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Molecular Analysis of AQP2 Promoter. I. cAMP-dependent Regulation of Mouse AQP2 Gene

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To determine molecular mechanisms of Aquaporin-CD (AQP2) gene regulation, the promoter region of the AQP2 gene was examined by transiently transfecting a promoter-luciferase reporter fusion gene into mouse renal collecting duct cell lines such as mIMCD-3, mIMCD-K2, and M-1 cells, and NIH3T3 mouse embryo fibroblast cells. PCR-Southern analysis reveals that mIMCD-3 and mIMCD-K2 cells express AQP2, but M-1 and NIH3T3 cells do not, and that the treatment with cpt-cAMP (400 μM) or forskolin/isobutylmethylxanthine (IBMX) increased the AQP2 expression in IMCD cells. In both IMCD and NIH3T3 cells, the constructs containing the promoter of AQP2 gene showed promoter activities, indicating lack of tissue-specific element in the 1.4 kb 5'-flanking region of the mouse AQP2 gene. Luciferase activity in the IMCD cells transfected with the construct containing 5-flanking region showed responsiveness to cpt-cAMP, indicating that the 1.4 kb 5'-flanking region contains the element necessary for the regulatory mechanism by cAMP. The promoter-luciferase constructs which do not have a cAMP-responsible element (CRE) still showed the cAMP responsiveness in IMCD cells, but not in NIH3T3 cells. Increase in medium osmolarity did not affect AQP2 promoter activity in mIMCD-K2 cells. These results demonstrate that AQP2 gene transcription is increased with cAMP treatment through multiple motifs including CRE in the 5'-flanking region of the gene *in vitro*, and the regulatory mechanism may be important for *in vivo* regulation of AQP2 expression.

Key Words: AQP2 gene, cAMP, Collecting duct, Regulation

INTRODUCTION

Aquaporin-CD (AQP2) is a member of the major intrinsic protein (MIP) family (Fushimi et al, 1993) which localizes to the apical membrane of collecting ducts in kidney, and it plays a pivotal role in urinary concentration (Fushimi et al, 1993; Nielsen et al, 1993). Nonfunctional mutations of human AQP2 gene result in nephrogenic diabetes insipidus (Deen et al, 1994). These reports demonstrate that the AQP2 is the vasopressin-regulated water channel and plays a key role in the regulation of urine concentration. The water permeability of collecting ducts is significantly increased by vasopressin, which is attained by the insertion of AQP2 in endosomes into the apical membrane of collecting ducts (Nielsen et al, 1995; Yamamoto et al, 1995). This is caused by the increase of intracellular cyclic AMP (cAMP) concentration by vasopressin via V2 receptor. (Orloff & Handler, 1962). In addition to this rapid regulation of water permeability of collecting ducts by vasopressin, it has been reported that the AQP2 mRNA and protein abundance were increased in dehydrated rats (Nielsen, 1993). Since it may take at least several hours for AQP2 mRNA to increase, this gene regulation might not account for the early response of vasopressin-induced increase in water permeability in collecting ducts, and instead may be involved in the long-term regulation of urinary concentrating capacity. Stimu-

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lated transcription of AQP2 gene may be necessary for abundant supply of AQP2 protein into the apical membrane.

It is well known that increased intracellular cAMP can enhance the transcription level of various genes (Roesler et al, 1988). Thus AVP-induced increases in intracellular cAMP could stimulate the gene transcription of AQP2. Recent promoter studies using human AQP2 gene demonstrated that cAMP increased the activity of AQP2 promoter, and that cAMP responsive element (CRE) played an important role in the increase (Hozawa et al, 1996; Yasui et al, 1997). Their studies also showed that additional regulatory elements in the AQP2 promoter may be involved in the process. Hozawa et al (1996) reported that a Sp1 site between -160 and -150 from the translation initiation site of human AQP2 gene besides CRE may participate in the cAMP-induced increase of AQP2 promoter activity. Yasui et al (1997) showed that a AP-1 element located at -547 may play a role of the cAMP response. Recently, Jung et al (1997) and Rai et al (1997) showed that the sequence of the mouse AQP2 promoter did not have the AP-1 and Sp1 element at the corresponding position of the human AQP2 gene, suggesting that these elements may not be critical for the transcriptional regulation of AQP2 gene.

It has been reported that hypertonicity itself alters transcription rate of some genes such as aldose reductase gene (Ferraris et al, 1994) and the sodium-dependent transporter genes (Takenaka et al, 1994) through tonicity-responsible elements in the 5′-flanking region of the genes. Therefore, there is a possibility that dehydration-induced increase in the transcription of AQP2 gene may be resulted from the changes in osmolarity itself as well as increase in ADH secretion. However, this possibility has not been examined yet.

mIMCD-3 cells (Rauchman et al, 1993) and mIMCD-K2 (Kizer et al, 1995) were derived from the terminal inner medullar collecting duct (IMCD) of mice overexpressing the early region of simian virus SV40 (large T antigen), and retained many differentiated characteristics of this nephron segment. M-1 cells were derived from the outer cortical collecting duct of mice overexpressing the early region of simian virus SV40 (large T antigen) (Stoos et al, 1991) and exhibited both principal and intercalated cells functions (Fejes-Toth & Naray-Fejes-Toth, 1992).

This study was designed to determine *cis* elements which are involved in cAMP-induced regulation of the AQP2 gene. We made the promoter-reporter constructs using the mouse AQP2 gene, and assayed their activities in three kinds of mouse collecting duct cells or mouse NIH3T3 fibroblasts by transient transfection. We also determined the effect of hypertonic medium on the activity of AQP2 promoter to examine whether change in osmolarity itself affects the activity of AQP2 promoter.

METHODS

PCR-Southern analysis of AQP2 mRNA abundance in cultured mIMCD cells

Total RNAs were prepared from IMCD cells treated for 24 hours with cpt-cAMP (400 μM) or forskolin (10 μM)/isobutylmethylxanthine (IBMX) (10 μM) and reverse transcribed with MMLV transcriptase (Promega, Madison, WI, U.S.A.) using oligo dT primers. Polymerase chain reaction (PCR) was carried out to amplify mouse AQP2 and β-actin as an internal standard in the following profile: 94°C 1 min, 60°C 1 min, and 72°C 1 min for 30 cycles. PCR primers for mouse AQP2 are 5′-ATGTGGGAACTC-CGGTCCATA-3′ and 5′-GCTACCCAGGTTGTCA-CTGC-3′. Since the sense primer is located in exon 1 and the anti-sense primer in exon 2, the PCR product is not the result of amplification from genomic DNA. The PCR products were transferred to nylon membrane, and hybridized with ^{32}P-labeled AQP2 probe. Hybridization was performed at 65°C overnight in solution containing 6 × SSC, 5 × Denhardt's solution, 0.5% SDS, and 0.1 mg/ml denatured Herring sperm DNA. The intensity of the hybridized bands was measured using an image analyzer (Biorad, U.S.A.).

Plasmid construction

To identify promoter activity, pGL2-basic vector (Promega) was utilized as an expression vector. The 4 kb *Kpn* I fragment of mouse AQP2 gene (Jung et al, 1997) was digested with *Eco47* III to remove the first ATG of mouse AQP2 cDNA. The proximal 1.4 kb 5'-flanking region was subcloned into a pGL2-basic vector (Promega) (Kpn construct). A series of deletion mutants of proximal 5′-flanks was constructed using native restriction endonucleases (*Xho* I,

Pst I, *Aat* II, *Nco* I, *Eco*72 I) sites, which were located at about -1000, -306, -210, -101 and -57 bp apart from the transcription initiation site of the mouse AQP2 gene, respectively (Fig. 1). After digesting Kpn-construct with each restriction endonuclease, the ends of digested DNA were made blunt with T4 DNA polymerase (Life technologies, Baltimore, MD, U.S.A.) and self-ligated. To confirm 5′-end of constructs, DNAs were sequenced with Sequenase ver 2.0 (USB).

Cell culture and transient transfection

mIMCD-3, M-1 and NIH3T3 cells were purchased from American Type Culture Collection (Manassas, VA, U.S.A.). mIMCD-K2 cells are a generous gift from Dr. Stanton. Cells were grown in DMEM/F12 medium supplemented with 10% fetal bovine serum, 100 units/ml penicillin, and 100 μg/ml streptomycin in 5% CO_2 atmosphere. Approximately 10^6 Cells were harvested with trypsin/EDTA and plated into 12 well plates (Corning, U.S.A) the day before transfection. Plasmids were transfected into cultured cells by Lipofectamine (Life Technologies). Optimal transfection efficiency was obtained by the addition of 1 μg total plasmid DNA to each well of a 12 well plate followed by incubation for 6 hr. For assessing transfection efficiency, 1 μg of PCMV-GAL, a plasmid containing the β-galactosidase was transfected into cultured cells. Twenty-four hours after cotransfection, the cells were either maintained in the normal culture medium for another 24 hr, switched to a medium containing 400 μM cpt-cAMP, or switched to hypertonic medium containing 200 mM urea or 200 mM raffinose. Luciferase and β-galactosidase activities were determined with commercial kits (Promega) at 48 hr after cotransfection.

Results are expressed as percent increase $\pm$ SE in luciferase activity of the construct vs. pGL2-basic normalized for β-galactosidase activity. Data were analyzed by paired Student's t-test.

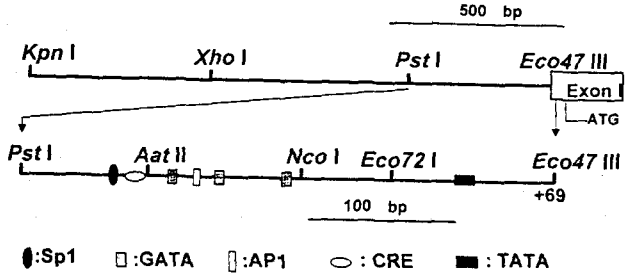

Fig. 1. The restriction map of the mouse AQP2 promoter.

Materials

Luciferase and β-galactosidase assay system were purchased from Promega (Madison, WI). Protein assay kit was purchased from Bio Rad. cpt-cAMP, forskolin, and IBMX were purchased from Sigma (St. Louis, MO).

RESULTS

Regulation of AQP2 expression in the IMCD cells by cAMP

To check whether mouse collecting duct cells express AQP2, Northern blot was performed using total RNA isolated from cells, but any positive signals were not detected. To increase detection sensitivity, PCR-Southern technique was used. Total RNAs isolated from cells were reverse transcribed and amplified with mouse AQP2 primers. PCR products were analyzed by Southern blot. Positive signals were only

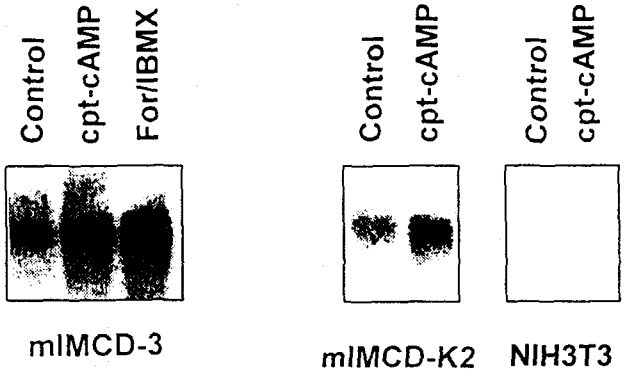

Fig. 2. Regulation of AQP2 expression in IMCD cells by cpt-cAMP or forskolin/IBMX. Total RNA was harvested from control, cpt-cAMP (400 μM) or forskolin/IBMX (10 μM)-treated IMCD and NIH3T3 cells. After reverse transcription of the total RNA, PCR was performed using mouse AQP2-specific and β-actin specific primers. PCR products were resolved in 2% gel, transferred to nylon membrane, and hybridized using the AQP2-specific probes. Lower pannel represents quantitation of Southern signals by image analyzer. The Southern signals were normalized with the β-actin signal of the corresponding samples. Data are shown as the ratio to the control values and are mean $\pm$ SE (n=3).

detected in mIMCD-3 and mIMCD-K2 cells, not in NIH3T3 cells (Fig. 2) and M-1 cells (data not shown). Treatment with cpt-cAMP (400 μM) or forskolin/IBMX increased the AQP2 expression about 2-3 folds in mIMCD-3 and mIMCD-K2 cell (Fig. 2). The abundance of β-actin in the IMCD cells co-amplified with AQP2 was not affected significantly by the treatment with cpt-cAMP (Data not shown). These results indicate that AQP2 gene expression can be regulated by cAMP and that IMCD cells can be used as the recipient cells in performing functional assay for the promoter of the mouse AQP2 gene.

Basal promoter activity of 5'-flanking region of mAQP2 gene is regulated by cAMP

To compare transfection efficiency of the cells to be used in the experiment, cells were tranfected with 1 μg of β-galactosidase construct (pCMV-GAL) and β-galactosidase activities were measured at the 2nd day after transfection. The transfection efficiency of NIH3T3 cells and M-1 cells were 5-6 folds higher than two IMCD cell lines (data not shown).

To determine whether the 5'-flanking region of mouse AQP2 gene contains tissue-specific promoter activity, the proximal 1.4 kb of 5'-flanking region was cloned into pGL2-basic vector and transiently transfected into two kinds of IMCD, M-1 and NIH3T3 cells. The promoter-luciferase constructs showed measurably greater luciferase activity than the control (pGL2-basic) in two IMCD cells and NIH3T3 cells, but not in M-1 cells (Fig. 3).

To examine the role of putative *cis*-acting con-

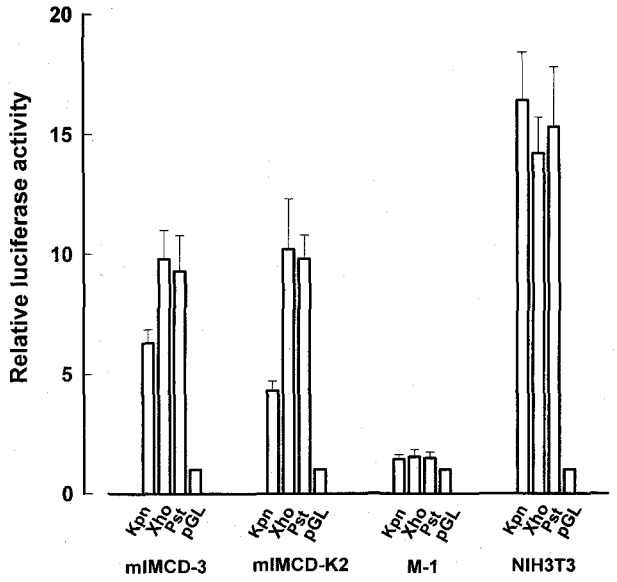

Fig. 3. Basal promoter activities of AQP2-promoter-luciferase fusion genes in renal collecting duct cells or NIH3T3 cells. 5-deletion mutants of AQP2 gene promoter (1.4 kb to 0.3 kb) were cloned in front of luciferase reporter construct and transfected into mIMCD-3, mIMCD-K2, M-1 and NIH3T3 cells. Luciferase activity was measured 48 hours after transfection. Luciferase activity results were normalized to β-galactosidase activity and expressed as increases in light units to pGL2-basic vector, which is assigned a value of 1.0. All data are the means $\pm$SE of 5 independent experiments.

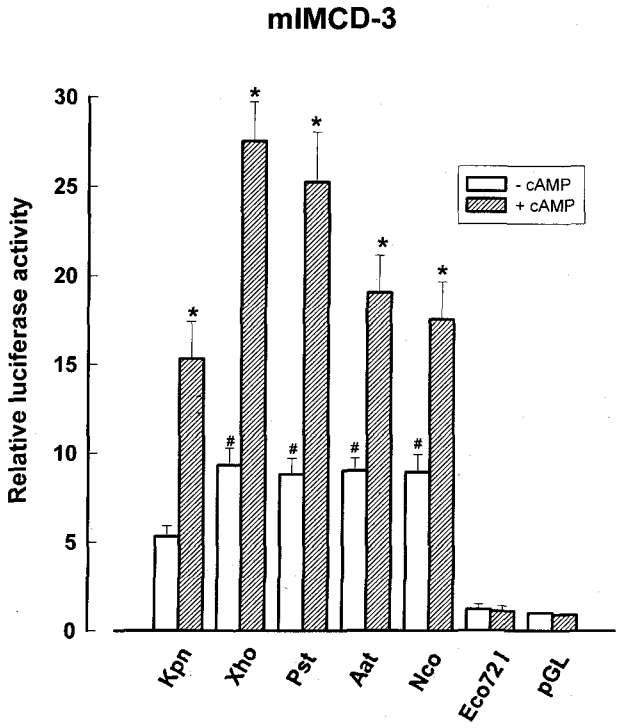

Fig. 4. Regulation of AQP2 promoter by cpt-cAMP in mIMCD-3 cells. 5'-deletion mutants of AQP2 gene promoter (1.4 kb to 57 bp) were cloned in front of luciferase reporter construct and transfected into mIMCD-3 cells. Transfected cells were seeded in 12 well plates and maintained for 24 hours. The cells in each of the two pairs of wells in 12-well plates were either maintained in normal medium for another 24 hours, or switched to a medium containing 400 μM cpt-cAMP. After 24 hours, the cells were harvested for luciferase assay. Data were presented as the ratio to the luciferase activity in cells transfected with pGL2-basic vector. All data are the mean $\pm$SE of 5 experiments. *, significantly different from the data in the absence of cpt-cAMP (<0.01). #, significantly different from the data of the Kpn constructs (<0.05)

sensus sequences in the transcription of the AQP2 gene by intracellular cAMP, mIMCD-3 cells were transfected with various AQP2 promoter reporter constructs and exposed to 400 μM cpt-cAMP. In mIMCD-3 cells the Nco construct, which has 5'-flanking region of 101 bps proximal to the transcription initiation site, still had considerable promoter activity. Further 44 bps deletion of the construct by the treatment of *Eco72* I reduced the promoter activity to the baseline value. The promoter activity of the Kpn construct was significanlty lower than those of the Xho, Pst, Aat and Nco constructs ($p<0.05$). The Aat and Nco constructs, which do not have a CRE in the promoter region, still had the responsiveness to the addition of cpt-cAMP in luciferase activities, although the responsiveness of these constructs to the addition of cpt-cAMP (2.11 ± 0.02 and 1.96 ± 0.03 fold increase in Aat and Nco constructs) was smaller than those in the Kpn, Xho or Pst constructs which contain a CRE (2.88 ± 0.03, 2.95 ± 0.02, and 2.86 ± 0.02 fold increase, respectively) ($p<0.05$) (Fig. 4). The luciferase activity of *Eco72* I construct was affected by the treatment of cAMP. Effect of cpt-cAMP on luciferease activity of the reporter constructs in mIMCD-K2 cells was similar to that in mIMCD-3 cells (data not shown).

AQP2 gene promoter-luciferase constructs were introduced into NIH3T3 cells using the same procedure as with mouse mIMCD-3 cells. The Kpn, Xho, Pst, Aat and Nco constructs in NIH3T3 cells in the absence of cpt-cAMP showed significant basal promoter activities, and showed almost identical activities unlike mIMCD cells. In the case of the Kpn, Xho and Pst constructs, cpt-cAMP increased promoter-luciferase activities like in IMCD cells, but failed to increase luciferase activities in the case of

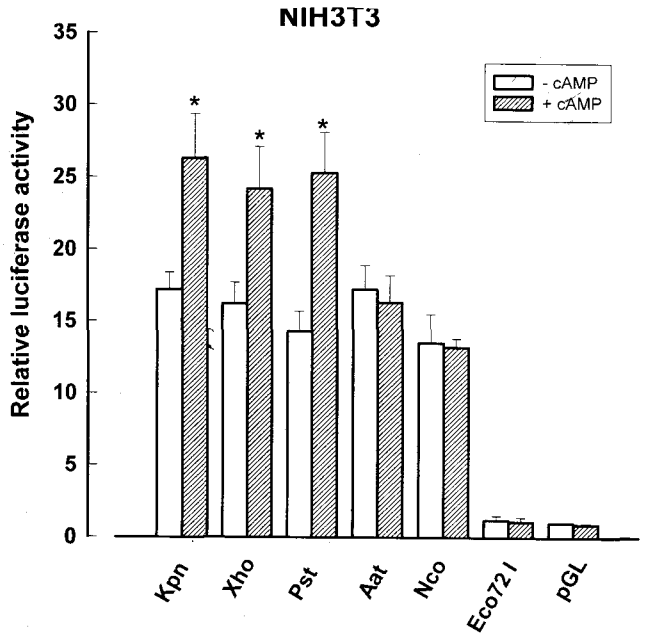

Fig. 5. Regulation of AQP2 promoter by cpt-cAMP in NIH3T3 cells. 5'-deletion mutants of AQP2 gene promoter (1.4 kb to 57 bp) were cloned in front of luciferase reporter construct and transfected into NIH3T3 cells. Transfected cells were seeded in 12 well plates and maintained for 24 hours. The cells in each of the two pairs of wells in 12-well plates were either maintained in normal medium for another 24 hours, or switched to a medium containing 400 μM cpt-cAMP. After 24 hours, the cells were harvested for luciferase assay. Data were presented as the ratio to the luciferase activity in cells transfected with pGL2-basic vector. All data are the mean $\pm$SE of 5 experiments. *, significantly different from the data in the absence of cpt-cAMP (<0.05)

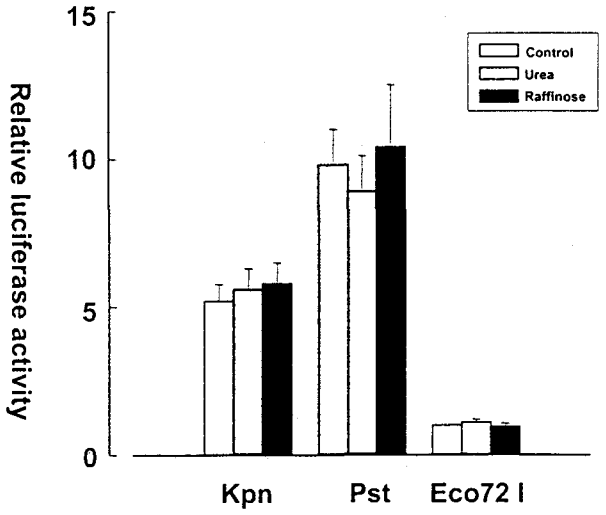

Fig. 6. Regulation of AQP2 promoter by hypertonicity in mIMCD-K2 cells. 5'-deletion mutants of AQP2 gene promoter (1.4 kb or 0.3 kb) were cloned in front of luciferase reporter construct and transfected into mIMCD-K2. Transfected cells were seeded in 12-well plates and maintained for 24 hours. The cells in each of the two pairs of wells in 12-well plates were either maintained in normal medium for another 24 hours, or switched to a medium each containing 200 mM urea or raffinose. After 24 hours, the cells were harvested for luciferase and protein assay. Data were presented as the ratio to the luciferase activity in cells transfected with PGL-Basic vector itself. All data are the mean$\pm$SE of 5 experiments.

the Aat and Nco-constructs (Fig. 5).

To determine if hypertonicity itself induces transcriptional activation of the mouse AQP2 gene, the Kpn, Xho or Pst constructs were transfected into mIMCD-K2 cells. It has been reported that cellular responses to hypertonicity depend on solute to be used for raising osmolarity (Zhang & Cohen, 1996). Therefore, medium osmolarity was increased by the addition of each 200 mM urea or raffinose. In this experiment, IMCD-K2 cells were used, because mIMCD-3 cells were not tolerable in the raffinose medium. As shown in Fig. 6, increase of medium osmolarity did not affect the luciferase activities in mIMCD-K2 cells.

DISCUSSION

AQP2 is a water channel, which is translocated to the apical pole of collecting duct principal cells after AVP stimulation (Fushimi et al, 1993). The mechanism of increasing the amount of AQP2 protein at apical plasma membranes of target cells by AVP stimulation may be the result of a redistribution of the protein both by vesicle shuttling and by increasing protein synthesis after stimulation of gene expression. The 5′-flanking promoter region of mouse AQP2 gene, which was used in this experiment, was active in NIH3T3 cells which do not express the AQP2 protein. These data suggest that the 1.4 kb 5′-flanking promoter region of the AQP2 gene does not include information to allow for tissue-specific expression, possibly because the regulatory promoter region for tissue-specific expression is nonfunctional in this cell line or, more likely, strong tissue-specific promoter-enhancers may exist elsewhere in other regions of the AQP2 gene. A recent study using transgenic mice revealed that 14 kb of 5′-flanking region contained *cis* elements that are sufficient for tissue-specific expression of AQP2 (Nelson et al, 1998). The Kpn construct showed lower promoter activity than the Xho, Pst, Aat and Nco construct, suggesting the presence of negative regulatory elements between the *Kpn* I and *Xho* I site in the 1.4 kb 5′-flanking region of AQP2 gene. This possibility should be confirmed by additional experiments such as footprinting and gel shift assay.

This study showed that mIMCD-3 and mIMCK-K2 cells expressed AQP2, although its expression was low, and that its mRNA abundance could be regulated by cAMP. Recently, the increase in AQP2 protein by dehydration in the kidney was shown to be blocked by V2 receptor antagonist, indicating that cAMP-mediated signal is likely to be important for the AQP2 promoter. Since Jung et al (1997) have found previously a CRE in the 5′-flanking region of the mouse AQP2 gene, the role of CRE may be important in cAMP-induced transcriptional activation of the AQP2 gene. This study showed that M-1 cells did not express AQP2, altough M-1 cells were derived from outer cortical collecting ducts cells which normally express AQP2. This may be resulted from the loss of important activators of AQP2 gene during cell line development. Comparison of DNA footprinting profiles between M-1 and mIMCD-3/mIMCD-K2 cells may provide clues about important regulatory factors of AQP2 gene.

Reporter gene assay clearly demonstrated that cAMP-mediated transcriptional activation of the AQP2 gene was dependent on other transcriptional factors as well as CRE in IMCD cells, because the Aat and Nco constructs still showed the responsiveness to cAMP. This study also showed that binding sites of additional transcriptional factors exist in between -101 and -57 bp sequence of mouse AQP2 gene. Previous studies using human AQP2 promoters reported that AP1 or SP2 site in the human AQP2 gene may be involved in cAMP-induced increase of human AQP2 promoter activity (Hozawa et al, 1996; Yasui et al, 1997). However, both sites are not conserved in the mouse AQP2 gene (Jung et al, 1997; Rai et al, 1997). It has been reported that putative CRE, AP2 sites, CCAAT box, and SP1 sites can confer cAMP inducibility independently in a variety of promoters (Boularand et al, 1995; Kagawa & Waterman, 1990; Roesler et al, 1988). Multiple cis- acting elements acting in concert with CRE have also been shown to regulate the gene transcription by cAMP (Anderson et al, 1990; Bustros et al, 1992; Comb et al, 1986; Muro et al, 1992). However, we could not find any consensus sequence in this region of mouse AQP2 gene, suggesting a possibility that novel transcription factors may participate in the process. The loss of cAMP-induced response by deletion of the CRE in the AQP2 promoter in NIH3T3 cells suggests that requirement of additional factors for cAMP-induced increase in the promoter activity of AQP2 gene in IMCD cells may be cell-specific.

In addition to the regulation by cAMP, the regulation of AQP2 gene by changes in medium osmo-

larity was determined. The transcription of osmolyte transporters in Madin Darby canine kidney-derived (MDCK) cells was shown to be regulated by medium hypertonicity (Uchida et al, 1993). The tonicity-responsive element (TonE) in the 5′-flanking region of Na^+-dependent betaine/GABA transporter gene was identified (Takenaka et al, 1994). However, increase in medium osmolarity by the addition of urea or raffinose did not affect AQP2 promoter activity in mIMCD-K2 cells, indicating that changes in osmolarity itself did not affect AQP2 pomoter activity.

These results indicate that the dehydration-induced increase in the transcription of AQP2 gene is mostly resulted from the changes in intracellular cAMP levels by dehydration-induced vasopressin release and that the interaction of multiple transcriptional factors is involved in the responsiveness of AQP2 gene to cAMP in collecting duct cells.

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REFERENCES

Anderson B, Kennedy GC, Hamernik DL, Bokar JA, Bohinski R, Nilson DL. Amplification of the transcriptional signal mediated by the tandem cAMP response elements of the glycoprotein hormone α-subunit gene occurs through several distinct mechanisms. *Mol Endocrinol* 4: 573−582, 1990

Boularand S, Darmon MC, Ravassard P, Mallet J. Characterization of the human tryptophan hydroxylase gene promoter. *J Biol Chem* 270: 3757−3764, 1995

Bustros A, de Ball DW, Peters R, Compton D, Nelkin BD. Regulation of human calcitonin gene transcription by cyclic AMP. *Biochem Biophys Res Commun* 189: 1157−1164, 1992

Comb M, Birnberg NC, Seaholtz A, Herbert E, Goodman HM. A cyclic AMP- and phorbol ester-inducible DNA element. *Nature Lond* 323: 353−356, 1986

Deen PMT, Verdijk MAJ, Knoers NVAM, Wieringga B, Monnens LAH, van Os CH, van Oost BA. Requirement of human renal water channel aquaporin-2 for vasopressin-dependent concentration of urine. *Science Wash DC* 264: 92−95, 1994

Fejes-Toth G, Naray-Fejes-Toth A. Differentiation of renal beta-intercalated cells to alpha-intercalated and principal cells in culture. *Proc Natl Acad Sci USA* 89(12): 5487−5491, 1992

Ferraris JD, Williams CK, Martin BM, Burg MB, Garcia-Perez A. Cloning, genomic organization, and osmotic response of the aldose reductase gene. *Proc Natl Acad Sci USA* 91: 10742−10746, 1994

Fushimi K, Uchida S, Hara Y, Hiraha Y, Marumo F, Sasaki S. Cloning and expression of apical membrane water channel of rat kidney collecting tubule. *Nature Lond* 361: 549−552, 1993

Hozawa S, Holtzman EJ, Ausiello DA. cAMP motifs regulating transcription in the aquaporin 2 gene. *Am J Physiol* 270(6 Pt 1): C1695−1702, 1996

Jung JS, Kim JI, Park MY, Bae HR, Lee SH. Cloning of mouse AQP-CD gene. *Kor J Physiol Pharmacol* 1: 195−200, 1997

Kagawa N, Waterman MR. cAMP-dependent transcription of the human CYP21B (P-450C21) gene requires a cis-regulatory element distinct from the consensus cAMP-regulatory element. *J Biol Chem* 265: 11299−11305, 1990

Kizer NL, Vandorpe D, Lewis B, Bunting B, Russell J, Stanton BA. Vasopressin and cAMP stimulate electrogenic chloride secretion in an IMCD cell line. *Am J Physiol* 268: F854−F861, 1995

Muro AF, Bernath VA, Kornblihtt AR. Interaction of the 170 cyclic AMP response element with the adjacent CCAAT box in the human fibronectin gene promoter. *J Biol Chem* 267: 12767−12774, 1992

Nelson RD, Stricklett P, Gustafson C, Stevens A, Ausiello D, Brown D, Kohan DE. Expression of an AQP2 Cre recombinase transgene in kidney and male reproductive system of transgenic mice. *Am J Physiol* 275: C216−C226, 1998

Nielsen S, DiGiovanni S, Christensen EI, Knepper MA, Harris HW. Cellular and subcellular immunolocalization of vasopressin-regulated water channel in rat kidney. *Proc Natl Acad Sci USA* 90: 11663−11667, 1993

Nielsen S, Chou CL, Marples D, Christensen EI, Kishore BK, Knepper MA. Vasopressin increases water permeability of kidney collecting duct by inducing translocation of aquaporin-CD water channels to plasma membrane. *Proc Natl Acad Sci USA* 92: 1013−1017, 1995

Orloff J, Handler JS. The similarity of effect of vasopressin, adenosine-3,5-phosphate (cyclic AMP) and theophylline on the toad bladder. *J Clin Invest* 41: 702−709, 1962

Rai T, Uchida S, Marumo F, Sasaki S. Cloning of rat and mouse aquaporin-2 gene promoters and identification of a negative cis-regulatory element. *Am J Physiol* 273: F264−F273, 1997

Rauchman MI, Nigam SK, Delpire E, Gullans SR. An osmotically tolerant inner medullary collecting duct cell line from an SV40 transgenic mouse. *Am J Physiol* 265: F416−F424, 1993

Roesler WJ, Vandenbark GR, Hanson RW. Cyclic AMP and the induction of eukaryotic gene transcription. *J Biol Chem* 263: 9063−9066, 1988

Stoos BA, Naray-Fejes-Toth A, Carretero OA, Ito S, Fejes-Toth G. Characterization of a mouse cortical collecting duct cell line. *Kidney Int* 39(6):1168−1175, 1991

Takenaka M, Bagnasco SM, Preston AS, Uchida S, Yamaguchi A, Kwon HM, Handler JS. The canine betaine c-amino-n-butyric acid transporter gene: diverse mRNA isoforms are regulated by hypertonicity and are expressed in a tissue-specific manner. *Proc Natl Acad Sci* 92: 1072−1076, 1995

Takenaka M, Preston AS, Kwon HM, Handler JS. The tonicity-sensitive element that mediates increased transcription of the betaine transporter gene in response to hypertonic stress. *J Biol Chem* 269: 29379−29381, 1994

Uchida S, Yamauchi A, Preston AS, Kwon HM, Handler JS. Medium tonicity regulates expression of the Na and Cl-dependent betaine transporter in Madin-Darby canine kidney cells by increasing transcription of the transporter gene. *J Clin Invest* 91: 1604−1607, 1993

Yamamoto T, Sasaki S, Fushimi K, Ishibashi K, Yaoita E, Kawasaki K, Marumo F, Kihara I. Vasopressin increases AQP-CD water channel in apical membrane of collecting duct cells in Brattleboro rats. *Am J Physiol* 268: C1546−C1551, 1995

Yasui M, Zelenin SM, Celsi G, Aperia A. Adenylate cyclase-coupled vasopressin receptor activates AQP2 promoter via a dual effect on CRE and AP1 elements. *Am J Physiol* 272(4 Pt 2): F443−F450, 1997

Zhang Z, Cohen DM. NaCl but not urea activates p38 and jun kinase in mIMCD3 murine inner medullary cells. *Am J Physiol* 271: F1234−F1238, 1996