Hypertonicity regulates the aquaporin-2 promoter independently of arginine vasopressin

Keizo Kasono1,3, Tomoyuki Saito1, Takako Saito1, Hiroyuki Tamemoto1, Chieko Yanagidate1, Shinichi Uchida2, Masanobu Kawakami1, Sei Sasaki2 and San-e Ishikawa1

1Department of Medicine, Jichi Medical School, Omiya Medical Center, Saitama, 2Department of Nephrology, Graduate School, School of Medicine, Tokyo Medical and Dental University, Tokyo and 3Laboratory of Clinical Nutrition, Department of Clinical Dietetics and Human Nutrition, Faculty of Pharmaceutical Sciences, Josai University, Saitama, Japan

Abstract
Background. Aquaporin-2 (AQP-2) is an arginine vasopressin (AVP)-regulated water channel in kidney collecting duct cells. The present study was undertaken to determine whether a change in tonicity could directly regulate the AQP-2 gene in an in vitro experiment.

Methods. Various fragments of the 5'-flanking region of the murine AQP-2 gene up to 9.5 kb were cloned into a luciferase (Luc) reporter plasmid, and they were transiently transfected into Madin–Darby canine kidney cells.

Results. Hypertonicity significantly increased the Luc activity of the constructs containing >6.1 kb of the 5'-flanking region of the AQP-2 gene (–6.1AQP2). However, promoter regions <4.3 kb in length containing the tonicity-responsive enhancer (TonE) at bp −570 to −560 were not stimulated by hypertonicity. The TonE-deleted construct which contains 9.5 to –1.1 kb of the 5' side of the AQP-2 gene, 8.4AQP2, was also stimulated by hypertonicity. Mitogen-activated protein (MAP) kinase inhibitors SB203580 and U0126 did not affect the Luc activity of –6.1AQP2 induced by hypertonicity. In addition, the vector expressing dominant-negative TonE-binding protein (TonEBP) did not affect the hypertonicity-induced Luc activity of –6.1AQP2. The Luc activity of –6.1AQP2 was stimulated by the overexpression of TonEBP. Hypertonicity further increased the Luc activity of –6.1AQP2 under the overexpression of TonEBP.

Conclusion. These findings indicate that hypertonicity regulates AQP-2 promoter activity via an AVP-independent mechanism, and that the tonicity-responsive element resides between the −6.1 and −4.3 kb 5'-flanking region of the AQP-2 gene, in which the structure and mechanism of response to hypertonicity could be distinct from those of TonE.

Keywords: aquaporin-2; gene regulation; hypertonicity; kidney; tonicity-responsive enhancer

Introduction

Aquaporin-2 (AQP-2) is an arginine vasopressin (AVP)-regulated water channel in renal collecting duct cells [1,2]. The subsequent activation of vasopressin V2 receptors (V2Rs) initiates the translocation of AQP-2-bearing vesicles from the cytosol to the plasma membrane within minutes, in which AQP-2 is inserted by an exocytosis-like process (short-term regulation) [3]. AVP also induces AQP-2 transcription by the cAMP-responsive element (CRE), and regulates the abundance of AQP-2 protein [4,5]. This is called long-term regulation. Therefore, AVP plays a pivotal role in the on–off regulation of the cellular trafficking of AQP-2 and the synthesis of AQP-2 in collecting duct cells.

However, there is no evidence that tonicity directly regulates the expression of AQP-2. In a state of chronic AVP excess (SIADH) [6,7], the antidiuretic action of V2R is attenuated, resulting in some water diuresis. This state has been termed as renal escape from AVP-induced antidiuresis. Several studies including our in vivo experiment recently have demonstrated that the upregulation of AQP-2 mRNA and protein expression was significantly attenuated in experimental rats with SIADH, compared with rats with an excess of AVP in the absence of volume expansion [8,9]. Therefore, either hypotonicity or volume expansion...
may directly regulate the post-receptor signalling of AVP in renal collecting duct cells [9].

A recent in vitro study may suggest the presence of osmotic control of AQP-2 transcription in primary culture of inner medullary collecting duct cells [10], which was probably mediated through the toxicity response enhancer element (TonE), (C/T)GGAGAnnn (C/T)m(C/T), located at bp −570 to −560 of the AQP-2 promoter [11,12]. However, the exact mechanism of transcriptional regulation by hypertonicity of the AQP-2 promoter has not been determined yet. Among the AQP family, it is evident that the expression of AQP-1, AQP-4 and AQP-9 was controlled by hypertonicity not via the TonE sequence [13,14].

In this study, we confirmed AVP-independent regulation of the AQP-2 promoter by hypertonicity, and investigated the site for hyperosmotic stimulation in the gene regulatory element of murine AQP-2. We found that the −6.1 to −4.3 kb 5′-flanking region of the AQP-2 promoter is the main hypertonicity-responsive region rather than TonE located at nucleotides −570 to −560.

Materials and methods

Cell culture

Madin-Darby canine kidney (MDCK) (CCL34) cells were purchased from the American Type Culture Collection (Manassas, VA). The number of passage of the cells was 49. Cells were maintained in Dulbecco’s modified Eagle’s medium (DMEM) supplemented with 5 mmol/l D-glucose, penicillin G (100 U/ml), streptomycin (100 μg/ml) and 10% fetal bovine serum (FBS). To clarify the effects of hypertonicity, all experiments were performed in medium containing a low concentration of FBS (1%).

Preparation of hypertonic medium

The hyperosmotic medium (600 mosmol/kg) was prepared by adding NaCl and urea at a molar ratio of 1:2 into the isotonic medium [15], which is close to the physiological condition in the renal medulla. Urea is a permeable solute, and does not make an osmotic gradient. Therefore, the medium showed ~450 mosmol/kg as the hypertonicity achieved by NaCl. The advantage is based on good viability and stability of cultured cells under long-term exposure to hypertonic conditions. Hyperosmotic medium (600 mosmol/kg) achieved by adding only urea was also used in some experiments.

Plasmid constructions for reporter assay

To make SfiI and EcoRI sites in the multiple cloning site of pGL3-basic vector (Promega, Madison, WI) as well as pGL3-basic vector, and annealed synthetic oligonucleotide fragments, 5′-TCGAGGGCCT GTACCGGCTCCTCGGAAATTTCCGATACTA-3′ and 5′-AGCTGTATATCGAATTCTTCTCGCAGGCGCTACAGGCC-3′, were inserted between the XhoI and HindIII restriction sites, and named modified-pGL3-basic vector. Then we cloned the SfiI and EcoRI sites into the multiple cloning site of pGL3-promoter vector (Promega, Madison, WI) as well as pGL3-basic vector, and annealed synthetic oligonucleotide fragments, 5′-TCGAGGGCCT GTACCGGCTCCTCGGAAATTTCCGATACTA-3′ and 5′-TCGAGGAGATCTCGTGGC-3′, were inserted between the XhoI restriction site, and named modified-pGL3-promoter vector. We isolated and characterized the murine AQP-2 gene promoter region. Approximately 5 x 105 phage clones from a mouse genomic library (Straytagene, La Jolla, CA) were screened with exon 1 of murine AQP-2 cDNA labelled with [α-32P]dCTP by random printing. Positive clones were purified and characterized by restriction endonuclease mapping and Southern blot analysis. Positive DNA fragments were subcloned into pSPORT1 vector (Life Technologies). We selected the longest clone and sequenced it near the 5′ side by Taq Dye Terminator Cycle Sequencing Kit (Perkin-Elmer, Culver City, CA). The murine AQP-2 gene and a total of 14 kb of flanking sequence (~10.5 kb from the 5′ side and 3.5 kb from the 3′ side of the initiation codon) into pSPORT1 (Invitrogen) from overlapping λ clones. In this study, all primers for polymerase chain reaction (PCR) amplifications of the murine AQP-2 promoter region were constructed according to the sequencing data of the murine AQP-2 gene reported by Nelson et al. (National Center for Biotechnology, National Institute of Health, accession number. AY055468). Introduction of SfiI and EcoRI sites was performed by PCR using upper primers 5′-ACTCGAATGGCCTGTACCAGGAAATTTCCGATACTACAC TTAGGAAAG-3′ (murine AQP-2 promoter from bp −9516 to −9497), 5′-ACTCGAATGGCCTGTACCAGGAAATTTCCGATACTACAC TTAGGAAAG-3′ (murine AQP-2 promoter from bp −6041 to −6022), 5′-ACTCGAATGGCCTGTACCAGGAAATTTCCGATACTACAC TTAGGAAAG-3′ (murine AQP-2 promoter from bp −4327 to −4304), 5′-ACT CGAATTCGCTGTACCAGGAAATTTCCGATACTACAC TTAGGAAAG-3′ (murine AQP-2 promoter from bp −356 to −337), and lower primer 5′-CTAGC CAGAATTCATGTGCCTCGAACCTTCTGGAC-3′ (from bp 1 to −20). After cutting these PCR products with SfiI and EcoRI, the fragments were cloned into the modified-pGL3-basic vector. These constructs contained 9.5, 6.1, 4.3, 2.6 and 0.36 kb 5′-flanking regions of the AQP-2 promoter, and were named −9.5AQP2, −6.1AQP2, −4.3 AQP2, −2.6 AQP2 and −0.36AQP2, respectively. We also cut out 1.1 kb of 5′-flanking region between the HindIII site from the −9.5AQP2, and inserted this fragment into the HindIII site of modified-pGL3-basic vector (−1.1AQP2). To delete the TonE-binding protein (TonEBP)-binding site at bp −570, we cloned −9.5 to −1.1 kb of the 5′ side of the murine AQP-2 gene. Introduction of SfiI and EcoRI sites was performed by PCR using the upper primer 5′-ACTCGAATGGCCTGTACCAGGAAATTTCCGATACTACAC TTAGGAAAG-3′ (from bp −9516 to −9497) and the lower primer 5′-CTAGC CAGAATTCATGTGCCTCGAACCTTCTGGAC-3′ (from bp −1103 to −1122). After cutting these PCR products with SfiI and EcoRI, the fragments were cloned into the modified-pGL3-promoter vector, and named 8.4AQP2. This vector contains approximately 5 kb of AQP2 and 2.6 kb of the 5′-flanking region between the HindIII site from the −9.5AQP2, and inserted this fragment into the HindIII site of modified-pGL3-basic vector (−1.1AQP2). To delete the TonE-binding protein (TonEBP)-binding site at bp −570, we cloned −9.5 to −1.1 kb of the 5′ side of the murine AQP-2 gene. Introduction of SfiI and EcoRI sites was performed by PCR using the upper primer 5′-ACTCGAATGGCCTGTACCAGGAAATTTCCGATACTACAC TTAGGAAAG-3′ (from bp −9516 to −9497) and the lower primer 5′-CTAGC CAGAATTCATGTGCCTCGAACCTTCTGGAC-3′ (from bp −1103 to −1122). After cutting these PCR products with SfiI and EcoRI, the fragments were cloned into the modified-pGL3-promoter vector, and named 8.4AQP2. This vector contains −9.5 to −1.1 kb of the 5′ side of the murine AQP-2 gene. Therefore, the TonEBP-binding site and CRE were deleted. The cDNA of human TonEBP was cloned by Miyakawa et al. [16], and the gene had a large open reading frame (ORF) of 1455 amino acids. The protein has a bipartite structure, a DNA-binding domain in the N-terminus and a
transactivation domain toward the C-terminus. A truncated TonEBP containing only the DNA-binding site (N-terminal 472 amino acids) showed dominant-negative activity on TonE-mediated stimulation of reporter gene expression [16,17]. TonEBP and the truncated TonEBP cloned into the multiple cloning site of pcDNA3, and named pTonEBP and pDNTonEBP, respectively, were kindly provided by Professor H. M. Kwon, Department of Medicine, Johns Hopkins University, Baltimore, MD.

Transient transfections
MDCK cells (5 × 10⁴) were seeded into 24-well tissue culture plates (16 mm well diameter) and grown for 24 h. The cells were transfected with 0.5 µg of DNA/well of the vectors described above. Transfections were performed using SuperFect (QIAGEN, Hilden, Germany) according to the manufacturer’s instructions. Hypertonic stress was induced by the 600 mosmol/kg medium. Regular DMEM was added to achieve the indicated concentrations 1 h before the hypertonic conditions were achieved. DMSO was also added to the controls to ensure that all wells had the same concentration of DMSO.

Luciferase and protein assay
Luciferase (Luc) activities were measured after 48 h incubation. In some experiments, 0.5 mmol/l of dibutyryl cAMP (DBcAMP) (Sigma-Aldrich, St Louis, MO) was added to the medium 6 h before the Luc assay. Cell lysis buffer (100 µl/well) was added to each well. A 10 µl aliquot of the lysate was used for protein assay using Bio-Rad protein assay reagent (Bio-Rad, Hercules, CA). The rest of the lysate was analysed for Luc activity using the Luciferase Assay System (Promega) and a luminometer TD-2020 (Turner Designs, Sunnyvale, CA). Luc activity was standardized by the amount of protein and presented as fold induction usually compared with the isotonic control wells cultured in the absence of DBcAMP.

Statistical analysis
Results were expressed as the mean ± SE. Statistical significance among experimental groups was assessed by analysis of variance (two-way ANOVA) and Student’s t-test, using a threshold for significance of P < 0.05.

Results
AQP-2 promoter is stimulated by DBcAMP and hypertonicity
A 6 h exposure of cells to 0.5 mmol/l DBcAMP significantly increased the fold induction of Luc activities of −1.1AQP2 and −9.5AQP2 by 1.79 ± 0.47 and 1.58 ± 0.37, respectively. Hypertonicity (600 mosmol/kg) significantly increased the fold induction of Luc activity of −9.5AQP2 by 1.64 ± 0.18. However, the Luc activity of −1.1AQP2, which has both TonE at bp −570 to −560 and CRE at bp −310 to −304, was unexpectedly not stimulated by hypertonicity (Figure 1). 8.4AQP2 does not include TonE and CRE. However, hypertonicity still activated the Luc activity of 8.4AQP2 by 1.77 ± 0.18. Hypertonic stimulation upregulated −9.5AQP2 in a dose-dependent manner. Hypertonicity >500 mosmol/kg significantly increased the Luc activity of −9.5AQP2 (data not shown). Hyperosmolality (not hypertonicity) of 600 mosmol/kg achieved only by the permeable solute urea did not affect the Luc activity of −9.5AQP2 (data not shown). Then, we examined the effects of both hypertonicity and DBcAMP on the Luc activity (Figure 2).

Synergistic effects of DBcAMP and hypertonicity on AQP-2 promoter activity. The constructs −1.1AQP2 and −9.5AQP2 were transfected into MDCK cells by the SuperFect transfection reagent. The transfected cells were incubated in isotonic (300 mosmol/kg) or hypertonic medium (600 mosmol/kg) for 48 h. DBcAMP (0.5 mmol/l) was added to the cells cultured in the isotonic medium at 42 h. cAMP(+)/(-) Hyper/Iso = cAMP(+)/(-) Hyper/Iso. Hyper/Iso = hypertonic medium/isotonic medium. *P < 0.05 vs DBcAMP(-) or the isotonic condition. Values are means ± SE (n = 4).

Fig. 1. Activity of the AQP-2 promoter in response to DBcAMP or hypertonicity. The constructs −1.1AQP2 and −9.5AQP2 were transfected into MDCK cells by the SuperFect transfection reagent. The transfected cells were incubated in isotonic (300 mosmol/kg) or hypertonic medium (600 mosmol/kg) for 48 h. DBcAMP (0.5 mmol/l) was added to the cells cultured in the isotonic medium at 42 h. A 10 µl aliquot of the lysate was used for protein assay using Bio-Rad protein assay reagent (Bio-Rad, Hercules, CA). The rest of the lysate was analysed for Luc activity using the Luciferase Assay System (Promega) and a luminometer TD-2020 (Turner Designs, Sunnyvale, CA). Luc activity was standardized by the amount of protein and presented as fold induction usually compared with the control wells cultured in the isotonic medium in the absence of DBcAMP.

Fig. 2. Synergistic effects of DBcAMP and hypertonicity on AQP-2 promoter activity. The constructs −1.1AQP2 and −9.5AQP2 were transfected into MDCK cells, and cells were incubated in isotonic (300 mosmol/kg) or hypertonic medium (600 mosmol/kg) for 48 h. DBcAMP (0.5 mmol/l) was added to the cells cultured in the isotonic and hypertonic medium at 42 h. A 10 µl aliquot of the lysate was used for protein assay using Bio-Rad protein assay reagent (Bio-Rad, Hercules, CA). The rest of the lysate was analysed for Luc activity using the Luciferase Assay System (Promega) and a luminometer TD-2020 (Turner Designs, Sunnyvale, CA). Luc activity was standardized by the amount of protein and presented as fold induction usually compared with the control wells cultured in the isotonic medium in the absence of DBcAMP.
fold induction of Luc activities of -9.5AQP2 by 1.25 ± 0.05 and 1.40 ± 0.16, respectively. Co-stimulation of hypertonicity and DBcAMP synergistically increased the Luc activity of -9.5AQP2 by 1.89 ± 0.11, but the Luc activity of -1.1AQP2 induced by DBcAMP was not significantly altered by the co-existence of hypertonicity. These results indicated that hypertonicity directly stimulated the AQP-2 promoter region in -9.5AQP2. In our experimental conditions, TonE was not significantly activated by hypertonicity.

Location of the hypertonicity-responsive region in the AQP-2 promoter

The promoter of the murine AQP-2 gene contains binding sites for CREB and TonEBP, and these elements are located at nucleotides -310 to -304 (GACGTCA) and -570 to -560 (TGGAAATTTGT), respectively. We made several constructs, which were named -0.36, -1.1, -2.6, -4.3, -6.1 and -9.5AQP2. All of the constructs transfected in MDCK cells usually showed >100-fold greater Luc activity than pGL3-basic vector (data not shown). Figure 3 shows the fold induction of Luc activities under hypertonic conditions (600 mosmol/kg) compared with isotonic conditions. The fold induction of Luc activities of -0.36, -1.1, -2.6, -4.3, -6.1 and -9.5AQP2 were 1.03 ± 0.35, 0.80 ± 0.13, 1.08 ± 0.13, 0.83 ± 0.07, 1.69 ± 0.29 and 1.62 ± 0.36, respectively. Only the Luc activities of -6.1 and -9.5AQP2 were significantly increased. These results suggested that the main hypertonicity-responsive region or regions were located in -6.1 through -4.3 kb of the 5′-flanking region of the murine AQP-2 promoter.

Effects of MAP kinase inhibitors and DNTonEBP on AQP-2 promoter activity

We next examined the effects of MAP kinase inhibitors on the promoter activity of -6.1AQP2. Neither SB203580 (p38 MAP kinase inhibitor) nor U0126 (MEK inhibitor) affected the Luc activity of -6.1AQP2 (Figure 4). Hypertonicity significantly increased the fold induction of Luc activity in MDCK cells co-transfected with -6.1AQP2 (0.5 µg/well) and control plasmid, pcDNA3 (1 µg/well), by 1.57 ± 0.15. Co-transfection with -6.1AQP2 (0.5 µg/well) and pDNTonEBP (1 µg/well) did not affect the fold induction of the hypertonicity-induced Luc activity of -6.1AQP2 (Figure 5). We cloned the double TonE sequence into -0.36AQP2 (pTonE2). Hypertonicity significantly increased the fold induction of Luc activity in MDCK cells co-transfected with pTonE2 and pcDNA3. Co-transfection of pDNTonEBP with pTonE2 significantly reduced the hypertonicity-induced Luc activity (data not shown).

Effect of TonEBP on AQP-2 promoter activity

To achieve the overexpression of TonEBP, we performed the transfection of -1.1AQP2 or -6.1AQP2 with pTonEBP. The promoter of pTonEBP is the cytomegalovirus (CMV) promoter, therefore the transcription of TonEBP may not be affected by
hypertonicity. Co-transfection with −1.1AQP2 (0.5 µg/well) and pTonEBP (0.5 or 1 µg/well) in the isotonic medium remarkably augmented the Luc activity by 1.65 ± 0.06 and 4.09 ± 0.47, respectively. In the same experiment, the co-existence of hypertonicity and pTonEBP did not stimulate the Luc activity of −1.1AQP2. pTonEBP (0.5 or 1 µg/well) in the isotonic medium also increased the fold induction of Luc activity of −6.1AQP2 by 1.92 ± 0.23 and 6.42 ± 1.24, respectively. In this case, hypertonicity further enhanced the Luc activity of −6.1AQP2 under the overexpression of TonEBP (P < 0.05; Figure 6).

Discussion

The 5′-flanking region of AQP-2 has a CRE located at bp −310 to −304 (GACGTCA) and a TonE located at bp −570 to −560 (TGGAAATTTGT) [4,10]. It is therefore speculated that AQP-2 transcription is regulated by hypertonicity.

The present study demonstrated the hypertonicity-responsive transcriptional regulation of the AQP-2 gene, by analysing the Luc assay of the reporter constructs containing the 5′-flanking region of the AQP-2 gene transfected into MDCK cells. We examined the 5′-flanking region of the AQP-2 gene for up to −9.5 kb from the initial codon of AQP-2. Hypertonic stimulation did not significantly activate the AQP-2 promoter with < 4.3 kb of the 5′-flanking region, though TonE is contained in the AQP-2 promoter at nucleotides −570 to −560. Furthermore, 8.4AQP2, the construct which contains no TonE and CRE, was still activated by hypertonic stimulation as well as −6.1AQP2. The TonE consensus sequence, (C/T) GGAA(nnn)C/T(n)(C/T), initially was identified as the cis-acting element responsible for hypertonic regulation [18]. TonE mediates the transcriptional regulation of several genes, including sodium/myo-inositol co-transporter, sodium/chloride/betaine co-transporter, and aldose reductase in response to hypertonicity [10,11,19]. TonEBP, a transcription factor that stimulates TonE, has been cloned using the yeast one-hybrid assay [16]. It is possible that MDCK cells do not produce a sufficient amount of TonEBP to stimulate TonE in our experimental conditions, or that TonE in the murine AQP-2 promoter region has low sensitivity to the action of TonEBP.

Rim et al. [12] reported that five TonEs were scattered over 50 kb in the gene regulatory element of the sodium/myo-inositol co-transporter gene. The exposure of cells to hypertonicity increased the binding of TonEBP to these TonE sites, and together they stimulated transcription under hypertonic conditions. Although we have no data regarding the nucleotide sequence further than 10.5 kb of the 5′-flanking region of the AQP-2 gene, there is only one TonE located in the 5′-flanking region...
of AQP-2 up to 10.5 kb. The present results suggested the presence of an additional toxicity-responsive element in addition to TonE in the 5′-flanking region of the AQP-2 gene.

Canine MDCK cells may not be the ideal model system to study gene regulation of murine AQP2. When the series of experiments was started, the transfected constructs were not well expressed in mouse IMCD3 cells, and the transfection ratio of mouse IMCD3 cells was <1% using SuperFect. Thus MDCK cells were employed to evaluate the AQP-2 promoter activity. The transfection ratio of MDCK cells was >20%. In our experimental system using MDCK cells, human TonEBP stimulated the Luc activity of constructs containing TonE well. Therefore, we speculate that the regulation of gene expression by hypertonicity may use the common pathway between many species.

The study using the fusion genes of various lengths of the AQP-2 promoter with the Luc reporter gene showed that constructs containing >6.1 kb of the 5′-flanking region had hypertonicity-induced Luc activity. The hypertonicity-induced Luc activity of −6.1AQP2 was found in hypertonic medium, by adding NaCl, but not urea. Urea penetrates the cell membrane, and does not produce a sufficient toxicity gradient. The osmolality of the medium was increased by adding NaCl and urea at a molar ratio of 1:2 in most of the present experiments [15]. The cells retained their viability well in the hypertonic medium containing both NaCl and urea. Additionally, this hypertonic medium showed a stable effect on the promoter activity of −6.1AQP2 and −9.5AQP2 rather than the hypertonic medium achieved by only NaCl.

We also examined the effect of DNTonEBP on the AQP-2 promoter. When the vector expressing DNTonEBP (pDNTonEBP) was co-transfected with −6.1AQP2, the Luc activity remained elevated after the cells were exposed to the hypertonic medium. Overexpression of TonEBP strongly stimulated the promoter activity of both −1.1AQP2 and −6.1AQP2. Hypertonicity further increased the Luc activity of −6.1 AQP2, but not that of −1.1 AQP2, under the over expression of TonEBP. In addition, the inhibitors of p38 MAP kinase and MEK, i.e. SB203580 and U0126, did not alter the hypertonicity-induced promoter activity of −6.1AQP2. As previously reported [20], p38 MAP kinase and MEK participate in cellular signal transduction to the hypertonicity-induced AQP-1 promoter activity. The AQP-2 promoter for up to −10.5 kb does not include the toxicity-responsive element (GCTCCCCCCC) located in the AQP-1 promoter. The present findings therefore indicate that the structure of the toxicity-responsive region at kb −6.1 to −4.3 is different from that of classical TonE or the toxicity-responsive element in the AQP-1 promoter. Further studies will be necessary to elucidate the exact sequence of such a TonE-independent toxicity-responsive element in the 5′-flanking region of the AQP-2 gene.

Since MDCK cells had no receptor for AVP, the effect of DBcAMP mimics the AVP-regulated transcription of the AQP-2 gene. The DBcAMP-induced Luc activity of −9.5AQP2 was augmented synergistically by hypertonicity, but it was not found in −1.1AQP2. The observation suggests that the toxicity-responsive region in −9.5AQP2 facilitates the synergism with CRE.

In conclusion, we demonstrated that there is an additional toxicity-responsive region in the 5′-flanking region of the AQP-2 gene located at kb −6.1 to −4.3 as well as the classical TonE. Hypertonic stimulation participates in a synergistic effect on AVP-induced AQP-2 transcription.

Acknowledgements. We thank Professor H. M. Kwon, Johns Hopkins University, for providing TonEBP and DNTonEBP. The present study was presented in part at the Annual Meeting of the American Society of Nephrology, November 13–17, 2003, in San Diego, California. The study was supported by grants from The Ministry of Education, Science and Culture of Japan (Nos 13671160 and 13137208), The Ministry of Health, Welfare and Labor of Japan and The Uehara Memorial Foundation.

Conflict of interest statement. None declared.

References

Hypertonicity regulates the AQP-2 promoter


Received for publication: 14.6.04
Accepted in revised form: 8.12.04