Downregulation of the V2 vasopressin receptor in dehydration: mechanisms and role of renal prostaglandin synthesis

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Submitted 2 May 2006; accepted in final form 22 December 2006

The main role of the kidney is to maintain body fluid homeostasis. The kidney is a target site of many hormones such as arginine vasopressin (AVP), endothelin, atrial natriuretic peptide (ANP), parathyroid hormone (PTH), glucagon, and aldosterone. AVP plays a key role in urine concentration in the case of dehydration (18). AVP has two types of receptors in the kidney, V1a and V2 receptors. V1a receptors mediate the vasopressor effects of AVP and are localized in renal vessels and in the thick ascending limbs and the collecting ducts (26, 28, 34). In contrast, V2 receptors mediate the antidiuretic action of AVP and are localized in the thick ascending limbs and the collecting ducts (9, 16, 25, 34). In the collecting ducts, the V2 receptor is located in the basolateral membrane of principal cells, while V1a receptor is mainly in the luminal membrane of both principal and intercalated cells (9, 25, 33). Urine concentration is caused by the binding of AVP to the V2 receptor and by the subsequent activation of aquaporin-2 (AQP2) (13, 14, 19, 23). The V2 receptor acts as Gs and stimulates adenylate cyclase. Nephrogenic diabetes insipidus is caused by an abnormality in the V2 receptor or AQP2 (20). AQP2 is present in the luminal membrane and intracellular vesicles of principal cells (22). The V1a receptor activates Gq/11 and subsequently phosphoinositide turnover (1).

In dehydration, trafficking of AQP2 in the intracellular vesicles to the apical membrane occurs as a short-term regulation (23). In long-term regulation, the expression of AQP2 is stimulated in chronic dehydration (35). In contrast, the expression of V2 receptor in collecting ducts is downregulated in dehydration (27), which is the opposite of the conservation of water and sodium in dehydration. Urine osmolality and plasma concentration of AVP are increased in dehydration, suggesting that hyperosmolality or increased plasma level of AVP may downregulate the expression of the V2 receptor. The production of other substances such as cytokines and prostaglandins is known to increase in dehydration or by vasopressin treatment (3, 31, 38). Those substances may also play roles in downregulation of the V2 receptor in dehydration.

METHODS

Materials. AVP, collagenase, PGE2, 8-(4-chlorophenylthio) adenosine 3’5’-cyclic monophosphate (CPT-cAMP), indomethacin, and bovine serum albumin (BSA) were purchased from Sigma (St. Louis, MO). The DNA synthesis kit and PCR master were obtained from Roche Molecular Biochemicals (Mannheim, Germany). Vanadyl ribonucleotide complex (VRC) was from GIBCO (Gaithersburg, MD). Primary antibody against AQP2 was a kind gift from Drs. S. Nielsen (University of Aarhus, Denmark) and M. A. Knepper (National Institutes of Health, Bethesda, MD).

Renal tubule microdissection. Microdissection of renal tubule segments was performed as described previously (21, 25). Our protocol of animal experiments was approved by the committee of the Kumamoto University Institute of Resource Development and Analysis (15-026, 16-063, 17-013, 18-127), where the animals were housed. In brief, male Sprague-Dawley (SD) rats weighing 75–100 g were anesthetized by pentobarbital sodium (50 mg/kg). The aorta was...
cannulated with polyethylene tubing below the left kidney, and the left kidney was perfused in vivo. The kidney was perfused initially with 10 ml of ice-cold dissection solution [solution A (in mM): 130 NaCl, 5 KCl, 1 NaH₂PO₄, 1 MgSO₄, 1 Ca lactate, 2 Na acetate, 5.5 glucose, and 10 HEPES (pH 7.4 by NaOH)] and then with 10 ml of the same solution A containing 1 mg/ml collagenase and 1 mg/ml BSA (solution B). The left kidney was removed, and coronal sections that contained the entire corticopapillary axis were made. These pieces were transferred into tubes containing 3 ml of solution B containing 150 μl VRC, a potent RNase inhibitor. The tubes were incubated for 30 min at 37°C in a shaking water bath. The solutions were bubbled with 100% oxygen during the incubation. Next, tissues were transferred to a digestion dish that contained 10 mM VRC. Two-millimeter lengths of inner medullary collecting duct (IMCD) were microdissected for each sample. IMCD was mainly dissected from the middle third of the inner medulla (IMCD2). Microdissected IMCDs were washed with solution A in separate wash dishes.

Tabule incubation study: These IMCDs were transferred into tubes that contained various solutions for incubation studies. For the examination of the effect of hyperosmolality, NaCl or urea was added to solution A. Hyperosmotic solutions of 490, 690, 890, and 1,090 mosmol/kgH₂O were made. AVP or CPT-cAMP was dissolved in solution A. In the PGE₂ incubation study, PGE₂, AVP and vehicle were incubated in isotonic (C) or hypertonic medium (N; 490 mosmol/kgH₂O by adding NaCl) for 1 to 6 h at 37°C. Typical: top panel, the effect of the ethidium bromide-stained gel. Top and bottom bands show the PCR product of V2 receptor mRNA (578 bp) and the competitor 453 bp, respectively. Bottom panel, V2 receptor mRNA expression after the incubation in isotonic and hypertonic medium (C and N, respectively). V2 receptor mRNA expression was stimulated by hyperosmolality after 2- to 3-h incubation, n = 4. *P < 0.05 vs. control (isotonic incubation).

0.05°C for 5 min, and then the reaction tubes were placed on ice until PCR studies were made.

P CR. Specific primers for the V2 receptor and AQP2 were designed. The sequence of sense and antisense V2 receptor primers were defined by bases 349–368 (5′-TACCTCGACATGTGGGGCAT-3′) and bases 910–929 (5′-AGCACACAAAGGGGGTTCT-3′), respectively (9, 20). This corresponds the long form of V2 receptor mRNA in Firsov’s classification (9). The predicted size of amplified cDNA was 578 bp. Sense and antisense AQP2 primers were defined by bases 232–252 (5′-TGCGCATGGATCTTCT-3′) and bases 765–784 (5′-AGCCTTCTTCAAGCCTT-3′), respectively (10). The predicted size of amplified cDNA was 553 bp.

Competitive PCR. V2 receptor and AQP2 mRNA expressions were quantified using competitive PCR. The DNA competitor for the competitive PCR was synthesized using overlap extension PCR as described previously (21). The inner sense and inner antisense primers for V2 receptor were defined by bases 786 to 803 (5′-TTTGCGGACCCTGGGTCAACAGCGACACCA-3′) and bases 640 to 657 (5′-TTGGCCATTGTCTGGATTAGGATGACCACCAAA-3′), respectively (the complementary sequence added to the 5′ tail is underlined) (20). The inner sense and inner antisense primers for AQP2 were defined by bases 545 to 564 (5′-GTCGAGAAGACATATCGGAC-3′) and bases 422 to 441 (5′-AGGCCGATCCAGGTTCTTT-3′), respectively (10). The sizes of the DNA competitor for V2 receptor and AQP2 were 453 and 450 bp, respectively.

Ethidium bromide staining and Southern blotting. The PCR products were ethanol precipitated and electrophoresed in 2% agarose gel in TAE. PCR products were visualized by ethidium bromide staining. For quantitative measurements of V2 receptor and AQP2 mRNA expression, the intensity of the bands was measured using a densitometer (Atto, Tokyo, Japan). In some experiments, the PCR products were sequenced to check the amplification of V2 and AQP2 mRNAs. TAE buffer was composed of (in mM) 40 Tris, 1 EDTA (pH 8.3 by acetic acid).

Isolation of membrane fraction. The membrane fraction of the inner medulla was obtained from rats as described previously (25). After both kidneys were removed, the inner medulla was dissected out and minced using a razor blade and homogenized by five strokes at 1,000 rpm with a glass homogenizer at 4°C. After centrifugation at 7,600 rpm for 15 min, the supernatant was collected and centrifuged

RT. RT was performed by adding 3.5 μl of 2% Triton X-100, containing 0.4 U/ml of RNase inhibitor and 11 mM DTT, and 4.4 μl of RT master mix. The reaction tubes were incubated at 42°C for 60 min in the block incubator. The reaction was stopped by heating at 90°C for 5 min, and then the reaction tubes were placed on ice until PCR studies were made.

V2R cDNA

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Fig. 1. Standard curve for quantification of V2 receptor mRNA expression. V2 receptor mRNA expression was examined using RT-competitive PCR. A series of dilutions of V2 receptor cDNA (0, 2, 4, 8, or 12 fg/tube) was coamplified with a constant amount of the competitor (4 fg/tube). The ratio of V2 receptor cDNA to the competitor was corrected for the difference in molecular weight. There was a linear relationship between the V2 receptor cDNA and V2 receptor cDNA/competitor ratio.

Data from http://ajrenal.org by 10.220.33.6 on June 22, 2017
Tubule suspensions were prepared for Western blotting as described previously (24). Male Sprague-Dawley rats weighing 150–250 g were anesthetized, and kidneys were perfused via the aorta. Renal outer and inner medulla were used for tubule suspension. 240 ml of suspension contained a total of 500 ml of solution (10 ml of tubule suspension, 1 ml of solution A with 0.20% BSA, and 250 ml of various kinds of hyperosmotic or AVP solutions made by adding NaCl, urea, or AVP). After incubation, tubule suspensions were centrifuged, and the supernatant was discarded. Ten microliters of 2× lysis buffer were added to the pellet and stored at −30°C until SDS-PAGE and Western blotting.

SDS-PAGE and Western blot analysis. SDS-PAGE was performed as described previously (21, 25). Tubule suspension or isolated IMCD was mixed with sample buffer (final concentration: 10% glycerol, 2.3% SDS, 5% b-mercaptoethanol, 65 mM DTT, 0.005% bromophenol blue, 62.5 mM Tris·HCl, pH 6.8) and running buffer (25 mM Tris, 192 mM glycine, 0.1% SDS). Samples were denatured for 5 min at 95°C, electrophoresed by polyacrylamide gel plate (4–20% gradation), and transferred onto the membrane (Immobilon-P, Millipore, Bedford, MA) electrophoretically. After blocking by TBS-T with 5% milk, membrane was incubated overnight with primary antibody for V2 receptor and AQP2 in TBS-T with 5% milk at 4°C. A polyclonal antibody against the synthetic peptide corresponding to the third intracellular loop (RRRGRRTGSP) of rat V2 receptor was newly raised as described previously (25). An antibody against AQP2 was a kind gift from Drs. S. Nielsen (University of Aarhus, Denmark) and M. A. Knepper (National Institutes of Health, Bethesda, MD). After hybridization, the membrane was washed with TBS-T. Next, a horseradish peroxidase-linked anti-rabbit Ig (Fab')2 fragment (from sheep) was hybridized for 1 h at room temperature. The membrane was washed in TBS-T again, and specific protein expression was visualized using ECL Western blotting detection reagents. The composition of TBS was as follows: 20 mM Tris, 137 mM NaCl (pH 7.6).

Indomethacin study. In the indomethacin study, SD rats (80–150 g body wt) were divided into four groups. Group 1 consisted of rats with free access to water without indomethacin injection; group 2 consisted of rats with free access to water with intraperitoneal indomethacin injection; group 3 consisted of rats with water deprivation without indomethacin injection; and group 4 consisted of rats with water deprivation with intraperitoneal indomethacin injection. Indomethacin was intraperitoneally injected at 0.5 mg·100 g body wt−1·day−1 for 2 days. Since indomethacin was dissolved in DMSO, the two groups without indomethacin were intraperitoneally injected with 50 μl·100 g body wt−1·day−1 of DMSO for 2 days. After 3, 6, 24, and 48 h, membrane fractions of inner medulla and microdissected IMCDs from these rats were obtained. For urine collection, rats were put in the metabolic cages from 1 day before the indomethacin or DMSO injection.

Fig. 4. Effects of hyperosmolality on V2 receptor protein expression in IMCD. Tubule suspension of inner medulla was incubated with various substances for 24 h. Top: typical example of Western blot. V2 receptor was detected at 41 kDa. Bottom: V2 receptor expression after incubation with various substances. V2 receptor expression was stimulated by hyperosmolality by NaCl but not by urea. Very small but significant stimulation was observed by high-dose AVP, n = 7. *P < 0.05 vs. control (isotonic incubation).
Immunohistochemical study. An immunohistochemical study was performed using the antibody to AQP2 as described previously (25). Inner medulla was obtained from dehydrated rats. Indomethacin or DMSO was administered for 2 days as described above. Fluorescence microscope (Olympus BX50 with BH2-RFL-T3) and image analyzing system (Olympus DP70) were used for immunohistochemical study.

Statistics. Results are expressed as means ± SE. Statistical analysis was performed using Student’s t-test for two groups or ANOVA (or Kruskal Wallis analysis) followed by Dunnett’s, Fisher’s, or Bonferroni’s (Dunn’s) multiple comparison for more than three groups, as appropriate. A P < 0.05 was considered to be statistically significant.

RESULTS

Standard curve for quantification of V2 receptor mRNA expression. Quantification of V2 receptor and AQP2 mRNA expression was performed using RT-competitive PCR. Figure 1 shows the standard curve for the quantification of V2 receptor mRNA expression.
tor mRNA. The figure shows the linear relationship between the V2 receptor cDNA and V2 receptor cDNA/competitor ratio.

Effects of hyperosmolality on mRNA and protein expression of V2 receptor. First, we examined the effects of hyperosmolality made by NaCl on V2 receptor mRNA expression. Incubation of IMCD in hypertonic solution (490 mosmol/kgH2O) stimulated V2 receptor mRNA expression at 2 to 3 h (Fig. 2). After 4.5-h incubation, the expression returned to almost the control level. Therefore, we performed 3-h incubation studies. We change the osmolality from 290 to 1,090 mosmol/kgH2O and found that V2 receptor mRNA expression was osmolality dependently stimulated (Fig. 3A). Maximal stimulation by NaCl was observed at 1,090 mosmol/kgH2O. Hypertonicity by urea also stimulated V2 receptor mRNA expression to a lesser extent (Fig. 3B).

Next, we examined the effects of hyperosmolality on V2 protein expression. The antibody against V2 receptor recognized a single band at 41 kDa, which is compatible with its predicted size of 40.5 kDa (11). This band was absorbed by addition of the corresponding peptide (data not shown). NaCl (690 mosmol/kgH2O) stimulated V2 receptor protein expression by 150% (Fig. 4). In contrast, hypertonicity by urea did not stimulate V2 receptor protein expression.

Effects of AVP on mRNA and protein expression of V2 receptor. The effect of AVP was examined by the incubation of IMCD in isotonic medium. AVP dose dependently stimulated V2 receptor mRNA expression (Fig. 5A). Since the second messenger of AVP in diuretic action is cAMP, we examined the effects of CPT-cAMP on V2 receptor mRNA expression. CPT-cAMP of more than 10^{-5} M stimulated V2 receptor mRNA expression in IMCD (Fig. 5B).

Next, the effects of AVP on V2 protein expression were examined. Although lower doses of AVP (10^{-10} M) had no effect on V2 receptor protein expression, high-dose AVP (10^{-7} M) significantly stimulated V2 receptor expression (Fig. 4). From the results obtained above, we conclude that hyperosmolality and vasopressin stimulate mRNA and protein expression of the V2 receptor. These results suggest that hyperosmolality and AVP are not the cause of the downregulation of the V2 receptor and that some inhibitory factors may be present in dehydration. Since renal synthesis of PGE2 is very high in renal medulla and is stimulated by dehydration, we focussed on PGE2 in the following study.

Effects of PGE2 on V2 receptor mRNA expression. Incubation of IMCD with PGE2 (2.8 \times 10^{-3} M) did not inhibit V2 receptor mRNA expression but slightly increased the expression (Fig. 6A). In the presence of 10^{-7} M AVP, however, the addition of PGE2 significantly decreased V2 receptor mRNA expression. In contrast, PGE2 did not cause any effects on AQP2 mRNA expression in either the absence or presence of AVP (Fig. 6B).

Effects of indomethacin on urine volume, urine osmolality, urinary PGE2 excretion, and mRNA expression of V2 receptor and AQP2. We investigated the effects of indomethacin on urine volume, urine osmolality, and urinary PGE2 excretion in control and dehydrated rats. In control rats, indomethacin decreased urine volume and urinary excretion of PGE2 (urine volume: 17.3 ± 2.6 and 7.2 ± 1.9 ml/day, urinary PGE2 24.9 ± 9.1 and 7.5 ± 1.1 µg/day in control and control with

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Fig. 7. Effects of indomethacin on urine volume, urine osmolality, and urinary PGE2 excretion in control (left) and dehydrated rats (right). Indomethacin was intraperitoneally injected into control and dehydrated rats for 2 days, and urine volume, urine osmolality, and urinary excretion of PGE2 were examined. Top: urine osmolality in control and dehydrated rats with or without indomethacin. Middle: urine volume in control and dehydrated rats with or without indomethacin. Bottom: urinary PGE2 excretion in control and dehydrated rats with or without indomethacin. Indomethacin decreased urine volume and urinary PGE2 excretion in control rats. In contrast, indomethacin increased urine volume while decreasing urinary PGE2 excretion, n = 5–7. *P < 0.05 vs. control or dehydrated rats without indomethacin.

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indomethacin rats, respectively, n = 6–8, *P < 0.05 vs. control; Fig. 7). In contrast, indomethacin in dehydrated rats significantly increased urine volume while decreased urinary excretion of PGE_2 (urine volume: 0.7 ± 0.3 and 1.5 ± 0.3 ml/day, urinary PGE_2: 94.0 ± 39.4 and 27.7 ± 18.3 μg/day in dehydration and dehydration with indomethacin rats, respectively, n = 5–7, *P < 0.05 vs. dehydration; Fig. 7). Urine osmolality showed reciprocal changes with urine volume and PGE_2 (1,102 ± 118 and 1,978 ± 137 ml/day in control and control with indomethacin rats, respectively, n = 6; 3,042 ± 317 and 1,559 ± 405 ml/day in dehydration and dehydration with indomethacin, respectively, n = 5, *P < 0.05 vs. dehydration; Fig. 7).

Then, time course of the effects of indomethacin on V2 receptor and AQP2 mRNA expression was examined. Indomethacin caused transient increase at 3 h and then sustained decrease at 24–48 h in V2 receptor mRNA expression in dehydrated rats (Fig. 8A). Same changes were observed in AQP2 mRNA expression (Fig. 8B). In control rats, indomethacin increased both V2 receptor and AQP2 mRNA expression at 48 h (Fig. 9, A and B).

**Effects of indomethacin on V2 receptor and AQP2 protein expression.** At first, time course of the effects of indomethacin on V2 receptor and AQP2 expression in dehydrated rats was examined. Indomethacin in dehydrated rats caused a transient decrease at 3 h and sustained increase at 24–48 h in V2 receptor expression (Fig. 8A). AQP2 expression was decreased by indomethacin in dehydrated rats from 3 to 48 h (Fig. 8B). In contrast, indomethacin reduced V2 receptor protein expression in control rats at 24–48 h (Fig. 10A). The changes of V2 receptor expression by indomethacin are opposite in control and dehydrated rats. In contrast, indomethacin decreased AQP2 protein expression in both the control and dehydrated rats at 48 h (Fig. 10B).

**Immunohistochemical study.** Finally, immunohistochemical study was performed using dehydrated rats. Indomethacin in dehydrated rats decreased AQP2 expression in the luminal membrane of IMCD (Fig. 11).

**DISCUSSION**

Dehydration is one of the most common physiological conditions, and the role of the kidney in dehydration is to store water and sodium chloride (18). It is interesting that the expression of the V2 receptor is downregulated in dehydration (27). This downregulation is the opposite of what we would expect. Our in vitro experiments showed that hyperosmolality and AVP stimulated V2 receptor mRNA and protein expression, suggesting the presence of factors inhibiting the V2 receptor in dehydration. We focused on PGE_2, since PGE_2 production is high in renal medulla and is increased in dehydration (3, 38). PGE_2 did not inhibit V2 receptor mRNA expression, except in the presence of AVP. Blockade of PGE_2 synthesis by indomethacin increased V2 receptor expression in renal medulla in dehydrated rats. These data suggest that prostaglandin plays a key role in urine concentration in dehydration.

Water preservation by the kidney, especially in dehydration, is controlled by the V2 receptor-AQP2 system (18, 19). Both the V2 receptor and AQP2 are localized in the principal cells in the collecting ducts. The V2 receptor is in the basolateral membrane, and AQP2 is in the luminal membrane of the
principal cells (22, 25). To affect short-term regulation of water balance in dehydration, AQP2 in the intracellular vesicles is moved to the apical membrane (23). Stimulation of mRNA and protein expression of AQP2 then occurs to affect long-term regulation (13, 35). The V2 receptor is downregulated in dehydration (27). Upregulation of AQP2 may cause downregulation of the V2 receptor as a negative feedback in dehydration. In our experiments, reduction of PGE₂ synthesis by indomethacin for 48 h in control rats caused decreased expression of AQP2 mRNA (27). Indomethacin increased V2 receptor mRNA expression in control rats after 48 h and decreased it in dehydrated rats, n = 8. B, top: typical example of the ethidium bromide-stained gel. Bottom: AQP2 mRNA expression. Dehydration increased AQP2 mRNA expression in IMCD. Indomethacin increased AQP2 mRNA expression in control rats and decreased it in dehydrated rats, n = 7. *P < 0.05 vs. control. #P < 0.05 vs. dehydration.

Fig. 9. Effects of indomethacin on V2 receptor (A) or AQP2 (B) mRNA expression in IMCD in control and dehydrated rats. V2 receptor (A) or AQP2 (B) mRNA expression was examined in rats with or without indomethacin in control and dehydrated rats. A, top: typical example of the ethidium bromide-stained gel. Bottom: V2 receptor mRNA. Dehydration decreased V2 receptor mRNA expression in IMCD. Indomethacin increased V2 receptor mRNA expression in control rats after 48 h and decreased it in dehydrated rats, n = 8. B, top: typical example of the ethidium bromide-stained gel. Bottom: AQP2 mRNA expression. Dehydration increased AQP2 mRNA expression in IMCD. Indomethacin increased AQP2 mRNA expression in control rats and decreased it in dehydrated rats, n = 7. *P < 0.05 vs. control. #P < 0.05 vs. dehydration.

Fig. 10. Effects of indomethacin on V2 receptor (A) or AQP2 (B) protein expression in IMCD in control and dehydrated rats. V2 receptor (A) or AQP2 (B) protein expression was examined in rats with or without indomethacin in control and dehydrated rats. A, top: typical example of Western blot. Bottom: V2 receptor expression. Dehydration decreased V2 receptor protein expression in IMCD. Inhibition of PGE₂ synthesis by indomethacin decreased V2 receptor protein expression in control rats. In contrast, indomethacin increased V2 receptor protein expression in IMCD in dehydrated rats, n = 6. *P < 0.05 vs. control or dehydration. B, top: typical example of Western blot. AQP2 protein was detected at 29 and 35 kDa. Bottom: AQP2 expression. Dehydration increased AQP2 protein expression in IMCD. Indomethacin decreased AQP2 protein expression in both control and dehydrated rats, n = 6. *P < 0.05, **P < 0.01, ***P < 0.001.
both AQP2 and V2 receptor proteins (Figs. 8 and 10). In contrast, the same PGE2 blockade for 48 h in dehydrated rats reduced AQP2 expression while stimulating V2 receptor expression. Thus the expression of AQP2 and the V2 receptor did not show reciprocal changes, suggesting that upregulation of AQP2 may not play a key role in downregulation of the V2 receptor in dehydration. Stimulation of AQP2 may suppress the promoter region of the V2 receptor. However, the V2 receptor regulates not only water balance but also sodium and acid-base balance through many ion transporters, channels, and exchangers. Therefore, it is unlikely that upregulation of AQP2 suppresses the V2 promoter region. Blockade of PGE2 by indomethacin decreased urine volume in control rats as previously reported (2, 8). However, in contrast, PGE2 blockade increased urine volume in dehydrated rats (Fig. 7). These data show that PGE2 may suppress V2 vasopressin action in control condition and strengthen vasopressin in dehydration. Effects of PGE2 on vasopressin action are different between control and dehydration. Our data support the clinical warning of the use of NSAIDs in patients with dehydration.

IMCD is the last segment of the nephron, and the osmolality in the interstitium may increase up to 3,000 mosmol/kgH2O, especially in dehydrated rats. Since dehydration causes increased osmolality in renal medulla and plasma levels of vasopressin, we examined the direct effects of hyperosmolality and AVP on V2 receptor expression. We found that both hyperosmolality and AVP stimulated V2 receptor mRNA and protein expression, which makes sense with regard to the lessening of water and sodium loss. These data suggest that neither AVP nor hyperosmolality is a causative factor in the downregulation of the V2 receptor in dehydration. Since the expression of the V2 receptor is regulated by many factors, the presence of some inhibitory factors is suggested.

It is well known that synthesis of many cytokines and autacoids is increased in dehydration. We focused on PGE2, since the production of PGE2 is higher in deeper medulla and is increased in dehydration and by AVP treatment (3, 31, 38). Increased plasma levels of vasopressin in dehydration stimulate PGE2 synthesis in renal medulla (17, 37). PGE2 slightly stimulated sodium absorption in the absence of vasopressin (13). In contrast, PGE2 inhibits AVP-dependent cAMP synthesis in IMCD and AVP-stimulated water and sodium transport in CCD and IMCD (7, 10, 15, 29, 30, 36).

At least four types of PGE2 receptor (EP) are known (6). EP1 acts as Gq/11 by activating phosphoinositide breakdown, and EP3 acts as Gi by decreasing in cAMP formation (12, 30). EP1 mediates the inhibitory effects of PGE2 in the absence of vasopressin via the increase in intracellular calcium (12). On the other hand, EP3 has been thought to mediate the inhibition of AVP action in IMCD. Thus, from the viewpoint of physiology, increased PGE2 synthesis in dehydration works through EP3 as a negative feedback against the antidiuretic action of AVP. We examined this theory with regard to gene regulation. Incubation of IMCD with PGE2 did not inhibit V2 receptor mRNA expression in our study. Slight stimulation of V2 receptor mRNA expression may have some role in slight stimulation of sodium reabsorption by PGE2 in collecting ducts (15). However, incubation of IMCD with PGE2 in the presence of 10−9 M AVP inhibits V2 receptor mRNA expression compared with AVP alone. Since AVP is always present in the blood, the latter experimental condition is close to the physiological condition. Blockade of PGE2 synthesis for 24–48 h by indomethacin in dehydrated rats increased V2 receptor expression by stimulating V2 receptor mRNA expression at an early stage (3 h). Our data clearly confirm that increased PGE2 synthesis in renal medulla plays a main role in downregulation of V2 receptor in dehydration from the aspect of gene regulation. It is interesting that the effects of PGE2 on AVP action are different between control and dehydration.

In summary, the downregulation of the V2 receptor in dehydration is not caused by hyperosmolality or by increased plasma levels of AVP. Rather, increased PGE2 production in renal medulla in dehydration plays a key role in the downregulation of V2 receptor expression. PGE2 works as a negative feedback system against the antidiuretic action of AVP not only acutely but also chronically through gene regulation.

ACKNOWLEDGMENTS

We thank Drs. S. Nielsen (University of Aarhus, Aarhus, Denmark) and M. A. Knepper (National Institutes of Health, Bethesda, MD) for providing the antibody against AQP2.

GRANTS

This work was supported by Grants-in-Aid for Scientific Research from the Ministry of Education, Culture, Sports, Science and Technology in Japan (17590833, 167904660, 16590792, 16590791, 16390246, 15590852, 14370321, and 13671121) and the Mochida Memorial Foundation for Medical and Pharmaceutical Research.
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