Osmotic Response Element-binding Protein (OREBP) Is an Essential Regulator of the Urine Concentrating Mechanism*

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OREBP (osmotic response element-binding protein), also called TonEBP or NFAT5, is thought to induce the expression of genes that increase the accumulation of organic osmolytes to protect cells against a hypertonic environment. To investigate the consequences of lacking OREBP activity, transgenic (Tg) mice that overexpress OREBPdn (dominant negative form of OREBP) specifically in the epithelial cells of the renal collecting tubules were generated. These mice showed impairment in their urine concentrating mechanism, most likely due to reduced expression of the aquaporin AQP2 and the urea transporter UT-A1 and UT-A2 mRNAs. When deprived of water or after the administration of a vasopressin analogue, urine osmolality of the Tg mice was significantly increased but not to the same extent as that of the wild type mice. The expression of AQP2 and UT-A1, but not UT-A2 mRNAs, was increased to the same level as that of the wild type mice in the urine dehydration state, indicating that the vasopressin regulatory mechanism was not affected by OREBPdn. These data indicate that in addition to vasopressin, OREBP is another essential regulator of the urine concentrating mechanism. Furthermore, the OREBPdn Tg mice developed progressive hydronephrosis soon after weaning, confirming the osmoprotective function of OREBP implicated by the in vitro experiments.

The mammalian kidney plays an important role in maintaining the homeostasis of osmolality and the electrolyte concentrations of the circulating fluid. The osmolality of the kidney inner medulla is highly hypertonic to facilitate the reabsorption of water from the urine. The cells in the collecting tubules guard against hypertonic stress by increasing the synthesis or import of several organic “compatible” osmolytes. These include sorbitol, which is synthesized by the enzyme aldose reductase, and betaine, myo-inositol, and taurine, which are imported by the betaine/γ-aminobutyric acid transporter, the Na+-dependent myo-inositol transporter, and taurine transporter, respectively (1). The transcription of these genes is induced by a hypertonic medium and regulated by a protein called the osmotic response element-binding protein (OREBP)1 (2) or toxicity element-binding protein (TonEBP) (3). This protein is also called NFAT5 because of its homology to the NFAT family of transcription factors (4).

OREBP consists of a nuclear localization signal near the N terminus followed by a DNA binding domain, a dimerization domain, and a transactivation domain at the carboxyl end. Upon stimulation by hypertonicity, it is rapidly translocated into the nucleus and binds to the osmotic response elements (OREs) (5) in the promoter region of the osmoprotective genes (2) to stimulate transcription. OREBP is highly expressed in the inner medulla as well as in the inner stripe of the outer medulla in the rat kidney (6). In response to water loading, OREBP in the partial portion of the inner medulla collecting ducts (IMCDs) is primarily located in the cytoplasm of the epithelial cells. In dehydrated animals, most of the protein is found in the nucleus of these cells, supporting its role as a hypertonicity-stimulated transcription enhancer for the osmolyte-accumulating genes. Interestingly, in the middle and terminal portions of the IMCD, OREBP is located in the nucleus of the cells in both euhydrated and dehydrated animals. This may be due to the fact that the osmolality in this region of the medulla is high even in the euhydrated state, and, hence, OREBP is constitutively activated.

Because the activity of OREBP is primarily deduced from cell culture experiments and its in vivo functions are inferred from correlation studies, we developed OREBP gene knock-out mice to determine its physiological functions. Unfortunately, these mice died at embryonic stages.2 Because the postulated role of OREBP is to induce the expression of genes that might provide protection against hypertonic stress in the kidney, we therefore developed transgenic mice (Tg) that overexpress, specifically in the renal collecting tubules, a dominant negative form of OREBP (OREBPdn) to determine its role in kidney function. These Tg mice developed polyuria and polydipsia, indicating that OREBP is an important regulator for the urine concentrating mechanism. These mice also developed progressive bilateral hydronephrosis, confirming the role of OREBP as a regulator of osmoprotective genes.

1 The abbreviations used are: OREBP, osmotic response element-binding protein; ORE, osmotic response element; OREBPdn, dominant negative form of OREBP; AVP, arginine vasopressin; dDAVP, 1-deamino-8-arginine vasopressin; AQP, aquaporin; CsA, cyclosporine A; DBA, Dolichos biflorus agglutinin; IMCD, inner medullary collecting ducts; Ntg, non-transgenic; Tg, transgenic; TRITC, tetra-methylrhodamine isothiocyanate; UT, urea transporter.

2 A. K. M. Lam, B. C. B. Ko, S. K. Chung, and S. S. M. Chung, unpublished results.
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EXPERIMENTAL PROCEDURES

Generation of OREBPdn Tg Mice—OREBPdn Tg mice were developed by injecting the vector DNA as shown in Fig. 1a into fertilized F1 (C57BL/6J X CBA hybrid) oocytes. Tg mice were identified by PCR amplification using a pair of PCR primers, pFLAG-1 (5'-TAATGCTGGGAGTAAGTCTA3') and OREBPdn-1 (5'-CATGGACTACAAAGACGATGACGACAAG-3'), and confirmed by Southern blot hybridization using the transgene as a probe. All Tg mice used in the experiments were heterogeneous Tg mice that had been backcrossed with the C57BL/6J strain for four generations. 

Animal Care and Metabolic Cages Studies—Mice were kept in daily 12-h light and 12-h dark cycles with free access to standard mouse chow and water. Mice were kept individually in metabolic cages for the measurement of water consumption and urine output. For water deprivation experiments, water bottles were removed before and after the 24 h water deprivation period. To study the effects of 1-deamino-8-D-arginine vasopressin (dDAVP) administration, urine samples were collected by spontaneous voiding just before injection and 2 h after intraperitoneal injection of dDAVP (0.4 μg/kg of body weight). Body weights were recorded before and after the 24-h water deprivation. 

Assays for Urine and Serum Electrolytes—Serum samples were collected by cardiac punctures. Serum sodium, potassium, calcium, chloride, urea, and creatinine levels were measured with a Hitachi-747 autoanalyzer (Roche Diagnostic). Urine samples were collected from the metabolic cages in a 24-h period, and sodium, potassium, calcium, chloride, urea, and creatinine levels were measured with the Synchron CX5 analyzer (Beckman-Coulter Inc.). Urine osmolality was measured by the vapor pressure method using the Vapro vapor pressure osmometer (Wescor Inc.). Serum osmolality was measured by the freezing depression method using the Advanced Micro-Osmometer 3300 (Advanced Instruments, Inc.). The urine arginine vasopressin (AVP) level was measured by a competitive enzyme assay method with an AVP enzyme immunoassay kit (Assay Designs Inc.).

Northern Blot Analysis—Total RNA (15 μg/lane) extracted from the medulla was separated by agarose-formaldehyde gel electrophoresis and transferred onto a Hybond-N+ nylon membrane. Hybridization was carried out using 32P-labeled cDNA probes. The OREBPdn DNA fragment was used as the probe for detecting the transgene, sections were incubated with the monoclonal anti-FLAG epitope along with 100 μg/ml of the anti-FLAG monoclonal antibodies (Invitrogen). Sections were counterstained with fluorescein-conjugated-DBA. The cells were mounted with Fluorsave Reagent (Calbiochem), and photomicrographs were taken.

RED INNER MEDULLARY EPITHELIAL CELL CULTURE—Epithelial cells from the inner medullary collecting duct were prepared as described (14). The renal collecting duct epithelial cells were confirmed by staining with a monoclonal epithelial-specific antibody antibody (1:200 dilution; clone VU-1D8, Sigma) and fluorescein-conjugated-DBA. The cells were mounted with Fluorsave™ Reagent and examined with a confocal microscope. Total RNA from the epithelial cell cultures was isolated using the Absolutely RNA reverse transcription PCR mini prep kit ( Stratagene).

Western Blot and Immunohistological Studies—For Western blot analysis, protein extracts from the inner medullae were analyzed as described previously (7) using affinity-purified, peptide-derived rabbit polyclonal antibodies against AQP1 (8), AQP2 (9), AQP4 (10), and AQP5 (10), and UT-A1 (11). Immunohistological analysis of the kidney section was performed as described previously (12, 13). To identify the cells expressing the transgene, sections were incubated with the monoclonal antibody against the FLAG epitope (Sigma) and visualized by the M.O.M. immunodetection kit (Vector Laboratories, Inc.). To determine whether the transgene was expressed in the renal tubule epithelial cells, kidney sections were incubated with primary antibody against the FLAG epitope along with 100 μg/ml of the fluorescein-conjugated-DBA or the antibody specific for the transcription factor OREBPdn (Dolichos biflorus agglutinin; DBA) (Vector Laboratories, Inc.) and subsequently with a TRITC-conjugated anti-mouse secondary antibody (1:200; Molecular Probes). The sections were mounted in Fluorsave™ Reagent (Calbiochem), and photomicrographs were taken.

RESULTS

Generation of Tg Mice That Overexpress the OREBPdn—Specifically in the Epithelial Cells of the Collecting Tubules—It has been shown that a mutant OREBP lacking the transactivation domain inhibits the function of a normal OREBP (3, 15). We therefore fused part of the mouse OREBP cDNA containing the amino acid residues 57–562 (and lacking 56 amino acids at the amino-terminal and the carboxyl-terminal transactivating domains) to the kidney-specific cadherin promoter that has been shown to direct the expression of heterologous genes specifically in the epithelial cells of the renal collecting tubules (16). A FLAG epitope was inserted at the amino terminus of OREBPdn to facilitate the detection of transgene expression (Fig. 1a). The Tg mice were identified by PCR and confirmed by Southern blot hybridization (data not shown). Ten lines of Tg mice were developed, and three lines were found to express detectable levels of the transgene. The Northern blot analysis showed that the expression of OREBPdn was only present in the kidney of the transgenic mice and not in the other tissues tested, including the brain, lung, liver, and heart (Fig. 1b). All three lines of mice had similar polyuric and polydipsic phenotypes (see below), and data from one of the lines (line 4449) are shown here. Immunocytochemical staining of OREBPdn (Fig. 1c) showed that the expression of OREBPdn was primarily localized in the medullary region of the kidney, and to a lesser extent, in the cortical region as well. To verify that the transgene was expressed in the collecting ducts, fluorescein-conjugated DBA, a lectin that specifically binds to the cells of the collecting duct, and antibodies against the FLAG epitope were used to confirm the site of the transgenic expression. As shown in Fig. 1d, cells expressing the transgene were also positive for fluorescein-DBA staining. Furthermore, there is no detectable transgenic expression in the glomeruli, proximal tubules, and loop of Henle. Collectively, these data demonstrated that the expression of the transgene was located exclusively in the collecting ducts of the kidney.

Phenotypic Analysis of the Tg Mice—The OREBPdn Tg mice were viable and fertile. However, ~9% of Tg mice died around 6–9 months. The body weights of the Tg mice were 15–30% less than that of their non-transgenic (Ntg) littermates. The average body weight of the 5-week-old Ntg mice was 19.01 ± 0.48 g and that of the age-matched Tg mice was 15.28 ± 0.46 g (p < 0.001). Tg mice exhibited marked polyuria and polydipsia. Measured at 5 weeks after birth, the volume of the urine excreted in 24 h was 0.088 ± 0.005 ml/g body weight for the Ntg mice and 0.418 ± 0.037 ml/g body weight for the Tg mice (p < 0.001), and the volume of water consumed in 24 h was 0.454 ± 0.024 ml/g body weight for the Ntg mice and 1.122 ± 0.052 ml/g body weight for the Tg mice (p < 0.001). Around 8 weeks after birth, the kidneys of most of the Tg mice began to develop progressive bilateral hydronephrosis (Fig. 2a). Histological examination revealed that the kidneys of the 5-week-old Tg mice appeared quite normal except that some had dilatation of the collecting tubules in the cortical region (Fig. 2b). Kidneys from the 8-week-old Tg mice typically showed atrophy of the renal
medulla and cortical thinning (Fig. 2b). Kidneys with severe hydronephrosis were enlarged and accompanied by the loss of the medulla structure. However, the severity of the kidney lesion varied even among the Tg siblings within the same litter. This may explain why some Tg mice died within a few months after birth, whereas others survived beyond 9 months.

**Urine Concentrating Ability of the Tg Mice**—To assess the urine concentrating ability of the Tg mice, urine from the 5-week-old Tg mice and their Ntg littermates was collected in a 24 h period, during which time they had free access to water. The osmolality of the urine, as well as the urinary concentrations of sodium, potassium, calcium, and chloride ions from the Tg mice, was significantly lower than that from the Ntg mice (Table I), indicating that the Tg mice may have developed primary polydipsia or that their urine concentrating mechanism was impaired. However, the serum osmolality and urea concentrations were significantly higher in the Tg mice, suggesting these mice develop a defect in their urine concentrating mechanism (Table I). There was no significant difference between the Tg and Ntg mice in the amounts of AVP excreted in 24-h period, suggesting that there is no significant difference in the levels of the circulating AVP between the Tg and Ntg mice.

To determine whether the AVP regulatory mechanism was impaired by OREBPdn, Tg mice were subjected to water deprivation. After 24 h of water deprivation, the Ntg mice lost 12.9 ± 0.83% of their body weight, whereas the Tg mice lost 20.89 ± 0.98% (p < 0.0001, n = 12), indicating that the water conservation mechanism in the Tg mice was not as efficient as that of the Ntg mice. However, the water-deprived Tg mice were able to partially concentrate their urine, as the osmolality
of the urine was significantly increased, suggesting that the kidneys in the Tg mice were responsive to AVP activation but not to the same extent as the kidneys from the Ntg mice (Fig. 3a). This was confirmed by intraperitoneal injection of dDAVP. The osmolality of the urine from Tg mice administered with dDAVP was significantly higher than that from mice receiving a bolus injection. Similar to the water deprivation situation, the increase in urine osmolality in the Tg mice was much less than those in the Ntg mice (Fig. 3c). These results demonstrated that the AVP-dependent water reabsorption system was functional in the Tg mice, although not as effective as that of the Ntg mice.

Expression of Aquaporin and Urea Transporter mRNAs in the Tg Mice under Water ad Libitum and Water-deprivation Conditions—UT-A, AQP2, and AQP3 are important for the water reabsorption in the kidney. To determine whether the impairment in the urine concentrating ability in the Tg mice was due to the abnormal expression of these genes, their mRNA levels in the kidney medulla were assessed by Northern blot hybridization. As shown in Fig. 4a under the condition when the mice had free access to water, the AQP2 mRNA level of the Tg mice was significantly down-regulated, whereas the expression of AQP3 mRNA (Fig. 4b) was not affected. Moreover, UT-A1 and UT-A2 mRNA levels were also significantly lower in the Tg mice (Fig. 4c). Western blot experiments confirmed that the AQP2 and UT-A1 protein levels in the inner medulla of the Tg mice were dramatically reduced under the water ad libitum condition (Fig. 4e). Interestingly, when the mice were deprived of water, the levels of AQP2 and UT-A1 mRNAs in the Tg mice were elevated to the same extent as that of their Ntg littermates (Fig. 4, a and c), indicating that the AVP regulatory system was not affected by OREBPdn.

Expression of Aquaporin and Urea Transporter mRNAs in Renal Epithelial Cells of the Tg Mice—It is possible that the down-regulation of AQP-2 and UT-A was an indirect effect of polyuria or a consequence of structural changes resulting from loss of OREBP activity and not due to the negative interference by OREBPdn. This possibility was tested by assessing the level of expression of these genes in renal epithelial cell cultures where the osmolalities of the culture media were kept constant and the hormonal effects of diuretics were absent. Similar to the in vivo situation, renal epithelial cells from the Tg mice showed a dramatic reduction in the expression of AQP2, UT-A1, and UT-A2 mRNA (Fig. 4d), indicating that the down-regulation of these genes was the direct effect of OREBPdn. In the epithelial cells from the Tg mice, the mRNA level of aldose reductase was reduced to 50% that of the Ntg mice (data not shown), indicating that OREBPdn did inhibit the expression of osmoprotective genes.

Effect of OPC-31260 on Urine Concentrating Mechanism in Tg Mice—To further confirm that OREBP and AVP are independent regulators of the water reabsorption mechanism in the kidney, OREBPdn Tg and Ntg mice were treated with OPC-31260, a V2R antagonist (17, 18), under water ad libitum and water-deprivation conditions. Under the water ad libitum condition in the Ntg mice, OPC-31260 dramatically lowered the urine osmolality as expected, indicating the significance of AVP in regulating water reabsorption (Fig. 3b). In the OREBPdn Tg mice the low urine osmolality was further reduced by the V2R antagonist, indicating that the AVP-V2R system was functional in the absence of OREBP activity. The urine osmolality of the OPC-31260-treated Tg mice was significantly lower than that of the OPC-31260-treated Ntg mice (135 ± 45.56 mosmol/kg versus 335.2 ± 42.32 mosmol/kg; p < 0.05), suggesting an additive effect of OREBP and the V2R antagonist in blocking water reabsorption in the kidney. Under the water deprivation condition, OPC-31260 also significantly lowered the urine osmolality in the Ntg and Tg mice (Fig. 3c). Similar to the situation in mice under ad libitum condition, under the water deprivation condition the osmolality of the OPC-31260-treated Tg mice was significantly lower than that of the OPC-31260-treated Ntg mice (656.3 ± 56.64 mosmol/kg versus 1864 ± 139.3 mosmol/kg; p < 0.001).
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**Fig. 3.** a, urine osmolality before treatment (C), after 24-h water deprivation (D), or after dDAVP administration (A) of the Ntg and Tg mice (n = 15). b and c, urine osmolality before treatment (C), vehicle treatment (V), and OPC-31260 treatment (O) of Ntg and Tg mice under the water *ad libitum* and water deprivation conditions, respectively (n = 6). d, relative levels of AQP-2 mRNA in the kidney medullae from Ntg and Tg mice. AC, mice under the water *ad libitum* condition; AO, mice treated with OPC-31260 under the water *ad libitum* condition; DC, mice under 12-h water deprivation condition; and DO, mice treated with OPC-31260 under the 12-h water deprivation condition (n = 3). This experiment was repeated with another cohort of n = 3 with similar results. Data are mean ± S.E., *p < 0.05; **p < 0.001. Statistical significance was determined by an unpaired Student’s *t* test.

**Fig. 4.** a–c, relative levels of AQP-2 (a), AQP3 (b), and UT-A (c) mRNAs in the water *ad libitum* (A) and water-deprived (D) Ntg and Tg mice. The mRNA levels were normalized by the expression of glyceraldehyde-3-phosphate dehydrogenase mRNA in Northern blots. Data are mean ± S.E. (n = 3). *, *p < 0.05; **, p < 0.01. Statistical significance was determined by an unpaired Student *t* test. d, Northern blot of AQP2, AQP3, and UT-A mRNAs from renal collecting duct epithelial cell primary culture. Experiments in panels a–d were repeated with a different set of cohorts (n = 3) with similar results. e, immunoblotting of kidney inner medulla protein homogenates from 5-week-old Ntg and Tg mice with free access to water. The numbers below each row indicate the relative intensity of the bands with the values of Ntg mice set at 100. Data are mean ± S.E. (n = 3). *, *p < 0.05. Statistical significance was determined by an unpaired Student’s *t* test.

The dual control of the urine concentrating mechanism by OREBP and AVP was reflected in the level of AQP2 mRNA. As shown in Fig. 3d, OPC-31260 significantly reduced the levels of AQP2 mRNA in the Ntg mice under *ad libitum* and water deprivation conditions, indicating that AVP is required for its expression under both conditions. In the OREBPdn Tg mice, similar to the results in the previous section, the AQP2 mRNA was greatly reduced in the water *ad libitum* state but restored to a level similar to that of the Ntg mice under the water deprivation condition, suggesting that OREBP is involved in the regulation of expression of this gene in the water *ad libitum* state but not in the water deprivation state.

**DISCUSSION**

We demonstrated that the inhibition of OREBP activity in the epithelial cells of the kidney collecting tubules dramatically impaired the urine concentrating mechanism. This was not due to hydronephrosis in the OREBPdn Tg mice, because all the urine and mRNA measurements were done on 5-week-old mice, which had no significant structural abnormality in their kidneys. The decrease in urine concentrating ability is due in part to the down-regulation of UT-A1, UT-A2, and AQP2 expressions. It is generally accepted that urea accumulation in the renal inner medulla is important for the maintenance of maximal urinary concentrating ability and that UT-A1 and UT-A2 play important roles in the urea accumulation process (19, 20). UT-A1, located in the terminal segment of the IMCD, transports urea from the lumen to the interstitium, whereas UT-A2, located in short and long descending limbs of the loops of Henle, transports urea from the interstitium back into the lumen of the duct to complete the cycle. Therefore, it is likely that down-regulation of these two genes would reduce urea recycling and impair the ability of the kidney to conserve water. Aquaporins are also crucial for water reabsorption. They greatly increase the water permeability of the collecting ducts. AQP2 in particular, is the major water channel in the apical membrane of the collecting duct’s principal cells. In humans, defective AQP2 causes hereditary diabetes insipidus (21). AQP2 gene knock-out mice also have a severe defect in water reabsorption, leading to early death (22). Thus, down-regulation of this gene would also cause impairment in urine concentration.

It is well established that AVP, acting through its receptor V2R, regulates water reabsorption in the kidney by regulating the expression of aquaporin (AQP) and urea transporter (UT-A) genes as well as the intracellular translocation of some of their gene products (23, 24). Interestingly, there is also evidence that some of these genes are regulated by a mechanism independent of AVP. UT-A2 is induced in mIMCD3 cells grown in hypertonic medium in the absence of AVP (25, 26). Furthermore, in the IMCD cell cultures hypertonicity evokes a large increase in AQP2 expression (27), and in the V2R antagonist-treated rat
cultures dehydration leads to an up-regulation of AQP2 protein and a partial restoration of the urine concentrating ability of the cells (28). There are suggestions that OREBP may be involved in the AVP-independent induction of these genes (26, 29, 31), but evidence is unclear. UT-A2 is one of the four isoforms of urea transporters. UT-A1, -A3 and -A4 are alternately spliced mRNAs of the same transcript initiating from the UT-Aa promoter at the 5′-end of the gene, whereas UT-A2 is transcribed from an internal UT-AB promoter of the same gene (26, 32, 33). An ORE consensus sequence is located in the UT-Aa promoter, but there is no ORE sequence in the internal UT-AB promoter responsible for UT-A2 transcription (32). An ORE consensus sequence is also present in the promoter region of AQP2 gene (27); however, it is not clear if it is functional.

Our findings that UT-A1 and AQP2 mRNA expression was down-regulated by OREBPdn suggests that the OREs in the promoters of these gene are functional. The down-regulation of UT-A2 is somewhat puzzling. Although its expression is also induced by hypertonicity independent of AVP (25), its promoter does not contain the ORE sequence. Moreover, it is predominately expressed in the descending limbs of the loops of Henle where the OREBPdn transgene was not expressed at a detectable level, suggesting that the down-regulation of UT-A2 may be an indirect effect of the overexpression of OREBPdn in the collecting ducts. Interestingly, the primary cultures of renal epithelial cells express UT-A2 mRNA, presumably due to de-differentiation. Nevertheless, the cells from the Tg mice also showed down-regulation of UT-A2 mRNA, indicating that OREBPdn could directly down-regulate its expression. It is possible that the expression of UT-A2 is regulated by OREBP via the ORE sequence located in the upstream UT-Aa promoter for UT-A1 or another enhancer site far from the UT-AB promoter (32). However, it is not clear why UT-A2 expression is down-regulated by an OREBPdn mutation in vivo. One possible explanation is that the transgenic OREBPdn was expressed at low levels in the descending loops of Henle, undetectable by immunocytochemical analysis but sufficient to interfere with the expression of UT-A2. It is also possible that the down-regulation of UT-A2 by OREBPdn is a consequence of polyuria or other indirect effects of OREBP suppression.

Our data show that in addition to AVP, OREBP is another essential regulator of the urine concentrating mechanism and that its action is independent of AVP. This finding was demonstrated by the fact that AVP and OREBP regulate the aquaporin and urea transporter genes differently. The osmolality of the urine from the OREBPdn Tg mice was increased when they were deprived of water for 24 h or when they were injected with dDAVP, but it was much lower than the osmolality of the urine from the similarly treated Ntg mice. Northern blot experiments revealed that water deprivation induced the expression of AQP2 and UT-A1 in the Tg mice to the same level as that of the Ntg mice, indicating that the AVP-V2R system was unaffected by OREBPdn. However, UT-A2 expression in the Tg mice was not increased by water deprivation, making them unable to concentrate their urine to the same osmolality as that of the Ntg mice. These results indicate that under the water ad libitum condition, OREBP is essential for the expression for AQP2, UT-A1 and UT-A2. In the water deprivation state the expression of AQP2 and UT-A1 is entirely under the control of AVP, whereas the expression of UT-A2 is induced by OREBP.

During the preparation of this manuscript, the development of NFAT5 null mutant mice was reported (34). Similar to what we observed for our OREBP knockout mice, the homozygous NFAT5 mutant mice died at the embryonic stages, except that a few managed to survive to adulthood (7 of the expected 205). These mice showed severe renal atrophy with loss of nephrons and marked reduction in the levels of aldose reductase, betaine transporter, and sodium-dependent myo-inositol transporter mRNAs. Renal atrophy in these NFAT5 mutant mice was attributed to the reduced expression of these osmoprotective genes. The morphology of the kidneys of the NFAT-5 null mutant mice is somewhat different from that of the OREBPdn Tg mice, presumably because the OREBPdn transgene was only expressed in the epithelial cells of the collecting ducts. The cause of hydronephrosis in the OREBPdn Tg mice needs further investigation. It could be the consequence of the reduced expression of osmoprotective genes. On the other hand, hydropenia may be a consequence of polyuria because other animals with defective urine concentrating mechanism also exhibit similar hydropic renal morphology (35, 36).

Chronic treatment with the powerful immunosuppressant cyclosporine A (CsA) often causes renal injuries as manifested by polyuria, tubular atrophy, and interstitial fibrosis (37). It has been shown that CsA blocks the nuclear translocation of OREBP and consequently inhibits the hypertonic activation of the ORE-mediated reporter gene transcription and induction of osmoprotective genes (38). A recent report shows that long-term administration of CsA to the rats decreases the expression of aquaporins and urea transporters in their kidneys, accounting for the polyuric effect of CsA (39). Furthermore, the urine volume and urine osmolality of the CsA-treated rats were normalized by dDAVP treatment, indicating that CsA did not affect the AVP-V2R regulatory system. These findings, together with our observation that OREBPdn down-regulates AQP2, UT-A1, and UT-A2 to impair the urine concentrating ability, suggest that the polyuric effect of CsA is in part due to its inhibition of OREBP. Because OREBPdn Tg mice develop severe kidney lesions, it will be interesting to determine whether other aspects of CsA nephrotoxicity such as tubular atrophy and interstitial fibrosis may also be the consequence of inhibition of OREBP.

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