capable of increasing their apical water permeability upon the binding of AVP to the V2 receptor on the basolateral membrane. The molecular identification of water channels or aquaporins (AQP), responsible for the osmotically driven water transport, has contributed to our understanding of the mechanism by which AVP increases the CD water permeability (1). The first functional correlation between AVP and AQP2 activity came from the finding that a natural mutation occurring in AQP2 gene was the defect underlying congenital nephrogenic diabetes insipidus (2). In vivo and in vitro studies have demonstrated that regulation of CD cell water permeability by AVP occurs by reversible translocation of AQP2 from intracellular vesicles to the apical plasma membrane (the shuttle hypothesis) (3–8). Two other water channels, AQP3 (9–11) and AQP4 (12,13), are also located in the principal cells of the CD. Both are constitutively expressed in the basolateral membrane and form the exit pathway for water toward the capillaries. The physiologic significance of the presence of two distinct water channels in the same cell remain to be established.

Besides the rapid action of AVP (30 to 40 min of exposure to the peptide) to increase the water permeability of the CD, a long-term regulation has also been described. Thus, in animals deprived of water for 24 h or more, a sustained increase of the CD water permeability was observed (14). Moreover, immunoblotting studies and Northern blot assays showed that the levels of mRNA and protein for AQP2 and AQP3 were increased in response to water deprivation (5,11,15). These results indicated that the water deprivation-induced increase in CD water permeability was due to an increase in AQP expression. Because AVP levels were increased by water deprivation, it was postulated that AVP also mediates long-term regulation.

However, controversial results regarding the role of AVP in the long-term regulation of CD water permeability can be found in the literature (14,16). The increased reabsorption of water observed in patients with severe edema is not always explained by increased AVP levels. For example, in 20% of patients with congestive heart failure and hyponatremic edema, the plasma levels of AVP were undetectable (17). Furthermore, we have reported (18) that homozygous Brattleboro rats, which have an inherited deficiency for synthesizing AVP, concentrate their urine to approximately 1137 ± 121 and 1510 ± 139 mosmol/kg water after 16 h and 40 h of water deprivation, respectively, showing that despite the lack of AVP, these rats can concentrate the urine during water restriction. Also, increased levels of AQP3 mRNA were found in these rats,
indicating that upregulation of the channel may in part be independent of AVP (18).

In the present work, we have studied whether AQP4 expression is regulated by water deprivation; whether not only dehydration, but also water overloading of the rats can modify the expression of the AQP2, -3, and -4 in CD cells; and the participation of V2 receptors in the upregulation of AQP expression during dehydration. By Northern blot analysis, we examined the levels of mRNA for AQP2, -3, and -4 in different conditions: control, water deprivation, water loading, and presence of vasopressin V2 receptor antagonist. The results indicated that the water balance of the animal up- or downregulates the expression of the three AQP.

Materials and Methods

Animal Treatment

Wistar rats (200 to 250 g) were kept in metabolic cages for 2 to 3 d for animals to become acclimated before the start of treatments. The four treatments described below lasted 2.5 d. In the control group, tap water was provided at libitum. Two other groups of animals were either deprived of water or induced to drink water abundantly by adding sucrose (20 g/100 ml) to their drinking water. A fourth group of animals was deprived of water and treated with OPC31260, an antagonist for the arginine vasopressin V2 receptors. Two doses of 5 mg OPC31260 were given every 12 h with food. These animals received 10 g of food per day to ensure the complete intake of the drug. The same food restriction was used for the four groups of animals to keep comparable conditions within the treatments. A total of five to six treatments was performed, and three rats per individual group were used in every treatment.

Plasma and Urine Parameters

Urine was collected every day, and its volume and osmolality (Osmometer Gonotec, Osmomat 030) were monitored throughout the treatment. Rats were anesthetized with ether and samples of blood were taken by cutting the animal neck and collecting the blood on heparinized tubes, which were centrifuged immediately at 4°C. Plasma AVP and aldosterone levels were measured by RIA at Cerba Laboratory (Barcelona, Spain).

Preparation of Tissue

Kidneys were removed and rinsed with ice-cold saline solution. The cortex and medulla were dissected apart and immediately dropped in liquid nitrogen and frozen at −80°C until use.

Preparation of Riboprobes

Partial cDNA clones of rat AQP1 and AQP2 were obtained by reverse transcription (RT)-PCR. Two micrograms of medulla mRNA were used in an RT reaction to synthesize the first strand cDNA, which, in turn, served as template in a PCR. Two degenerate primers designed on the basis of conserved amino acid sequences in the major intrinsic protein family were used in the amplification reaction. The sense primer (5'- TGG(C/G)T(C/G)(T/A)(C/G)(A/C/T)GG (A/C/G/T)G/G/C-3') and the antisense primer (5'-G-A(G/C)(A/C/G/T)G/G/C (A/C/G/T)A(A/G)- (A/C/G/T)C/G) (A/G/T)(A/G/C/T)(A/G/T)(A/C/G/T)GC(A/C/G/T)GG(A/G/T)(T/T/CT-3') used corresponded to amino acids 70 to 76 and 212 to 219, respectively, of the rat AQP1 sequence (19). Thirty PCR cycles were carried out (each comprising 1 min at 94°C, 2 min at 50°C, and 3 min at 72°C), and the last cycle ended with 10 min at 72°C. PCR products were directly cloned into the pCR II vector (Invitrogen, San Diego, CA) and, after transformation, colonies containing inserts of approximately 400 to 600 bp were selected and sequenced by the dideoxynucleotide chain termination method, using the Sequenase 2.0 kit (United States Biochemical, Cleveland, OH). cDNA fragments of AQP1, -2, and -3 were found after sequencing of these colonies. Also, a pSPORT plasmid (Life Technologies, BRL, Gaithersburg, MD) containing the full-length cDNA of AQP3 was available (9).

For AQP4, a pBluescript SK plasmid containing the full-length cDNA sequence (13) was purchased from the American Type Culture Collection (Rockville, MD). The plasmid pB1B3 containing the cDNA sequence of cyclophilin was used as reported (20).

Plasmids were linearized and antisense riboprobes were labeled with [α-32P]-UTP (800 Ci/mmol; Amersham International), using T7 or SP6 RNA polymerase (Promega, Madison, WI). β-Actin riboprobe was synthesized using the pTRI-β-actin-125-Rat template cDNA (Ambion, Austin, TX).

RNA Preparation and Northern Blot Analysis

Total RNA was extracted using the method described by Chomczynski and Sacchi (21). Poly(A)+ RNA was separated with an oligo(dT)-cellulose T7 column from Pharmacia Biotech Europe, using a previously described method (22). Poly(A)+ RNA, prepared separately from cortex or medulla, was used for Northern blot analysis (23). Equal amounts (5 μg/lane) of each mRNA was fractionated on 0.8% agarose-2.2 M formaldehyde gels, blotted onto nylon filters, and baked at 80°C for 2 h to link the RNA to the filters. Blots were prehybridized for 2 to 4 h at 68°C with a mixture containing: 5× SSC, 50 mM sodium phosphate, pH 6.7, 10× Denhardt’s reagent, 50% deionized formamide, 0.5% sodium dodecyl sulfate (SDS), and 0.5 mg/ml boiled salmon sperm DNA. The hybridization mixture was the same, except for the following changes: 1× Denhardt’s concentration, 20 mM sodium phosphate, pH 6.7, and 0.1 mg/ml salmon sperm DNA. The radiolabeled antisense oligonucleotide was added to the hybridization solution at a final concentration of 106 cpm/ml. The filters were hybridized overnight at 68°C and then washed twice with 2× SSC/0.1% SDS and twice with 0.2× SSC/0.1% SDS, at 68°C for 15 min each wash. After autoradiography, the filters were rehybridized with a radiolabeled riboprobe (128-mer) synthesized from a fragment of the rat cyclophilin cDNA (20). Cyclophilin mRNA is commonly used (20) as an internal standard for the amount of mRNA load in each lane.

The quantity of the different mRNA studied was determined with a Phosphorlmager system (Fuji Film Photo Film), using for quantification the PCBS program (Raytest). In each blot, levels of AQP mRNA were normalized to levels of cyclophilin mRNA, always using exposures in the linear range of the Phosphorlmager screen (Fuji Imaging Plate Type BAS-III).

Ribonuclease Protection Assay

An AQP4 antisense riboprobe of 451 bp was synthesized by T7 RNA polymerase with [α-32P]-UTP, using SspI linearized AQP4 cDNA plasmid. Transcription of the pTri-β-actin plasmid was done with T3 RNA polymerase to give a riboprobe of 160 bp total length. Total RNA (15 μg) prepared from cortex and medulla of control and water-deprived rats was mixed with 2× 106 cpm/min of AQP4 probe and 2× 106 cpm/min of β-actin probe in 20 μl of hybridization buffer (80% deionized formamide, 100 mM sodium citrate, pH 6.4, 300 mM sodium acetate, pH 6.4, 1 mM ethylenediaminetra-acetic acid) and incubated overnight at 42°C. Samples were then digested with a
mixture of ribonuclease A (RNase A; 2.5 U/ml) and RNase T1 (100 U/ml), according to procedure (RPA II™ Ribonuclease Protection Assay Kit; Ambion). After ethanol precipitation, protected fragments were analyzed by electrophoresis in a 6% polyacrylamide-8M urea gel.

**Chemicals**

All chemicals were purchased from Sigma Chemical Co. (St. Louis, MO) unless specified otherwise. The nonpeptide V2 receptor antagonist OPC31260 was generously provided by Otsuka America Pharmaceutical (Rockville, MD).

**Statistical Analyses**

Results are expressed as mean ± SEM. Statistical significance was evaluated by ANOVA using the Scheffe test. *P < 0.05* was considered significant.

**Results**

**Urine and Plasma Parameters of Rats**

Volume and osmolality of the urine, as well as plasma levels of AVP and aldosterone, were measured in the four groups of animals (Tables 1 and 2). Urine osmolality and volume were inversely related and could be used as good indicators of the effectiveness of the treatments. The values shown in Table 1 indicate that the control animals have a hydration state intermediate between water-deprived and water-overloaded animals. Compared with control conditions, the osmolality of the urine either dramatically increased or decreased depending on whether the animal was water-deprived or water-overloaded, respectively. Urine osmolality of water-deprived animals receiving OPC31260 was significantly increased compared with that measured in control animals, but it was approximately 20% smaller than that of water-deprived animals that did not receive the drug.

Table 2 shows that the plasma AVP levels were significantly higher in water-deprived animals than in control animals, regardless of whether the animals were treated or untreated with OPC31260. Contrary to what could be expected, the AVP levels of water-overloaded animals were similar to those measured in control animals. This observation could be due, however, to the difficulties inherent in the AVP measurements. Changes in levels of aldosterone parallel those of AVP. Aldosterone values were similar in control and water-loaded rats, and increased in water-deprived animals, regardless of the presence of OPC31260 in the food (Table 2).

**Water Intake and Renal AQP mRNA Levels**

Immunoblotting studies had shown that water deprivation doubles the amount of AQP2 (5) and AQP3 (11) proteins in the renal inner medulla, without affecting AQP4 protein (15). Here we test whether changes in water intake, in either direction (overloading and thirst), regulate the abundance of the three AQP present in the renal collecting tubule. Figure 1 shows Northern blot assays of rat kidney cortex and medulla hybridized with antisense riboprobes for AQP2, AQP3, and AQP4. Relative quantification of AQP RNA expression was determined by densitometry of blots. For a direct comparison of band densities, equal amounts of mRNA from cortex and medulla of treated rats were loaded into individual lanes of a single gel, and quantification was done as described in Materials and Methods. Table 3 summarizes the Northern blot data and the results are presented as ratios of mRNA levels after cyclophilin normalization found in two different conditions. AQP2 mRNA levels were increased by approximately 1.5-fold in cortex and medulla of water-deprived rats relative to control rats. AQP3 (2.8-fold) and AQP4 (2.1-fold) mRNA levels were also increased by water deprivation relative to control, but only in cortex. The abundance of AQP4 mRNA in cortex was very small, and its presence was detected and quantified thanks to the high sensitivity of the PhosphorImager system, which allows the direct exposure of the blot to the PhosphorImager screen. Exclusively for AQP4, we performed RNase protection assay, a more sensitive method to quantify AQP4 mRNA levels. The RNase protection assay shown in Figure 2 reveals a strong band in medulla and a barely detectable faint band in the cortex. The faint band, which corresponds to a 400-bp protected fragment of AQP4 mRNA, was more abundant in water-deprived animals than in control. Taken together, these results indicate that water deprivation induced the upregulation of the mRNA expression for the three aquaporins present in the principal collecting duct cells of the rat.

The levels of mRNA for AQP1 were also examined to confirm that the observed upregulation of the mRNA expression, induced by water deprivation, is a specific process restricted to some AQP. Figure 3 shows a representative Northern blot assay of rat kidney cortex and medulla mRNA from water-deprived and control animals hybridized with antisense riboprobes for AQP1. The levels of AQP1 mRNA were not changed by water deprivation, supporting the idea that the upregulatory effect of water deprivation is specific for the aquaporins of the collecting duct.

Figure 1 also shows the effect of water loading of the rats (animals induced to drink large amounts of water by giving them water containing 20% sucrose) on the AQP2, -3, and -4 mRNA expression. Water loading reduced the levels of mRNA for AQP2, -3, and -4 of renal cortex and medulla by approximately twofold compared with control animals (Table 3).

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### Table 1. Osmolality and volume of urine collected from rats maintained under different water intake conditions

<table>
<thead>
<tr>
<th>Group</th>
<th>Osmolality (mosmol/kg)</th>
<th>Volume (ml/24 h)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>906 ± 98</td>
<td>8.1 ± 2</td>
</tr>
<tr>
<td>H2O + 20% sucrose</td>
<td>380 ± 84b</td>
<td>28 ± 4b</td>
</tr>
<tr>
<td>Water-deprived</td>
<td>3,140 ± 88b</td>
<td>0.8 ± 0.3b</td>
</tr>
<tr>
<td>Water-deprived + OPC31260</td>
<td>2,515 ± 96b,c</td>
<td>0.9 ± 0.2b</td>
</tr>
</tbody>
</table>

* a Urine osmolality was measured after 36 h of treatment since the collection of urine from rats not receiving water often was impossible at a later time. For more details, see Materials and Methods. Values are means ± SEM of 12 animals.

b *P < 0.05* compared with control.

c *P < 0.05* compared with water-deprived animals.
downregulation of AQP4 mRNA in cortex was undetectable given the basal low abundance of the transcript in this tissue. Therefore, these results indicated that the expression of AQP of collecting duct principal cells is a dynamic process that can be induced or repressed depending on animal water balance. The levels of cyclophilin mRNA, which are used to normalize the AQP mRNA load in a particular lane, were not affected by the treatments.

**Table 2.** Plasma values of arginine vasopressin and aldosterone in rats after 2.5 d of treatments

<table>
<thead>
<tr>
<th>Group</th>
<th>H₂O + 20% Sucrose</th>
<th>Control</th>
<th>Dehydration</th>
<th>Dehydration + OPC31260</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>AVP (pg/ml)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>5.53 ± 0.6</td>
<td>5.38 ± 0.45</td>
<td>8.6 ± 0.5ᵇ</td>
<td>9.02 ± 0.71ᵇ</td>
</tr>
<tr>
<td></td>
<td>Aldosterone (pg/ml)</td>
<td></td>
<td>1.292 ± 332ᵇ</td>
<td>1.595 ± 291ᵇ</td>
</tr>
<tr>
<td></td>
<td>657 ± 124</td>
<td>537 ± 90</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

ᵃ Plasma hormone levels were measured using RIA as indicated in Materials and Methods. Values are means ± SEM of 4 to 9 rats. ᵇ P < 0.05 compared with control.

**Figure 1.** Northern blot analysis of aquaporin-2 (AQP2) (A), AQP3 (B), and AQP4 (C) mRNA in cortex and medulla of rats maintained for 2.5 d with different water restrictions: Control rats have free access to tap water; hydrated rats were drinking water with 20% sucrose during the entire treatment; thirsted rats were deprived of drinking water, and a group of water-deprived rats received with the food two doses (5 mg) per day of OPC31260. Five micrograms of poly(A⁺) RNA were loaded onto the gel per lane. In Panel A, besides the major transcript (1.5 kb), other bands corresponding to less abundant transcripts of AQP2 were detected. A single band of 1.8 kb and 5.5 kb was detected in Panels B and C, respectively. Levels of AQP4 in cortex are hardly detectable after 24 h of exposure (C). Band intensities were normalized relative to the amount of cyclophilin (0.8-kb band).

**Table 3.** Ratios of mRNA levels from densitometric quantification of Northern blot assays

<table>
<thead>
<tr>
<th>Group</th>
<th>Dehydration/Control</th>
<th>Control/H₂O + 20% Sucrose</th>
<th>Dehydration/H₂O + 20% Sucrose</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Cortex</td>
<td>Medulla</td>
<td>Cortex</td>
</tr>
<tr>
<td>AQP2</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AQP3</td>
<td>1.6 ± 0.3ᵇ</td>
<td>1.4 ± 0.2ᶜ</td>
<td>1.7 ± 0.4ᵇ</td>
</tr>
<tr>
<td>AQP4</td>
<td>1.0 ± 0.1</td>
<td></td>
<td>1.7 ± 0.2ᵇ</td>
</tr>
</tbody>
</table>

ᵃ Ratios were calculated by dividing the values of signal intensity, normalized by cyclophilin, of the two conditions that are compared. Values are means ± SEM of 3 to 6 independent blots. ND, not detectable. ᵇ P < 0.05 compared with a value ratio of 1. ᶜ P < 0.01 compared with a value ratio of 1.

**Effect of the V₂ Receptor Antagonist OPC31260**

To examine whether the water deprivation-induced increase in plasma AVP levels is responsible for the upregulation of AQP2, -3, and -4 transcription, the levels of these AQP mRNA were measured in water-deprived rats that received OPC31260, a vasopressin V₂ receptor antagonist. Northern blot analysis (Figure 1, Table 4) showed that OPC31260 attenuated by approximately 20 to 30% the water
deprivation-induced increase in levels of AQP2, AQP3 and AQP4 mRNA.

The observation that OPC31260 decreased, but did not abolish, the water deprivation-induced increase in the levels of AQP mRNA indicates that modulation of AQP expression is not mediated exclusively by V2 receptor. These results are consistent with previous studies by Hayashi et al. (5), showing that the amount of AQP2 protein is increased by water deprivation and that such increase was diminished, but not abolished, after treatment with the V2 receptor antagonist OPC31260.

Discussion

The current results demonstrate that water intake regulates the mRNA expression of AQP of the renal collecting duct. The expression of AQP2, -3, and -4 mRNA, but not that of AQP1, was upregulated by water deprivation, partially confirming previous results (15), and was downregulated by water loading. The latter observation has been overlooked as it will be discussed later.

Two and half days of water deprivation increased the abundance of the message for AQP2, -3, and -4 in the renal cortex. In contrast, in renal medulla exclusively AQP2 mRNA was significantly increased by water deprivation (Table 3, Dehydration versus Control column). The upregulation of AQP2 and AQP3 mRNA agrees with previous studies that showed increased quantity of these proteins in the renal cortex of water-deprived rats (5,11,15). However, Terris et al. (15) reported that the abundance of AQP2 and AQP3 proteins was increased in the cortex, as well as in inner and outer medulla. The reason for the discrepancy with our results might come from differences in the experimental conditions that are compared. Most studies, including that of Terris et al. (15), compared dehydrated animals against water-loaded animals and in doing so the authors have concluded that water deprivation elicits a long-term adaptation process responsible for the increased water permeability of collecting duct. The same conclusion could have been reached in the present study (see Table 3,

Figure 2. Protection assay of AQP4 RNA expressed in kidney cortex and medulla of thirsted and control rats. Fifteen micrograms of total RNA was hybridized with antisense [32P]-riboprobes of AQP4 and β-actin, then digested with ribonuclease A and T1, and protected fragments were electrophoresed on 6% denaturing gel. The size of protected RNA fragments was 400 bp and 126 bp, for AQP4 and β-actin, respectively.

Figure 3. Northern blot analysis of AQP1 in cortex and medulla of rats maintained either with free access to tap water (control) or deprived of drinking water (thirsted). Equal amounts (5 µg) of poly(A+) RNA were loaded onto the gel per lane. A 2.8-kb band corresponding to AQP1 RNA was detected. Band intensities were normalized relative to the amount of cyclophilin.

Table 4. Percentage (%) of inhibition of mRNA expression by the V2 antagonist OPC31260a

<table>
<thead>
<tr>
<th>Group</th>
<th>Cortex</th>
<th>Medulla</th>
</tr>
</thead>
<tbody>
<tr>
<td>AQP2</td>
<td>13 ± 6</td>
<td>21 ± 15</td>
</tr>
<tr>
<td>AQP3</td>
<td>31 ± 2</td>
<td>NE</td>
</tr>
<tr>
<td>AQP4</td>
<td>ND</td>
<td>30 ± 8</td>
</tr>
</tbody>
</table>

a All of the animals were dehydrated for 2.5 d, and some of them received 40 mg/kg of weight per day OPC31260. The inhibition by OPC31260 was calculated relative to the levels measured in the OPC31260-untreated, water-deprived group. Values are means ± SEM of 9 to 12 animals. ND, not detectable; NE, no effect.
Dehydration versus Water + 20% Sucrose) if the same comparison was the only one done. Thus, when levels of AQP2, -3, and -4 mRNA from thirsted rats were compared with those measured in water-loaded rats, an upregulation of the three AQP mRNA was observed both in cortex and in medulla. However, as the current results show, water loading of the animals also regulates the expression of AQP mRNA, reducing their levels when compared with control rats (Figure 1, Table 3). Therefore, the comparison of the levels of AQP proteins in water-deprived animals with those in well hydrated animals overestimates the thirsting effect. Whether the thirsting-induced increase or the overloading-induced decrease of AQP mRNA levels in the collecting duct is due to changes in either the transcription rates or the stability of the mRNA has not been explored.

The long-term adaptation process of AQP mRNA expression induced by changes in water intake agrees with the osmotic permeability (P_t) values measured in rat inner medullary CD by Lankford et al. (14). These authors found that P_t values increased in antidiuretic rats and decreased in diuretic rats (14). Thus, the P_t values changed according to the animals’ water intake, as it does AQP expression. This parallelism between P_t values and AQP expression suggests that the long-term regulation of urine osmolality depends directly on the transcriptional control of collecting duct AQP. Furthermore, the observed downregulation of AQP expression in water-loaded rats may explain the reduction in the response to either AVP stimulus or to thirsting for a short period of time, of rats that have been overhydrated previously for a prolonged period of time (24).

The long-term regulation associated with water intake seems to be restricted to AQP expressed in the collecting tubule, since the levels of mRNA for AQP1 were not affected at all (Figure 1). The same conclusion has been reached from immunoblotting experiments that showed no change in levels of AQP1 protein, either in cortex or medulla, of rats dehydrated for 48 h (15). At present, no explanation for this absence of AQP1 regulation can be offered.

The following observations suggest that, in addition to AVP, other factors may mediate the effect of changes in water intake on collecting duct AQP mRNA expression. (1) Water overloading significantly decreases cortical and medullar mRNA levels of the three AQP, whereas dehydration increases the levels of the three AQP mRNA in cortex but not in medulla. The different regulation that occurs in cortex and medulla, at least for AQP3 and AQP4, is not fully explained if only one water intake-depending mechanism, operating back and forward, would be responsible for the up- and downregulation here reported. (2) Levels of cortical AQP2, -3, and -4 mRNA were reduced by 1.5- to 1.7-fold in overhydrated rats compared with control rats, a change of the same order of magnitude but in the opposite direction from that observed in dehydration. However, whereas AVP levels were significantly augmented after 2.5 d of dehydration, water overloading did not significantly modify AVP levels (Table 2), suggesting that AVP is not fully responsible for the overhydration-induced decrease in the transcription of AQP2, -3, and -4. Furthermore, treatment with a V2 receptor antagonist, OPC31260, at a dose reported to block the V2 receptor (25), only inhibited by approximately 20 to 30% the water deprivation-induced increases in mRNA levels for AQP2, AQP3, and AQP4. Even though a complete effect of OPC31260 on V2 receptors cannot be guaranteed, the observation suggests that, as mentioned above, the upregulation of collecting duct AQP expression is not mediated exclusively by AVP via V2 receptors. Treatment with OPC31260 also diminished the urine concentrating capacity by about 20%. Although urine output was increased by approximately 10%, the values were not significantly different from those obtained from the OPC31260-un-treated, dehydrated animals. The small increase in urine output obtained from dehydrated animals treated with OPC31260 could be due to the difficulty in collecting the small volumes of urine obtained under those experimental conditions.

Previous reports support the view that the regulation of collecting duct AQP expression is not mediated exclusively by AVP via V2 receptors. Marples et al. (25) observed that in rats made diuretic by OPC31260 treatment, 12 h of thirsting completely reversed the urine osmolality and AQP2 protein values to those observed in control rats, indicating that other mechanism(s) independent of V2 receptors must be involved. It has also been reported (18) that 40 h of dehydration in Brattleboro rats increased AQP3 mRNA levels only slightly less than in AVP-secreting animals, confirming the limited role of AVP in the increased transcription of AQP3 mRNA. Although in Brattleboro rats (26,27) dehydration increases oxytocin, another hormone with antidiuretic properties, it is unclear whether oxytocin can fully substitute for AVP during 40 h of water deprivation in mediating the increased transcription of AQP3 mRNA via V2 receptor. Thus, whereas the promoter region of human AQP2 gene has a cAMP-responsive element (CRE) that probably participates in the V2-induced transcription of AQP2 (28,29), the promoter region of AQP3 lacks CRE, and a poor effect of cAMP on stimulating AQP3 promoter activity has been shown (30). Three AP2 sites that possibly participate in upregulation of AQP3 by tetracaneoylphorbol acetate were found after cloning the promoter region of AQP3 (30). Alternatively, oxytocin may stimulate transcription of AQP3 by acting on specific oxytocin receptors, V1 receptors, or on some other receptors. The partial inhibitory effect of OPC31260 on AQP3 expression reported here, however, may indicate that cAMP may have some role in the transcriptional control of AQP3. The cloning of the promoter region of AQP4 gene will allow better understanding of the factors that control its expression.

Other hormones, such as atrial natriuretic factor, angiotensin II, catecholamines, or physiologic variables are expected to change in diuresis and anti-diuresis conditions and contribute to the regulation of AQP expression.

As reported for the transcription of mRNA coding for the enzymes and transporters that synthesized and accumulate osmolites in the cells (31), another factor that may be involved in the increase of AQP mRNA expression is the renal medullary interstitial hypertonicity that is increased by water deprivation. The current results suggest that hypertonicity by itself
is not the stimulus that triggers the upregulatory response in dehydration state. Thus, water deprivation increases AQP2 mRNA levels in both cortex and medulla, whereas those of AQP3 and AQP4 increased exclusively in cortex, where interstitium osmolality is maintained close to isotonicity (300 mosmol/kg H2O).

One byproduct of this work is the observation that after dehydration, the cortical levels of AQP4 mRNA are remarkably low, even hard to detect with RNase protection assay, and undetectable after downregulation by overhydration. These results may be indicative of a poor contribution of this protein in water reabsorption in the cortical segment of collecting duct, and may suggest that AQP3, whose levels in cortex are considerably higher than those of AQP4, is the principal basolateral water channel involved in water transport in this portion of renal collecting duct.

In conclusion, the functional significance of the long-term regulation of AQP expression can be visualized as a process by which the water channel proteins present in the collecting duct are augmented or diminished in a coordinated manner, presumably to increase or reduce the water permeability in the apical and basolateral membranes of the principal cells, thereby further enhancing or lessening the transepithelial movement of water, and diluting or concentrating the urine, as the final response of the adaptation process to the water intake. The findings presented here pointed to a distinct transcriptional regulation of AQP2 and AQP3. Additional experiments are required to understand the mechanism(s) underlying the long-term regulation of aquaporin expression.

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