Vasopressin regulates the renin-angiotensin-aldosterone system via V1a receptors in macula densa cells

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ARGinine VASOPRESSIN (AVP) is a neuropeptide hormone that is involved in diverse functions including the regulation of body fluid homeostasis, osmoregulation, and ACTH release. These physiological effects are mediated by three subtypes of AVP receptors, designated V1a, V1b, and V2 (13, 17, 20). The V1a receptor (V1aR) is widely expressed, while the V1bR and V2R are predominantly expressed in the anterior pituitary gland and the kidney, respectively (7, 13, 17, 20). V1aR mediates vascular contraction, cellular proliferation, platelet aggregation, glycogenolysis, lipid metabolism, and glucose tolerance (1, 8, 10, 14). AVP stimulates translocation of aquaporin (AQP)-2 (AQP2) via the V2R in the collecting duct to reabsorb water (10). Since the V1aR is also detected in the juxtaglomerular apparatus, TAL, and the collecting duct in the kidney, both V1aR and V2R could be involved in regulating body fluid homeostasis (9, 37).

Renin secretion is stimulated by multiple factors such as sympatheomimetic stimuli, decreased intraglomerular pressure, relaxation of the afferent arteriole, and decreased NaCl delivery to the macula densa (MD) cells. The tubuloglomerular feedback (TGF) system regulates the balance of distal NaCl delivery and glomerular filtration rate (GFR) (14). Acting through the TGF system, nitric oxide (NO) and prostaglandin E2 (PGE2) produced in the MD cells stimulate renin production via increasing cAMP and cGMP generation in the granule cells (28, 29) and upregulate the GFR to return to a normal level (14). AVP stimulates the expression of neuronal nitric oxide synthase (nNOS) and cyclooxygenase (COX)-2, and then renin production. RAS stimulates production of aldosterone in the adrenal cortex as well as promotes mRNA expression of V2R and AVP-V2R-dependent water and urea reabsorption in the collecting ducts (11, 16, 32, 38). Consequently, increased plasma AVP could stimulate renin secretion and urine concentrating activity of AQP2 via the V1aR and V2R, and AVP-stimulated RAS could promote the V2R-AQP2 system, which leads to upregulation of water reabsorption.

We generated V1aR-deficient (V1aR−/−) mice and found that glucose, protein, and fat metabolism were altered in the mutant mice (1, 8). In addition, the circulating blood volume and blood pressure were decreased in V1aR−/− mice (15). V1aR−/− mice also showed decreased levels of plasma atrial natriuretic peptide (ANP) and aldosterone (1, 15). In contrast, hematocrit and blood urea nitrogen (BUN) increased in V1aR−/− mice (1). Since plasma volume was decreased in...
V1αR<sup>−/−</sup> mice, we hypothesized that the RAS and V2R-AQP2 system were altered due to lack of expression of V1αR.

In the present study, we assessed the activity of the V2R-AQP2 system and renal function and compared expression levels of renin, nNOS, and COX-2 in wild-type (WT) and V1αR<sup>−/−</sup> mouse kidneys. Furthermore, we examined the detailed expression profile of V1αR in the WT mouse kidney. Our results indicate that the abolishment of V1αR-mediated AVP function can result in suppression of the RAS, GFR, and V2R-AQP2 system, leading to hypotension with polyuria in V1αR<sup>−/−</sup> mice.

**MATERIALS AND METHODS**

**Animals.** The generation of V1αR<sup>−/−</sup> mice has been described previously (1). Mice littersmates that were not deficient in V1αR (i.e., WT) were used as age-matched controls for V1αR<sup>−/−</sup> mice. The genetic background of WT and V1αR<sup>−/−</sup> mice was a mixture of 129sv and C57Black/6j mice. Animals were used at 4–8 wk of age. All data presented here were obtained from male mice. All experimentation was performed under approved institutional guidelines (the Committee for Animal Experimentation of the National Research Institute for Child Health and Development and the Committee for Animal Experimentation, Kumamoto University Graduate School of Medical Sciences).

**Measurement of plasma and urine parameters.** Mice were kept in metabolic cages for 3 days before urine collection. Urine osmolality was measured using a microosmometer (Fiske, Needham Heights, MA). Urinary sodium, potassium, and chloride concentrations were measured at the SRL laboratory (Tokyo, Japan). Eight hundred microliters of blood were collected from the central vein, blood samples were centrifuged at 3,000 rpm for 10 min at 4°C, and the supernatants were stored at −80°C. The levels of angiotensin II and PGE<sub>2</sub> were determined using ELISA kits (angiotensin II, Phoenix Pharmaceuticals, Belmont, CA; PGE<sub>2</sub>, R&D Systems, Minneapolis, MN). Plasma renin activity (PRA) was measured as previously described (19). To determine PRA, plasma was incubated for 1 h at 37°C with 3 μl of plasma, prepared from nephrectomized rats 72 h after operation as a source of renin substrate, plus 3 μl of PRA buffer containing 0.1 M sodium phosphate (pH 6.5), 10 mM EDTA, and 1 mM PMSF. The reaction was stopped by boiling. The generated angiotensin I (AI) was measured by an enzyme immunoassay (Bachem America, Torrance, CA). PRA was expressed as AI generated per hour.

**Measurement of AVP-induced cAMP generation.** To examine the function of V2R-mediated antidiuretic action, AVP-induced cAMP generation was performed as described previously (23).

**Renal function studies.** Renal function was measured as previously described with some modification (12, 30). A catheter (a stretched PE-10 polyethylene tube connected to a PE-50 polyethylene tube, Becton Dickinson, Franklin Lakes, NJ) was inserted into the left internal jugular vein for fluid infusion and the common carotid artery for continuous measurement of mean arterial pressure (MAP). MAP was monitored with the Powerlab system (ADInstruments, Bella Vista NSW, Australia). The bladder was cannulated with PE-50 tubing for urine collection. During the surgery, 0.9% NaCl containing 6% BSA (Sigma-Aldrich, Tokyo, Japan) was infused intravenously at the rate of 2.5 μl/min. After the surgery, the intravenous infusion was changed to 0.9% NaCl containing 1% BSA, 5% inulin (Sigma), and 1.5% PAH (Sigma), and infused at the rate of 2.5 μl/min. After a 1-h equilibration period, two consecutive 30-min urine and tail vein blood samples were obtained to evaluate whole-kidney function and the hematocrit value. The hematocrit value was measured using a heparinized microcapillary tube (Drummond, Broomall, PA). Inulin and PAH concentration were measured by the anthrone and colorimetric methods, respectively. GFR and renal plasma flow (RPF) were determined by

the clearance of inulin and PAH, respectively. Renal blood flow (RBF) was calculated from RPF and the hematocrit. The filtration fraction was measured by RPF/GFR, and renal vascular resistance (RVR) was measured by MAP/RBF. Clearance data were normalized by the kidney weight.

**Immunohistochemistry and in situ hybridization.** Immunohistochemistry (IHC) analysis of nNOS, COX-2, and renin, and in situ hybridization (ISH) for V1αR were performed as follows. Two- and 6-μm paraffin sections of kidneys were used for IHC and ISH. After antigen unmasking and inactivation of endogenous peroxidase, the sections were blocked and diluted and an antibody (anti-renin antibody, 1:50, Santa Cruz Biotechnology, Santa Cruz, CA; anti-nNOS antibody, 1:100, Cell Signaling Technology, Danvers, MA; anti-COX-2 antibody, 1:50, Cell Signaling Technology) was added. The sections were incubated for 16 h at 4°C or 2 h at room temperature and washed. After washing, a diluted secondary antibody (anti-rabbit or anti-goat biotinylated antibody, GE Healthcare) was added to the sections and incubated at 4°C for 45 min. Then, ABC reagent (Vector Laboratories, Burlingame, CA) was added and incubated at room temperature for 30 min. Color was developed by addition of diaminobenzidine (DAB) substrate solution to sections (Dojinno, Kumamoto, Japan). The sections were counterstained with hematoxylin (Muto Chemicals, Tokyo, Japan). The numbers of nNOS-positive MD or renin-positive granular cells determined with IHC analysis were expressed as positive cells/glomerulus, as described previously (25).

For ISH of V1αR, an antisense or sense probe for the full-length mouse V1αR was prepared using DIG RNA labeling Mix (Roche Diagnostics, Basel, Switzerland). Hybridization was performed with the probe at 60°C for 16 h. After treatment with 0.5% blocking reagent (Roche Diagnostics) in Tris-buffered saline containing 0.1% Triton X-100 (TBS-T) for 30 min, the sections were incubated for 2 h with an anti-DIG alkaline phosphatase-conjugated antibody (Roche Diagnostics) diluted 1:1000 with TBS-T. Color development reactions were performed with BT/BCIP solution (Roche Diagnostics) overnight. The sections were counterstained with Kernethrotin stain solution (Muto Chemicals).

**RT-PCR.** RNA preparation and RT-PCR were performed as described previously (8). RT-PCR was performed using specific primer sets as follows: GAPDH, forward 5′-ggctactatcgcgcgcct-3′ and reverse 5′-ccaccccccgtgcggctgt-3′; V1αR, forward 5′-tcacgctcttgagcgaggtc-3′; renin, forward 5′-gacatcggtctgtccaggtgaag-3′; COX-2, reverse 5′-ccctgatcgcataatgctca-3′; nNOS, forward 5′-aagactggctcgcacgtgac-3′; reverse 5′-ggtcggagacactgtggaaagct-3′; COX-2, forward 5′-acactttcactctgactc-3′; reverse 5′-gaagcaccctctcactac-3′; and cpyl1b2, forward 5′-atctgcgttttcgcttgcttc-3′, reverse 5′-ctctcctgcgttcgcttc-3′.

**Western blotting.** Tissue lysates were prepared from microdissected outer medullary collecting duct (OMCD), inner medulla, and whole kidney, and Western blot analyses were performed with anti-V2R (a kind gift from Dr. Sören Nielsen, University of Aarhus, Aarhus, Denmark), anti-AQP2 (Santa Cruz Biotechnology), anti-renin, or anti-COX-2 antibody as described previously (6, 8, 12, 23).

**Statistical analysis.** All values are expressed as means ± SE. An unpaired Student’s t-test or unpaired Welch’s t-test for comparison between two groups was employed. The Dr. SPSS-II computer program (SPSS, Tokyo, Japan) was used for the analyses. A P value <0.05 was considered statistically significant.

**RESULTS**

**Urine production parameters in WT and V1αR<sup>−/−</sup> mice.** To investigate body fluid balance in V1αR<sup>−/−</sup> mice, we measured urine volume and electrolyte excretion using metabolic cages. Under free water access, V1αR<sup>−/−</sup> mice produced twofold greater urine volume with lower osmolality compared with WT mice (Fig. 1. A and B, middle). Greater urine volume and lower
Fig. 1. Twenty-four-hour urine was collected, and the following measurements were made: A: urine volumes under normal conditions with free water access, after treatment with 5% glucose, and after 1-day water restriction [n = 6–12 and n = 6–11 for wild-type (WT) and vasopressin V1a receptor-deficient (V1aR−/−) mice under the 3 conditions, respectively]. B: urine osmolality under the 3 conditions (n = 5–8 and n = 5–9 for WT and V1aR−/− mice under the 3 conditions, respectively). C: urinary excretion of sodium (n = 5 for WT and n = 8 for V1aR−/− mice). D: urinary secretion of chloride for WT (n = 5) and V1aR−/− (n = 8) mice under normal conditions. Concentrations of urine sodium and chloride were standardized by taking into account the concentration of urine creatinine. *P < 0.05, ***P < 0.001 vs. WT mice.

urine osmolality were also found in V1aR−/− mice compared with the WT when the mice were overhydrated by giving them 5% glucose in the drinking water (Fig. 1, A and B, right). Under 1-day water restriction, the differences in urine volume and urine osmolality between WT and V1aR−/− mice were not significant (Fig. 1, A and B, left). Urinary sodium and chloride excretion in V1aR−/− mice were significantly less than in WT mice (Fig. 1, C and D), while potassium excretion was not significantly different between WT and V1aR−/− mice (0.88 ± 0.03 vs. 0.84 ± 0.02 mmol/mg creatinine in WT and V1aR−/− mice, n = 5 and 8, respectively, P = 0.240). These results indicate that the antidiuretic function is particularly impaired when water is loaded in the kidney of V1aR−/− mice.

V2R functions in WT and V1aR−/− mice. To assess the AVP-V2R-mediated antidiuretic function in V1aR−/− mice, we measured expression of V2R by Western blotting. The expression of V2R in the inner medulla was significantly decreased in V1aR−/− mice under basal conditions (Fig. 2, A and B). In addition to expression of V2R, we measured AVP-dependent cAMP generation in the medullary TAL (mTAL) and OMCD and the expression of AQP2 in the whole kidney and OMCD. AVP-dependent cAMP generation in the mTAL and OMCD of V1aR−/− mice was significantly lower than that in WT mice (Fig. 2, C and D, Table 1). AQP2 expressions in the whole kidney and OMCD of V1aR−/− mice were also reduced to 33 ± 3 (P = 0.024) and 61 ± 12% (P = 0.045), respectively, of those in WT mice (Fig. 2, E and F). These data indicate that the V2R-mediated antidiuretic function is impaired in both the mTAL and collecting ducts of V1aR−/− mice.

Renal function in WT and V1aR−/− mice. Since V1aR−/− mice exhibited polyuria and decreased blood pressure and plasma volume (1, 15), we measured the RBF, GFR, filtration fraction, and RVR to evaluate the renal function in V1aR−/− mice. The RBF and GFR were significantly lower in V1aR−/− mice compared with WT mice (RBF: 6.56 ± 0.39 vs. 4.15 ± 0.44 ml/min·100 g−1 in WT and V1aR−/− mice, n = 9 and 8, respectively, P < 0.001; GFR was 1.07 ± 0.08 vs. 0.74 ± 0.06 ml/min·100 g−1 in WT and V1aR−/− mice, n = 9 and 8, respectively, P = 0.006; Fig. 3, A and B). There was no significant difference in the filtration fraction between WT and V1aR−/− mice (30.0 ± 2.0 vs. 33.0 ± 1.7% in WT and V1aR−/− mice, n = 9 and 8, respectively, P = 0.283). RVR

Table 1. Measurement of AVP-dependent cAMP generation

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<th>Vehicle</th>
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<th>AVP 10−7 M</th>
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<td>mTAL</td>
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<tr>
<td>WT</td>
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<tr>
<td>V1aR−/−</td>
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<tr>
<td>WT</td>
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<tr>
<td>V1aR−/−</td>
<td>0.12±0.08</td>
<td>36.52±4.60</td>
<td>46.83±8.64</td>
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Values are means ± SE. mTAL, medullary thick ascending limb of Henle’s loop; OMCD, outer medullary collecting duct; WT, wild-type mice; V1aR−/−, vasopressin V1a receptor-deficient mice. Data represent cAMP generation (fmol·min−1·3 min−1) in Fig. 2, C and D. *P < 0.05, †P < 0.01 vs. WT mice.
and V1aR-impaired V2R function, which are promoted by RAS (1, 12, 17, 39), we evaluated PRA and angiotensin II levels in WT and V1aR−/− mice. PRA and plasma angiotensin II levels were lower in V1aR−/− mice than in WT mice under normal (0 h) and 24-h water deprivation (PRA under basal conditions 50.6 ± 5.7 vs. 32.8 ± 6.1 ng Al·ml−1·h−1 in WT and V1aR−/− mice, n = 10, respectively, P = 0.0496; PRA under water-deprived conditions 61.5 ± 6.8 vs. 43.4 ± 4.0 ng Al·ml−1·h−1 in WT and V1aR−/− mice, n = 10, respectively, P = 0.025; angiotensin II under basal conditions 6.31 ± 1.03 vs. 3.47 ± 0.63 ng/ml in WT and V1aR−/− mice, n = 10, respectively, P = 0.042; and angiotensin II under water-deprived conditions 11.04 ± 0.94 vs. 7.88 ± 0.97 ng/ml in WT and V1aR−/− mice, n = 10, respectively, P = 0.035) (Fig. 4, A and B). These data indicate that the renin production and response to dehydration are decreased in V1aR−/− mice, which resulted in decreased a plasma angiotensin II level in V1aR−/− mice.

Renin production in WT and V1aR−/− mouse kidney. Since the plasma renin level was decreased in V1aR−/− mice, we examined its expression profile in the kidney of WT and V1aR−/− mice. IHC analysis showed that renin was expressed in both the granule cells and renal tubules of the WT mouse kidney (Fig. 4C, left). In V1aR−/− mice, renin staining was weaker in both the granule cells and renal tubules than in WT mice (Fig. 4C, right), and the number of renin-positive cells in the
juxtaglomerular apparatus was significantly decreased under normal conditions (1.16 ± 0.15 vs. 0.71 ± 0.11 cells/glomerulus in WT and V1aR−/− mice, n = 4 kidney sections, respectively, P = 0.012) (Fig. 4D). Expressions of renin mRNA and protein in the whole kidney were decreased in V1aR−/− mice under both normal and water-deprived conditions (Fig. 4, E and F). These results indicate that renin production in V1aR−/− mice is significantly decreased and that blockade of the V1aR leads to decreased RAS activity and consequently to lowering of the plasma aldosterone level (1).

Since renin expression is controlled by COX-2 and nNOS, which are expressed in the MD cell and/or TAL (14), we next examined expression profiles of nNOS and COX-2 in the kidney of WT or V1aR−/− mice. Staining with anti-COX-2 antibody in TAL and MD cells was weaker in V1aR−/− mice than in WT mice (Fig. 5A, top and middle). Since PGE2 synthesis in the kidney is mediated mainly by COX-2 (3), we measured the plasma and urine PGE2 level to evaluate COX-2 activity in V1aR−/− mice. Under normal conditions, the plasma and urine PGE2 levels were significantly lower in V1aR−/− mice than WT mice (plasma PGE2, 216 ± 7 vs. 170 ± 7 ng/ml in WT and V1aR−/− mice, n = 10, respectively, P < 0.001; urine PGE2, 1,106 ± 102 vs. 791 ± 59 pg/mg creatinine in WT and V1aR−/− mice, n = 5 and 8, respectively, P = 0.018) (Fig. 5, B and C). Furthermore, RT-PCR and Western blot analysis showed a lower level of expression for COX-2 in V1aR−/− mice compared with WT mice under normal conditions (Fig. 5, E and F). As with COX-2, nNOS was also decreased in V1aR−/− mice. IHC analysis revealed nNOS-positive cells in the MD of the WT mouse kidneys as reported previously (31) (Fig. 5A, bottom left). Under normal conditions, the number of nNOS-positive cells was significantly less in V1aR−/− mice than WT mice (1.25 ± 0.14 vs. 0.65 ± 0.10 cells/glomerulus in WT and V1aR−/− mice, n = 4 kidney sections, respectively, P < 0.001) (Fig. 5A, bottom, and D). Expression of nNOS mRNA in the V1aR−/− mouse kidneys was lower under both normal and water-deprived conditions (Fig. 5E). Taken together, nNOS as well as COX-2 expressions are suppressed in the MD cells of V1aR−/− mouse kidney, which could lead to reduced renin production.

Expression of V1aR in MD and granular cells. Since the expressions of nNOS and COX-2 was decreased in the V1aR−/− mouse kidneys, we performed ISH for the V1aR in the WT mouse kidney to determine whether V1aR was coexpressed with nNOS or COX-2. The antisense probe for the mouse V1aR produced positive signals in the TAL, collecting ducts, and renal vascular cells, as reported previously (4, 37), whereas the sense probe did not show any signals (data not shown). V1aR was detected in the MD cells and was colocal-
ized with nNOS and COX-2 (Fig. 6, A and B). Furthermore, V1aR was colocalized with COX-2 in the TAL cell (Fig. 6B).

**DISCUSSION**

Body fluid homeostasis is maintained via two major hormonal pathways, the osmoregulatory AVP and volume-regulatory RAS (10, 29). Since the plasma volume and aldosterone levels were decreased in V1aR−/− mice (1), we hypothesized that the altered body fluid homeostasis and resulting hypotension observed in V1aR−/− mice could be caused by impairment of both the AVP and RAS systems (15). V1aR−/− mice showed suppressed V2R-mediated antidiuretic action. The GFR was also decreased in V1aR−/− mice. Chronic polyuria, due to any cause, can reduce the amount of cAMP generated in response to vasopressin (21). Thus the reduction in vasopressin-stimulated cAMP production in the V1a knockout mice may be due to chronic polyuria as well as the reduced V2R expression. Since the V2R-AQP2 system and GFR are controlled by RAS (11, 14, 16, 38), we analyzed renin and angiotensin II in V1aR−/− mice. PRA, angiotensin II level, and renin-stimulated cAMP production in the V1a knockout mice may be due to the lack of AVP stimulation of the RAS system (15), which has been shown to be downstream of AVP (11, 14). We found that PRA, angiotensin II level, and renin expression in the kidney were decreased in V1aR−/− mice. The expression of COX-2 and nNOS was decreased in V1aR−/− mice. V1aR mRNA was specifically expressed in the MD cells of WT mice. Thus the AVP/V1aR plays a crucial role as a gatekeeper in regulating RAS in the MD cells, and inhibition of RAS in V1aR−/− mice leads to the impaired V2R-mediated antidiuretic action, consequently resulting in polyuria, lower plasma volume, and hypotension (11, 15, 35, 39).

AVP is known to regulate body fluid homeostasis via the V2R-AQP2 system in the kidney. In contrast, the functional role of the V1aR in the kidney has not been fully elucidated, even though localized in several regions of the kidney such as the TAL, collecting ducts, and renal vascular cells (4, 37). The V1aR has been considered to play a role in regulation of acid-base balance and water/NaCl reabsorption (4, 9). In the present study, we determined the expression V1aR in the MD cell of WT mice. Coexpression of the V1aR with nNOS and/or COX-2 in the MD cell suggests that AVP controls nNOS and COX-2 expression via the V1aR, which stimulates the production of NO and PGE2, and subsequently regulates renin production. V1aR was expressed in the MD cells and also on the distal nephron and was colocalized with COX-2 and renin, which suggests that AVP stimulates renin production via the V1aR in the distal nephron by stimulating the expression of COX-2. In fact, the expressions of renin and COX-2 were decreased in granular cells and distal tubules of V1aR−/− mice. Thus V1aR is coexpressed with nNOS and/or COX-2 in the MD and distal tubules of the mice kidney where AVP/V1aR controls renin production by stimulation of production of NO and/or PGE2.

Whereas we demonstrated the functional role of AVP/V1aR for renin secretion in this study, renin secretion is stimulated by other factors such as sympathomimetic stimuli, decreased intraglomerular pressure, relaxation of afferent arteriole, and the TGF system (14). In the MD cells, decreased urine osmolality and/or decreased NaCl delivery due to decreased GFR stimulates signals of nNOS/NO and COX-2/PGE2, which induce the dilation of afferent arterioles directly and contraction of efferent arterioles via activation of RAS (2, 14). In addition, the reduction of GFR also suppresses the release of adenosine or ATP, two molecules known to contract afferent arterioles in the MD cells, resulting in reduced contraction of afferent arterioles (14, 26). These mechanisms in the TGF system would lead to an increase in GFR (2, 14, 26). We found that the expression of nNOS and COX-2 in the MD cells, renin production, and GFR were decreased in V1aR−/− mice. Thus renin is crucial for the TGF system and AVP/V1aR is involved in regulation of the renin-stimulating factors such as NO and PGE2. Therefore, disrupting the AVP/V1aR system in the MD cells leads to decreased nNOS and COX-2 activity, resulting in reduced renin production and subsequent impairment of the TGF system in V1aR−/− mice.

We found that V1aR−/− mice had a twofold greater urine volume than WT mice under the free water access condition. The difference in urine volume was more evident when mice were loaded with 5% glucose and was not evident when mice were water restricted. Thus the defect in urine concentration in V1aR−/− mice becomes evident under water-loaded conditions. When water intake is low, such as in the water-restricted condition, the reduced V2R-AQP2 function can reabsorb water in V1aR−/− mice. The urine volume in V1aR−/− mice, however, was not as high as what might be expected in the classic type of renal diabetes insipidus (27), suggesting that the lack of
V1aR in the kidney resulted in a mild type of nephrogenic diabetes insipidus rather than classic diabetes insipidus. The polyuria observed in V1aR−/− mice was similar to the phenotype of mutant mice lacking renin, type 1a angiotensin II receptor, or triply neuronal/inducible/endothelial NOS (21, 24, 39). Several possible mechanisms could be involved in the polyuria in V1aR−/− mice, an impaired V2R-AQP2 system or an impaired TGF system, both of which could be caused by the impaired RAS. Angiotensin II promotes AVP-stimulated fluid and urea permeability (11, 16). Thus the V2R-AQP2 function, as well as aldosterone synthesis, is promoted by RAS (11, 16, 32). Therefore, the decreased renin and angiotensin II levels might cause the altered V2R-AQP2 function and aldosterone production, leading to polyuria. According to the analysis of the urine concentrating mechanism by Stephenson et al. (33), there are four major factors that determine the osmolality of the urine relative to the blood: 1) rate of active NaCl transport in the mTAL, 2) delivery of fluid to the mTAL, 3) water permeability of the medullary collecting ducts, and 4) rate of delivery of fluid to the medullary collecting ducts. It is thought that the cause of polyuria is delivery of excess fluid to the medullary collecting ducts due to the failure of the TGF system in MD cells, which regulates distal fluid delivery (33). Disruption of the steady-state GFR due to reduced functioning of the TGF system can also contribute to polyuria in V1aR−/− mice (22).

In the present study, we have demonstrated that the V2-AQP2 system and RAS were simultaneously impaired, leading to an increased urine volume in V1aR−/− mice. Several studies have reported that the V1aR and V2R are expressed in the luminal and basolateral membranes, respectively, the TAL, and in collecting ducts (9, 23, 36), suggesting that AVP in the urine downregulates the expression of V2R via V1aR in the luminal membrane. This implies that suppression of the V1aR function leads to activation of the V2R function in the collecting ducts. Therefore, we had expected low urine output in the V1aR−/− mice. On the contrary, the lack of V1aR caused polyuria. Even with the lack of V1aR, AVP-dependent cAMP generation in the mTAL and OMCD was decreased in V1aR−/− mice. Although we cannot exclude the possibility that chronic polyuria may have caused reduced AVP-dependent cAMP generation, reduced V2R expression is thought to have caused the decreased AVP-dependent cAMP generation in V1aR−/− mice. These data suggested that V1aR-mediated RAS stimulation in MD cells has a functional role upstream of the pathway of V1aR-mediated suppression of V2R-mediated antidiuretic action in the collecting duct. These findings, taken together with previous reports, suggest that urine AVP, as well as urine chloride levels, could be novel regulators of the TGF system by stimulating V1aR in MD cells. Our findings imply that the V1aR in the MD cells plays an important role in activating the V2R-AQP2 as well as the TGF system.

In summary, we have shown that AVP regulates body fluid homeostasis and the GFR via the V1aR in MD cells by activating the TGF system and RAS, and subsequently the V2R-AQP2 system.

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