Different Localization and Regulation of Two Types of Vasopressin Receptor Messenger RNA in Microdissected Rat Nephron Segments Using Reverse Transcription Polymerase Chain Reaction

Yoshio Terada, Kimio Tomita, Hiroshi Nonoguchi, Tianxin Yang, and Fumiaki Marumo
Second Department of Internal Medicine, Tokyo Medical and Dental University, Tokyo, 113, Japan

Abstract

Recent studies have revealed that arginine vasopressin (AVP) has at least two types of receptors in the kidney: V1a receptor and V2 receptor. In this study, microlocalization of mRNA coding for V1a and V2 receptors was carried out in the rat kidney using a reverse transcription and polymerase chain reaction. Large signals for V1a receptor PCR product were detected in the glomerulus, initial cortical collecting duct, cortical collecting duct, outer medullary collecting duct, inner medullary collecting duct, and arcuate artery. Small but detectable signals were found in proximal convoluted and straight tubules, inner medullary thin limbs, and medullary thick ascending limbs. Large signals for V2 receptor mRNA were detected in the cortical collecting duct, outer medullary collecting duct, and inner medullary collecting duct. Small signals for V2 receptor were found in the inner medullary thick limbs, medullary thick ascending limbs, and initial cortical collecting duct. Next, we investigated V1a and V2 receptor mRNA regulation in the dehydrated state. During a 72-h water restriction state, the plasma AVP level increased and V2 receptor mRNA decreased in collecting ducts. In contrast, V1a receptor mRNA did not change significantly. Thus, the two AVP receptor subtypes are distributed differently along the nephron, and these mRNAs are regulated differently in the dehydrated state. (*J. Clin. Invest.* 92:2339–2345.) Key words: reverse transcription • polymerase chain reaction • messenger RNA • glomerulus • inner medullary collecting duct

Introduction

The antidiuretic hormone arginine vasopressin (AVP)[1] is known to influence the glomerular function and to increase the water permeability of the collecting duct and it has also been shown to act on other nephron segments, in the mammalian kidney (1, 2). AVP receptors are G protein–coupled and have been divided into at least three types: V1a (vascular/hepatic) and V1b (anterior pituitary) receptors, which act through phosphatidylinositol hydrolysis to mobilize intracellular Ca^{2+}; and V2 (kidney) receptor, which is coupled to adenylate cyclase (3). Recently, V1a and V2 receptor cDNAs were cloned (4–6). These cDNAs encode proteins with seven putative transmembrane domains and a similar structure to rhodopsin and other G protein–coupled receptors (4–6).

The distribution of V1a and V2 receptors in the kidney has been investigated by binding studies (7–11) and many physiological studies (12–17). The presence of V2 receptor in the collecting duct is well established (9, 11, 18). However, in MTAL and glomerulus, the presence of V2 receptor is controversial. It is also not well clarified whether V2 receptor is expressed or not in IMTL and the renal vascular system.

Recently, some reports revealed that V1a receptor is expressed in the glomerular mesangial cell (19), CCD (11, 16), OMCD (11), and IMCD (11). Ando et al. reported that AVP action from the apical side may be mediated via V1a receptor in rabbit CCD (20). On the other hand, binding studies could not detect V1a binding sites in glomerulus (7–10). Thus, the presence of V1a receptor in the kidney is still controversial. To understand clearly the physiological function of AVP in the kidney, it seems very important to know the precise localization of both types of receptor expressions along the nephron segments and in the renal vascular system.

AVP plays critical roles in maintaining body homeostasis in water restricted conditions. In the dehydrated state, the plasma AVP level increases and water and solute are reabsorbed in the collecting ducts (21, 22). Downregulation or desensitization of AVP receptor have been reported when the plasma AVP level increases (23, 24). The mechanism of this phenomenon is not well known. It has not been well investigated which type of receptor is regulated by the plasma AVP level. Therefore, we investigated the regulations of mRNA expressions of V1a and V2 receptors in water restricted rats.

Recently, Moriyama et al. (25) and Terada et al. (26) introduced a new method for measurement of relative levels of specific mRNA in single microdissected renal tubules, using the PCR coupled to reverse transcription (RT-PCR). Using this technique, relative quantitation of mRNA coding for peptide hormone receptor was performed from only 2-mm lengths of renal tubules (26).

In the present study, we used the RT-PCR technique for the precise localization and regulation of V1a and V2 receptor mRNAs in microdissected renal tubules, glomeruli, and arcuate arteries.

Methods

Renal tubule microdissection. After previously described techniques (26), male Sprague-Dawley rats weighing 75–100 g were killed by de-

Address correspondence and reprint requests to Yoshio Terada, M.D., Second Department of Internal Medicine, Tokyo Medical and Dental University, 5-45, Yushima 1-chome, Bunkyo-ku, Tokyo 113, Japan.

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1. *Abbreviations used in this paper:* AVP, arginine vasopressin; iCCD, initial cortical collecting duct; IMCD, inner medullary collecting duct; IMTL, inner medullary thin limb; MTAL, medullary thick ascending limb; OMCD, outer medullary collecting duct; PCT, proximal converted tubule; PST, proximal straight tubule; RT-PCR, reverse transcription PCR.

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capitation. 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 1) and then with 10 ml of the same solution containing 1 mg/ml collagenase (collagenase solution) (type I, 300 U/mg; Sigma Chemical Co., St. Louis, MO) and 1 mg/ml bovine serum albumin (Sigma Chemical Co.). The dissection solution (solution 1) contained the following (in mM): 135 NaCl, 1 Na_2SO_4, 1.2 $MgSO_4$, 5 KCl, 2 $CaCl_2$, 5.5 glucose, and 5 *N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid (Hepes) (pH 7.4). The left kidney was removed and a coronal section was made that contained the entire corticopapillary axis. This section was cut into three pieces: cortex, outer medulla, and inner medulla. These pieces were transferred into individual tubes containing 1 ml of the same collagenase solution that was used to perfuse the kidney. The tubes were incubated for 30 min (cortex and outer medulla) or 40 min (inner medulla) at 37°C in a shaking water bath. The solutions were bubbled with 100% oxygen during these incubations. Then tissues were transferred to the dissection solution containing 10 mM vanadyl ribonucleotide complex (Life Technologies, Inc., Gaithersburg, MD), a potent RNase inhibitor, and they were placed on ice until microdissection.

After previously described techniques (26), we microdissected the following structures: glomeruli (Glm), proximal convoluted tubule (PCT), proximal straight tubule (PST), inner medullary thin limb (IMTL), outer medullary thick ascending limb (MTAL), initial cortical connecting ducts (iCCD), cortical collecting ducts (CCD), outer medullary collecting duct (OMCD), inner medullary collecting duct (IMCD), and arcuate artery. Generally, five glomeruli or 2-mm lengths of the renal tubule segments or arcuate arteries were transferred to each assay tube, as indicated. The reason why we took five Glm as one sample is based on the protein contents. The protein contents of five Glm is 185.3±14.7 ng (n = 5, mean±SEM) is approximately equal to the protein contents of 2-mm of IMCD (27). The protein level of Glm was determined using protein assay reagent (Bio-Rad Laboratories, Richmond, CA).

Microdissected tubules, glomeruli, arcuate arteries were washed free of contaminating debris and vanadyl ribonucleotide complex in separate wash dishes. These structures were transferred into appropriate RT-PCR reaction tubes, which contained 10 μl of ice-cold dissection solution containing > 1 U/μl of human placental RNase inhibitor (Boehringer-Mannheim GmbH., Mannheim, Germany) and 5 mM dithiothreitol (Sigma Chemical Co.).

Reverse transcription. RT was performed using a cDNA synthesis kit (Boehringer-Mannheim GmbH.). The RNase-inhibitor solution was removed, and 9 μl of 2% Triton X-100, containing > 1 U/μl of RNase-inhibitor, 5 mM DTT, and 3 U RNase-free DNase (Pharmacia Fine Chemicals, Piscataway, NJ), was added to permeabilize the cells, followed by incubation at 37°C for 30 min to digest the genomic DNA. The samples were heated to 90°C for 5 min to inactivate the DNase. RT components were added to the reaction tubes as described previously (26): 4 μl of buffer I, 1 μl of RNase inhibitor, 2 μl of deoxynucleotide mixture, 2 μl of random primer, and 2 μl of avian myeloblastoma virus reverse transcriptase. Reaction tubes were incubated at 42°C for 60 min in the Programmed Tempcontrol System (Astec, Tokyo, Japan). At the end of the incubation period, the reaction was stopped by heating at 90°C for 5 min. This heat treatment also denatures RNA-cDNA hybrids and inactivates the reverse transcriptase. Then the reaction tubes were placed on ice until the addition of PCR reagents.

Polymerase chain reaction. PCR was performed using the GeneAmp DNA Amplification Reagent Kit (Perkin-Elmer Cetus, Norwalk, CT), with rat V1a and V2 receptor specific primers prepared on a DNA synthesizer (Applied Biosystems Inc., Tokyo, Japan). We designed specific primers 20–25 nucleotides in length with 50–60% GC composition. The resultant high calculated melting temperature (> 75°C) allows a stringent annealing temperature in the PCR cycle. V1a receptor primer 1 (antisense) was defined by bases 579–598, and primer 2 (sense) encompassed bases 1–21 (4). The sequence of V1a primer 1 was 5'-TAGTGCCATTGTTCACCTCG-3', primer 2 was 5'-ATGAGTTTCCCGCGAGGCTCC-3'. The predominant cDNA amplification product was predicted to be 598 bp in length (the distance between primers plus primer length). A third oligonucleotide was synthesized to serve as an amplification product-specific probe. This oligonucleotide (sense) included bases 444–463 of the cDNA, positioned between primer 1 and primer 2. The sequence of this oligonucleotide probe was 5'-TACATCGCCGTGTGCCACCC-3'.

V2 receptor primer 1 (antisense) was defined by bases 581–600, and primer 2 (sense) encompassed bases −24 to −3 (6). The sequence of V2 receptor primer 1 was 5'-CCATGGTTCTGCAAATCGGG-3', primer 2 was 5'-TAGGTCATCATCAACCACCCCA-3'. The predominant cDNA amplification product was predicted to be 625 bp in length. A third oligonucleotide was synthesized to serve as an amplification product-specific probe. This oligonucleotide (sense) included bases 307–326 of the cDNA, positioned between primer 1 and primer 2. The sequence of this oligonucleotide probe was 5'-ACCGCTTCCATGGCCCTGAT-3'.

RT and PCR of GAPDH served as a positive control. The primers were defined by the following cDNA base sequences (28): primer 1 (antisense), bases 794–813, sequence, 5'-AGATCCACAACGGATACATT-3'; primer 2 (sense), bases 506–525, sequence, 5'-TCCCTCAAGATTGTCAGCAA-3'. The cDNA amplification product was predicted to be 309 bp in length. A third oligonucleotide was synthesized to serve as an amplification product-specific probe. This oligonucleotide (sense) included bases 307–326 of the cDNA, positioned between primer 1 and primer 2. The sequence of this oligonucleotide probe was 5'-ACCGCTTCCATGGCCCTGAT-3'. When we used GAPDH as an internal control primer, after reverse transcription, we divided 20 μl samples into 15 μl for V1a or V2 receptor and 5 μl for GAPDH. The volume was adjusted to 20 μl with sterile water. Then we ran parallel PCR reactions with each set of primers.

To carry out the PCR, 80 μl of a PCR master mix was added to each tube directly. 50 pmol of each of primers 1 and 2 was used per reaction for both V1a and V2 receptor. Deoxynucleotides were added to a final concentration of 0.20 mM each. Reaction buffer (10×) was diluted ($^1/_{10}$) to have a final composition of: 10 mM Tris-HCl, pH 8.3, 50 mM KCl, 1.5 mM $MgCl_2$, and 0.001% (wt/vol) gelatin, 2.5 U of Taq DNA polymerase.

100 μl of mineral oil was overlayed to prevent evaporation during the high temperature incubations. The tubes were placed in the Programmed Tempcontrol System programmed as follows. First, incubation at 94°C for 3 min (initial melt). Then, 30 cycles of the following sequential steps: 94°C for 1 min (melt), 60°C for 1 min (anneal), and 72°C for 3 min (extend). And last, incubation at 72°C for 7 min (final extension). Then samples were kept at 4°C until the time of analysis.

PCR product analysis. 90 μl of the total reaction volume was ethanol precipitated (29). The PCR products were size-fractionated by 2% agarose gel electrophoresis. After electrophoresis and ethidium bromide staining, DNA bands were visualized with an ultraviolet transilluminator (Funakoshi, Tokyo, Japan).

For Southern blot analysis, gels were blotted onto a nitrocellulose filter (Funakoshi) essentially as described by Maniatis et al. (29). The synthetic oligonucleotide probes were end-labeled with ^{32}P as described previously (26). Prehybridization/hybridization washes were also the same as previously described (26).

To confirm that the PCR products were really V1a and V2 receptor cDNAs, the PCR products were sequenced. PCR products from glomeruli were separated by gel electrophoresis. PCR products of V1a and V2 receptors were subcloned into pGEM-3Zf(−) vector (Promega Biotec, Madison, WI) as described by Finney (30). We cut pGEM-3Zf(−) vector at SamI site, and thymine was attached at 3' end using Taq DNA polymerase (Perkin-Elmer Cetus), because Taq DNA polymerase has terminal transferase activity. The PCR product has adenine in its 3' end. We ligated PCR products and pGEM-3Zf(−), then sequenced using the dideoxynucleotide chain termination reaction of Sanger et al. (31).

Relative-quantitation of mRNA levels from autoradiographs. The relative amount of PCR products was determined by densitometer scanning of autoradiographs using a laser densitometer (Hoefer Scientific Instruments, Inc., San Francisco, CA). For relative quantitation, we used the densitometry value from the 2 mm of same nephron segments, and adjusted the densitometry values with the GAPDH band intensity.

To test the relationship between the quantity of starting material and that of amplification product as reflected by densitometry values, we compared amplification products from several lengths of renal tubules. We compared amplification products from several lengths of OMCD (0.2–2.0 mm, $n = 12$) for V1a receptor and IMCD (0.2–2.0 mm, $n = 12$) for V2 receptor. Linear regression analysis showed high correlation between densitometry values and the length of each tubule for V1a receptor ($r = 0.95$) and V2 receptor ($r = 0.95$) (data not shown).

Water restriction experiments. The water restricted experiments were performed using male Sprague-Dawley rats weighing 75–100 g ($n = 6$ per group). The normal rat group was given a standard diet and allowed free access to tap water. The dehydration experiment group was deprived of water for 6, 12, 24, 48, and 72 h before death. The rats were anesthetized intraperitoneally with pentobarbital sodium (50 mg/kg) and the blood samples were taken from the heart. Plasma AVP was measured by radioimmunoassay with specific AVP antibody (AVP-RIA kits, Mitsubishiyuka, Tokyo, Japan) as previously described (32). Briefly, the purification process is as follows: SepPak C18 cartridges (Waters Associates, Milford, MA) were prewetted with 10 ml methanol. A 1-ml plasma sample is acidified with 0.1 N HCl and applied to the column dropwise. The cartridge is rinsed with 10 ml 4% acetic acid, and then AVP is eluted with 1.5 ml ethanol. The samples were evaporated and then reconstituted with 1 ml of assay buffer (50 mM phosphate buffer [pH 7.4] containing 0.2% bovine serum albumin and 10 mM EDTA). The radioimmunoassay was carried out with specific AVP antibody as indicated in the kits. Plasma osmolality was measured with a vapor pressure osmometer (Wescor Inc., Logan, UT). The band intensity of V1a and V2 receptor was adjusted by the GAPDH band intensity of the same lane.

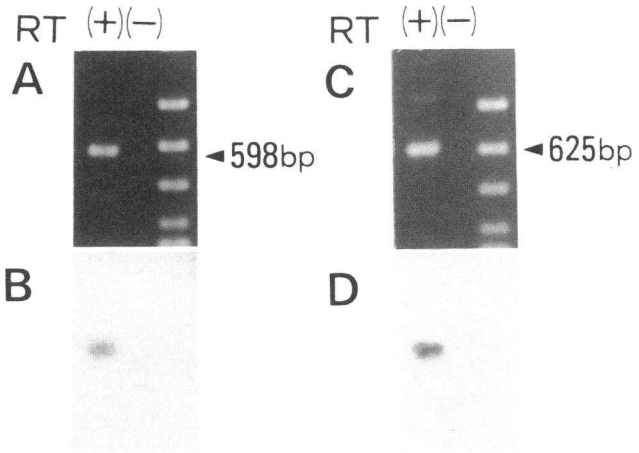

Figure 1. Effect of RT on V1a and V2 receptor mRNAs amplification. The PCR amplification (30 cycles) was performed from 2 mm of OMCD for V1a receptor or IMCD for V2 receptor with and without reverse transcription. (*A*) Ethidium bromide–stained agarose gels for V1a receptor. (*B*) Autoradiograms of corresponding Southern blots. The blots were probed with a ^{32}P-labeled oligonucleotide that localized between the V1a receptor primers. (*C*) Ethidium bromide–stained agarose gels for V2 receptor. (*D*) Autoradiograms of corresponding Southern blots. The blots were probed with a ^{32}P-labeled oligonucleotide that localized between the V2 receptor primers.

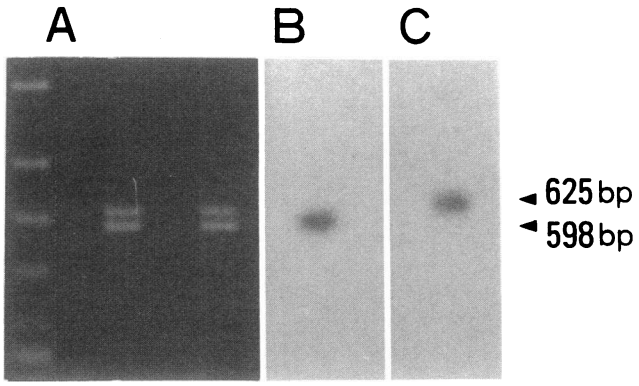

Figure 2. Specificity of V1a and V2 probes to PCR products. (*A*) PCR products amplified from OMCD in the presence of both V1a and V2 receptor primers (30 cycles of PCR amplification). (*B*) Southern hybridization with ^{32}P-labeled V1a probe to 598-bp band. (*C*) Southern hybridization with ^{32}P-labeled V2 probe to 625-bp band.

Statistics. The results were given as mean±SEM. The differences were tested using analysis of variance. $P < 0.05$ was considered significant.

Results

Effect of reverse transcription on V1a and V2 receptor mRNAs amplification (Fig. 1). With reverse transcription, we detected a clear single band, which was the predicted size of 598 bp for V1a receptor from OMCD (Fig. 1, *A* and *B*), and we also detected a clear single band, which was the predicted size of 625 bp for V2 receptor from IMCD (Fig. 1, *C* and *D*). When the PCR procedure was carried out in the absence of reverse transcriptase, the 598-bp band and the 625-bp band were not seen, and there was no other recognizable band. This indicated that the 598-bp and the 625-bp bands originated from mRNA, not from genomic DNA, which was presumably digested by DNase treatment. The Southern blots of the gels demonstrated that V1a specific probe binds to the 598-bp product, and V2 specific probe binds to the 625-bp product, confirming their identity.

Specificity of V1a and V2 probes to PCR products (Fig. 2). We performed PCR amplification in the presence of both V1a and V2 receptor primers from OMCD. As shown in Fig. 2 *A*, the 598-bp band and 625-bp band are clearly distinguished in 2% agarose gel. V1a probe hybridized to the 598-bp band specifically and did not hybridize to 625-bp band (Fig. 2 *B*). V2 probe hybridized to 625-bp band specifically and did not hybridize to 598-bp band (Fig. 2 *C*).

Distribution of V1a and V2 receptors mRNAs in microdissected structures (Fig. 3). Each reaction was performed using either 2-mm of tubule length, five glomeruli, or 2-mm length of arcuate artery. A single band of predicted size (598 bp) was consistently found from Glm, iCCD, CCD, OMCD, IMCD, and arcuate artery (Fig. 3 *A*). A faint but detectable band was found from PCT, PST, IMTL, and MTAL (Fig. 3 *B*). The Southern blots of the gels demonstrated specific binding of the oligonucleotide probe to the 598-bp product (Fig. 3 *B*). We confirmed that the PCR product had an identical sequence to V1a receptor cDNA by sequencing the subcloned PCR product. The largest signal for V1a receptor was detected in Glm.

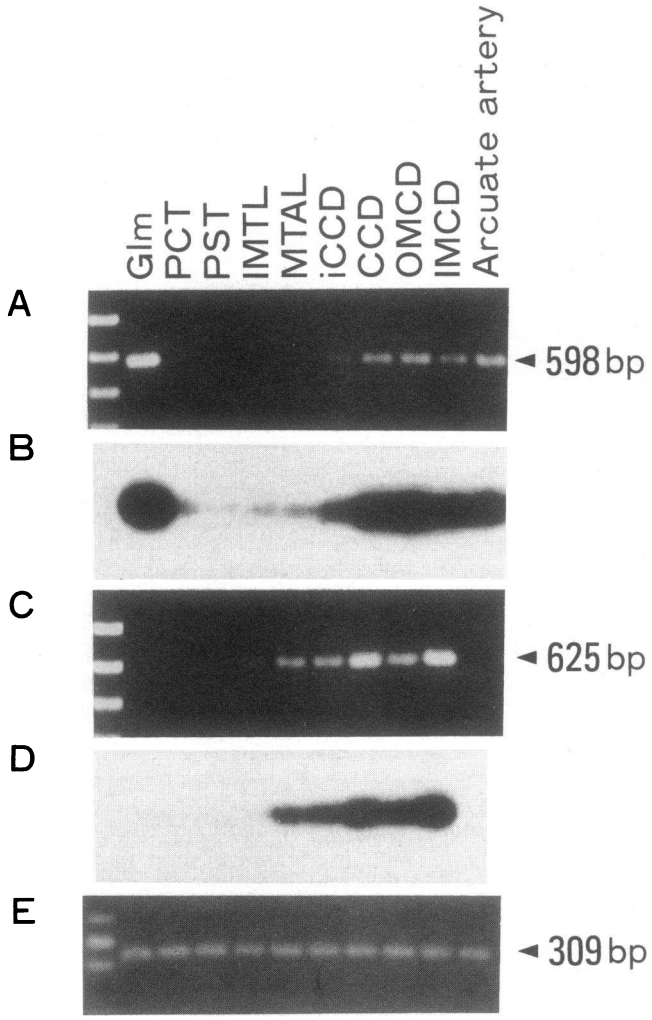

Figure 3. Detection of V1a and V2 receptors mRNAs in microdissected renal structures by RT-PCR. Each PCR amplification (30 cycles) was performed using either 2 mm of renal tubule, five glomeruli, or 2 mm length of arcuate artery. (*A*) Ethidium bromide–stained agarose gels for V1a receptor. The arrow indicates expected PCR product size (598 bp). (*B*) Autoradiograms of corresponding Southern blots for V1a receptor. The blots were probed with a ^{32}P-labeled oligonucleotide that localized between the PCR primers. (*C*) Ethidium bromide–stained agarose gels for V2 receptor. The arrow indicates expected PCR product size (625 bp). (*D*) Autoradiograms of corresponding Southern blots for V2 receptor. The blots were probed with a ^{32}P-labeled oligonucleotide that localized between the PCR primers. (*E*) Ethidium bromide–stained agarose gel of GAPDH as positive control primers. The arrow indicates expected PCR products size (309 bp).

Large signals were detected in OMCD, CCD, IMCD, iCCD, and arcuate artery.

A single band for V2 receptor mRNA was consistently found from IMCD, OMCD, CCD, iCCD, MTAL, and IMTL (Fig. 3 *C*). This band was the predicted size (625 bp). The Southern blots of the gels demonstrated specific binding of the oligonucleotide probe to the 625-bp product (Fig. 3 *D*). We confirmed that PCR product had an identical sequence to V2 receptor cDNA by sequencing the subcloned PCR product. Among renal tubule segments, the largest signal was consistently found in IMCD. Furthermore, large signals were consistently seen in CCD, OMCD, iCCD, and MTAL. A small but detectable band was observed from IMTL. We could not detect a band from Glm, PCT, PST, or arcuate artery. The amplification product of GAPDH was detected from all renal structures at the predicted size (309 bp) and served as a positive control for the RT-PCR reaction (Fig. 3 *E*). We obtained similar results for V1a and V2 receptors from five experiments of five rats.

V1a and V2 receptor mRNA regulation by dehydration in rat glomeruli and collecting ducts (Fig. 4). The state of dehydration of the rats was evaluated by comparing plasma osmolality and plasma AVP concentration among the two groups. Plasma osmolality was significantly elevated in the 72-h water restricted animals (306±2.5 mosmol/kg) compared with control values (290±1.1 mosmol/kg) ($P < 0.05$). Plasma AVP levels also were significantly increased, at 23.4-fold, in animals in the 72-h water restricted group (77.5±17.4 pg/ml) compared with control values (3.3±0.9 pg/ml). V1a receptor expressions did not significantly change between the two groups in glomeruli and collecting ducts (Fig. 4, *A* and *B*). On the other hand, V2 receptor mRNA expressions in collecting ducts decreased in water restricted rats (Fig. 4, *D* and *E*). In CCD of water restricted rats, PCR products for V2 receptor mRNA decreased to 58.5±8.2% of the PCR products of normal rat group. In OMCD and IMCD the signals for V2 receptor mRNA reduced to 67.1±9.4% and 42.3±11.0% of the normal rat group in water restricted rats, respectively. The values were corrected by the band intensity of GAPDH band intensity of the same sample.

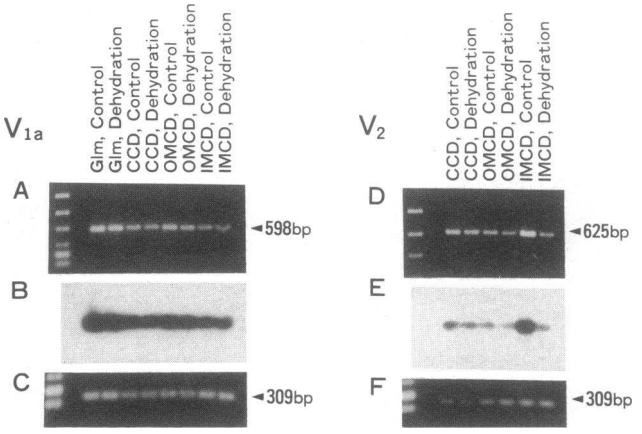

Figure 4. Effects of dehydration on V1a and V2 receptor mRNA expression in nephron segments. Each PCR amplification (30 cycles) was performed using either 2 mm of renal tubule. (*A*) Ethidium bromide–stained agarose gels for V1a receptor from control and dehydrated rats nephron segments. The arrow indicates expected PCR product size (598 bp). (*B*) Autoradiograms of corresponding Southern blots for V1a receptor. (*C*) Ethidium bromide–stained agarose gel of GAPDH as positive control primers. The arrow indicates expected PCR products size (309 bp). (*D*) Ethidium bromide–stained agarose gels for V2 receptor from control and dehydrated rats nephron segments. The arrow indicates expected PCR product size (625 bp). (*E*) Autoradiograms of corresponding Southern blots for V2 receptor. (*F*) Ethidium bromide–stained agarose gel of GAPDH as positive control primers. The arrow indicates expected PCR products size (309–bp).

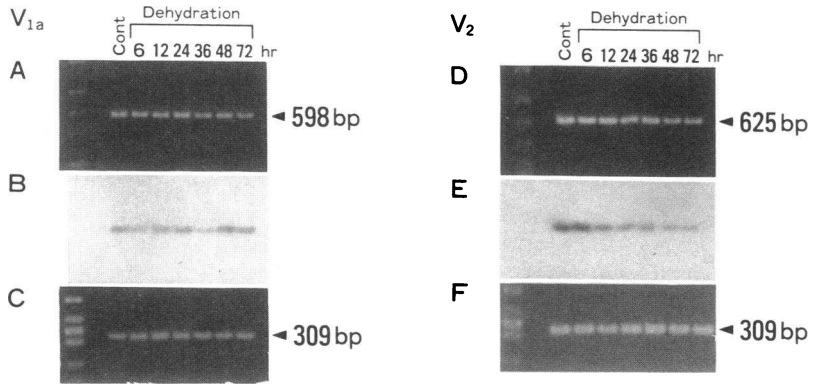

Figure 5. Time course on V1a and V2 receptor mRNA expression in IMCD during dehydration. Each PCR amplification (30 cycles) was performed using either 2 mm of renal tubule. (*A*) Ethidium bromide–stained agarose gels for V1a receptor from IMCD of control and dehydrated rats. The arrow indicates expected PCR product size (598 bp). (*B*) Autoradiograms of corresponding Southern blots for V1a receptor. (*C*) Ethidium bromide–stained agarose gel of GAPDH as positive control primers. The arrow indicates expected PCR products size (309 bp). (*D*) Ethidium bromide–stained agarose gels for V2 receptor from IMCD of control and dehydrated rats. The arrow indicates expected PCR product size (625 bp). (*E*) Autoradiograms of corresponding Southern blots for V2 receptor. (*F*) Ethidium bromide–stained agarose gel of GAPDH as positive control primers. The arrow indicates expected PCR products size (309 bp).

Time course of V2 and V1a receptor expression in IMCD during dehydration (Fig. 5). Plasma osmolality was significantly elevated from 24-h water restriction (299±2.5 mosmol/kg) compared with control values (290±1.1 mosmol/kg) (n = 5, mean±SEM, $P < 0.05$). Plasma AVP levels were also increased from control values (3.3±0.9 pg/ml) as follows: (10.7±2.5) at 6 h, (37.5±5.2) at 12 h, (44.0±3.8) at 24 h, (52.3±8.5) at 36 h, (58.5±10.3) at 48 h, and (88.5±19.2) at 72 h in water restricted rats. V1a receptor expressions did not significantly change during experiments in IMCD (Fig. 5, *A* and *B*). On the other hand, V2 receptor mRNA expressions in IMCD decreased from 12-h water restriction and gradually decreased to 72 h in water restricted rats (Fig. 5, *D* and *E*). The PCR products for V2 receptor decreased to 75.8±8.8% in 12 h (n = 5, vs control group), 66.3±9.4% in 24 h, 60.7±9.2% in 36 h, 53.8±7.9% in 48 h, and 43.8±11.2% in 72 h in water restriction rat group. The values were corrected by the band intensity of GAPDH band intensity of the same sample.

Discussion

AVP is reported to reduce the glomerular ultrafiltration permeability coefficient (K_f) and decrease the glomerular filtration rate (33). It has been shown that AVP stimulates contraction of cultured glomerular mesangial cells via a rise in intracellular Ca^{2+} (34). This effect is considered to be through the V1a receptor (19, 34). It is not known, however, if the native glomerulus expresses the V1a receptor. In fact, no autoradiographic studies have demonstrated V1a specific glomerular binding on native renal tissue (7–10). The presence of the V2 receptor in glomerulus is not well established either. Imbert et al. (35) showed that 10^{-10} M AVP produces a small but significant stimulatory effect on the adenylate cyclase activity in isolated rabbit glomeruli, but others did not (36, 37). Our data show that V1a, and not V2 receptor mRNA is expressed in rat glomerulus. Therefore, the action of AVP to influence glomerular function may be mediated by the V1a receptor.

Only a few studies have been carried out to investigate AVP effect on proximal tubules. Binding studies (7–10) and microdissection studies (12, 14) had no positive results for AVP effect in proximal tubule. We could not detect V2 receptor mRNA from PCT or PST. The V1a receptor mRNA levels in PCT and PST are very small compared with glomerulus or collecting ducts, and the physiological significance of this expression is unclear.

Imbert et al. (14) reported that there is a small but statistically significant cAMP increase induced by AVP in thin limbs. We detected a very low level of V1a and V2 mRNAs from IMTL, although it was impossible to discern whether the expression was in ascending or descending limbs. Physiological function of these receptor in thin limbs is unknown.

There is general agreement about the V2 receptors play an important regulatory role in the MTAL, but the possible role of V1a receptors is uncertain. Autoradiographic studies using ^{3}H-AVP and V1a and V2 antagonist show binding sites are present in outer and inner medulla (7–10). AVP has been reported to stimulate adenylate cyclase activities of MTAL of rabbit, rat, and mouse kidneys, but not of human and dog kidneys (12, 14, 38). In vitro microperfusion studies showed that AVP stimulates NaCl transport in rabbit, rat, and mouse kidneys (39, 40), possibly through the V2 receptor. Recently, Ammar et al. (11) reported, using V1a and V2 specific ligands, that not only V2 but also V1a receptor is present in MTAL. Our results show that both V2 and V1a receptor mRNAs are indeed expressed in MTAL. Nitschke et al. reported that the V1a receptor mediated intracellular Ca^{2+} increment in cortical thick ascending limbs (17). Prostaglandin E_2, which may be synthesized via V1a receptor (41), inhibits AVP-dependent stimulation of NaCl transport in mouse MTAL (42). Thus, V1a receptor may play some role in the self-inhibition of AVP in the MTAL.

Collecting tubules are the major target for physiological action of AVP in the kidney (43). AVP at the physiological concentration 10^{-11} M can increase the hydro-osmotic water permeability and adenylate cyclase activity in the collecting tubules (18). Recently, AVP was reported to increase intracellular Ca^{2+} in CCD (16), IMCD (44), and cultured IMCD cells (45). Initial observation in CCD indicated that AVP evokes a transient rise in intracellular Ca^{2+}, probably through V1a receptor (16). However more recent studies using IMCD cells showed that both AVP and 1-desamino-8-D-arginine vasopressin, on an equimolar basis, evoke a rise in intracellular Ca^{2+}, suggesting the mediation of V2 receptor (45) or possibly heretofore unknown receptor type (46). Teitelbaum et al. reported that the increment of intracellular inositol 1, 4,

5-trisphosphate evoked by AVP or V2 receptor agonist occurs via occupancy of the oxytocin receptor (47). Recently, Ando et al. (20) reported that luminal AVP interacts with the basolateral action of AVP, and suggesting that this luminal action of AVP may be mediated via V1a receptor. Thus, determination of the existence of V1a receptor in the collecting duct is very important to understand overall AVP action in the collecting duct. Our data show not only the expression of the V2 receptor gene but also show V1a receptor mRNA in rat collecting ducts. In situ hybridization study revealed high levels of V2 receptor mRNA in collecting ducts, but no evidence of V1a receptor mRNA (48). The discrepancy about the expression of V1a receptor mRNA may be due to the different sensitivity between in situ hybridization and RT-PCR.

Our study revealed that the arcuate artery expresses mainly V1a receptor mRNA, as in other vascular tissue. Functional roles of the V1a receptor in the arcuate artery have not yet been investigated.

Our results show that in the dehydrated state, V2 receptor mRNA expression decreases to 50–70% of the basal level, while V1a receptor mRNA does not change significantly in collecting ducts. V2 receptor PCR products in IMCD decreased from 12 h water restriction and decreased further during 72 h water restriction. In contrast, V1a receptor PCR products in the IMCD did not change significantly during 72-h water restriction. Downregulation or desensitization of vasopressin receptors has been reported when the plasma AVP level is elevated (21–24). Steiner et al. (21) and Baddouri et al. (22) reported that water restriction causes the elevation of plasma AVP level and a decrease of AVP binding sites. The mechanism of receptor downregulation remains poorly understood. The mechanisms are likely to be complex and involve alterations in the rates of receptor internalization, degradation, assembly, processing, mRNA stability, and alterations in the rates of receptor gene transcription (21, 24, 49, 50). Our data suggest that some part of this downregulation may be caused by a decrease of the V2 receptor mRNA level.

We believe that our method gives a relative measure of the amount of specific V1a and V2 receptor mRNA initially present in the cells. It is possible to normalize mRNA values by the contents of DNA, protein, or number of cells. Because many physiological studies (i.e., microdissection or microperfusion studies) have expressed hormonal action per tubule length (2, 10, 12–18, 20, 27, 39, 40, 42, 44), we expressed PCR products per tubule length to relate the data to physiological functions.

Protein synthesis is determined by both mRNA level and by the efficiency of mRNA transcription. The rate of V1a and V2 receptor mRNA translation has not been evaluated here, nor have the levels of protein expression been investigated. Therefore, it seems very difficult to speculate about the relationship between the amount of mRNA level and the number of receptors present in cell surface. The RT-PCR technique is so sensitive that some proteins may not be detectable in the cell despite the presence of mRNA according to RT-PCR (51). Thus the presence of V1a and V2 receptor mRNA does not imply necessarily the existence of its functional protein product in the nephron segments. In particular, it must be careful with regard to the presence of functional receptor protein, when we detect very small levels PCR product such as V1a receptor mRNA in PST and PCT.

In summary, our data show that mRNA coding for the two AVP receptor subtypes are distributed differently along the nephron and renal vascular system, and that these mRNAs are regulated differently in response to the dehydrated state. These results are consistent with the view that the two type receptors may play different roles in modulating renal functions.

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