Downregulation of AQP2 expression in the kidney of polydipsic STR/N mice

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Tsumura, Keiko, Xuefei Li, Kwartarini Murdiastuti, Most. Nahid Parvin, Tetsuya Akamatsu, Chenjuan Yao, Norio Kanamori, Kiyotoshi Inenaga, Hiroshi Yamashita, and Kazuo Hosoi. Downregulation of AQP2 expression in the kidney of polydipsic STR/N mice. Am J Physiol Renal Physiol 290: F478–F485, 2006. First published September 6, 2005; doi:10.1152/ajprenal.00029.2005.—Aquaporin-2 (AQP2) is responsible for the concentration of urine in the kidney collecting tubule under the regulation of vasopressin. The mRNA level of this water channel in polydipsic STR/N mice was extremely reduced compared with that in normal ICR mice. In male mice, reduction of the AQP2 mRNA level was not evident at 3 wk of age, at which time water intake was not increased. At 10 wk of age, however, the AQP2 mRNA level was reduced to 10% of that in control mice, whereas water intake was increased by 36%. At 44 wk, the water intake became five times that of the control ICR mice, and the AQP2 mRNA level in these polydipsic mice was only ~5% of control. Similar changes were observed in the AQP2 protein level, suggesting that the mRNA level of AQP2 reflects the protein level of AQP2. These inverse changes in the AQP2 mRNA level and water intake were also evident in female mice. The data imply that polydipsia in STR/N mice may have affected AQP2 mRNA transcription in the kidney, resulting in reduced AQP2 expression, which would contribute to a reduction in overretention of water.

Abbreviations: AVP, arginine vasopressin; AQP, aquaporin; ECL, enhanced chemiluminescence; ICR, inbred control; IGF-I, insulin-like growth factor-I; NHO, normohydrated; NICE, normal intermediate control; NO, nitric oxide; OMA, organ culture medium; PCR, polymerase chain reaction; qPCR, quantitative PCR; RT, reverse transcriptase; STR, spontaneously hypertensive; TBS, Tris-buffered saline; TCA, trichloroacetic acid; UV, ultraviolet; Z-actin, a smooth muscle actin isoform.

With respect to water intake, the existence of water channels throughout the animal kingdom has recently been established; these channels are composed of proteins called aquaporins (AQP), which selectively transport water and other compounds such as glycerol and urea (for review see Ref. 1). In the kidney, AQP2 is specifically expressed in the collecting tubule, where it is involved in water reabsorption or urine concentration (5). Trafficking of the cytoplasmic AQP2 vesicles is provoked by stimulation with AVP, resulting in an increase in the concentration of this water channel protein in the apical membrane (11). Because dilute urine is circulating in the collecting tubules and because water transport via AQP2 is based simply on the osmotic gradient (1), the water moves from the lumen into the tubule cells.

Polydipsic STR/N mice excrete extremely dilute urine (14). A large osmotic gradient in the collecting duct is, therefore, expected in the kidney of polydipsic mice. If the AQP2 level in the apical membrane is not reduced in these mice, much of their body water would be retained, potentially shortening their life span. However, STR/N mice actually survive for >1 yr, despite strong polyuria and hydrenephrosis (14), implying the existence of some unknown regulatory mechanism in the kidney water balance.

In the present study, therefore, we examined whether the expression of AQP channels in the kidney of polydipsic STR/N mice is normal or is affected by their strong polyuria. Our results indicate that AQP2 mRNA expression in the kidney of these mice was reduced to one-fifth to one-tenth that of normal ICR mice.

MATERIALS AND METHODS

Reagents. TRI-reagent was obtained from Sigma (St. Louis, MO), SuperScript One-Step RT-PCR System and Concert Rapid PCR Purification System from Gibco BRL (Gaithersburg, MD), Competitive DNA Construction Kit and Competitive RNA Translation Kit from Takara Shuzo (Kyoto, Japan), β-actin primers (XAH20 and XAH17) from Funakoshi (Tokyo, Japan), Nusieve agarose and Seakem agarose from FMC (Rockland, ME), AQP2 antibody from Santa Cruz Biotechnology (Santa Cruz, CA), Fuji medical X-ray film (Tokyo, Japan), horseradish peroxidase-conjugated goat anti-rabbit IgG and enhanced chemiluminescence detection system from Amersham Pharmacia Biotech (Buckinghamshire, UK), horse serum from Gibco BRL (New York, NY), and protein assay kit from Bio-Rad Laboratories (Hercules, CA)

Experimental animals. Male and female polydipsic STR/N mice were bred in the animal facility of the University of Occupational and Environmental Health School of Medicine and transferred to the Institute of Health Biosciences, The University of Tokushima. The mice were housed at ambient temperature with lights on from 8 AM to 8 PM, and food and water were given ad libitum. Male and female ICR mice (Japan SLC, Shizuoka, Japan), which were employed as controls, were kept under the same conditions as the STR/N polydipsic mice during the experimental period.

Water intake was measured once a day for 2–3 days, and the average of these values was calculated. Body weight was measured immediately before the animal was killed. In the water restriction experiment, mice were given 3 ml of water at 9 AM and 4 ml at 8 PM
RNA preparation and RT-PCR. TRI-reagent was used to isolate total RNA from the mouse kidney. The expression of mRNAs for AQP2 and β-actin was detected by RT-PCR using the SuperScript One-Step RT-PCR System following the manufacturer’s protocol. Complementary DNA was synthesized by incubation at 45°C for 30 min. Subsequently, the DNA amplification was carried out for 35 or 25 cycles in a DNA thermal cycler (model TP3000, Takara), with each cycle consisting of denaturation at 94°C for 15 s, primer annealing at 55–63°C (depending on the respective primer) for 30 s, and extension at 72°C for 1.5 min. The primers for amplification of AQPs were 5′-CTTGGTGGCTGATGTTCTCTG-3′ (sense) and 5′-ATTTCGGCAGGAGTATCCTC-3′ (antisense) for AQP1, 5′-AGTCTCTGCCAGCAGTCTTCT-3′ (sense) and 5′-TTCGCCGTTCCTCCAGCTGTTG-3′ (antisense) for AQP2, 5′-GAGATGGCCATCATCGCTAC-3′ (sense) and 5′-CACACAAATAAGGGCGTCTCTG-3′ (antisense) for AQP3, 5′-CTCTCGTGTGGACTCAGCATGTTG-3′ (sense) and 5′-TCTTCTTAGGCCGACCTTTTGAG-3′ (antisense) for AQP4, and 5′-ACCCACACTGTGCCCATCTA-3′ (sense) and 5′-CGGAACCCGCTATTGCC-3′ (antisense) for β-actin.

Synthesis of RNA competitor for quantitative RT-PCR. The AQP2 DNA competitor was prepared by PCR using a Competitive DNA Construction Kit according to the protocol supplied by the manufacturer. In this amplification, λDNA (supplied in the kit) was used as a template DNA, whereas synthetic DNAs, 5′-ATTATGGGACTATAGAATACGAGATGCTCCACATCCGCTAC-3′ (sense) and 5′-GACACTATAGAATACGAGATGCTCCACATCCGCTACGTAC-3′ (antisense) for AQP1, 5′-AGTCTCTGCCAGCAGTCTTCT-3′ (sense) and 5′-TTCGCCGTTCCTCCAGCTGTTG-3′ (antisense) for AQP2, 5′-GAGATGGCCATCATCGCTAC-3′ (sense) and 5′-CACACAAATAAGGGCGTCTCTG-3′ (antisense) for AQP3, 5′-CTCTCGTGTGGACTCAGCATGTTG-3′ (sense) and 5′-TCTTCTTAGGCCGACCTTTTGAG-3′ (antisense) for AQP4, and 5′-ACCCACACTGTGCCCATCTA-3′ (sense) and 5′-CGGAACCCGCTATTGCC-3′ (antisense) for β-actin.

Expression of AQP1, AQP2, AQP3, and AQP4 in the kidney of STR/N polydipsic mice. The reaction conditions employed for amplification of AQP2, AQP3, and β-actin mRNAs consisted of 1 cycle of cDNA synthesis and 35 cycles of DNA amplification. The cDNA was first synthesized by incubation at 45°C for 30 min and denatured by heating at 94°C for 2 min. The thermal reaction was then followed by denaturation at 94°C for 15 s, primer annealing at 55–63°C for 30 s, and extension at 72°C for 1.5 min. The reaction was finally incubated for one cycle at 72°C for 5 min. These reactions were carried out in a PCR Thermal Cycler MP (model TP3000, Takara).

The amplified product (5 μl aliquot) was separated by electrophoresis in a 3% agarose gel (3:1 Nusieve-SeaKem agarose) containing Tris acetate-EDTA buffer. The DNA that was separated was stained with 2 μg/ml ethidium bromide and photographed on Polaroid film. The electrophoresis image was analyzed by NIH Image (Bethesda, MD), and the band intensity was quantified.

Preparation of membrane fractions of kidney. The expression of AQP2 protein in the kidney was determined by Western blot analysis. The kidney tissue was homogenized in a 15-ml tared-type glass mortar fitted with a Teflon pestle (Wheaton Science Products, Millville, NJ) using 10 vol of homogenization buffer consisting of 80 mM sucrose, 20 mM Tris-HCl (pH 7.4), 1 mM EDTA, 1 mM phenylmethylsulfonyl fluoride, 5 μg/ml leupeptin, 5 μg/ml pepstatin, 5 mM MgCl₂, and 1 tablet of Complete EDTA-free protease inhibitor cocktail per 25 ml of buffer solution. The homogenate was centrifuged at 4,000 g for 10 min, and the supernatant was subjected to a second centrifugation at 200,000 g for 1 h; the pellet was suspended in the same buffer. The protein concentration of samples was measured with the Bio-Rad protein assay kit, and 10 μg of each sample were used for Western blotting for AQP2 analysis, as described previously (13).

Western blot analysis. The sample was incubated at room temperature (25°C) for 10 min in a sample-loading buffer (2% SDS, 62.5 mM Tris-HCl, 10% glycerol, 5% 2-mercaptoethanol, and 0.005% bromphenol blue) and subjected to SDS-PAGE (12% polyacrylamide gels). The separated proteins were electrophoretically transferred to nitrocellulose filters. The blot filter was blocked for 1 h with PBS containing 0.05% Tween 20 and 5% horse serum and washed with PBS containing 0.05% Tween 20. The filter was then reacted with anti-AQP2 antibody (1:500 dilution, 0.4 μg/ml), washed with PBS containing 0.05% Tween 20, and incubated with horseradish peroxidase-conjugated donkey anti-goat IgG (1:3,000 dilution). The filter was finally subjected to an enhanced chemiluminescence detection system and exposed to X-ray film.

For the peptide preabsorption experiment, 1 μl of anti-AQP2 antibody (0.2 μg) was mixed with 3 μl of its immunogen peptide (0.6 μg), incubated at room temperature for 1 h, and then diluted with washing buffer to give the same antibody concentration as nonpreabsorbed antibody solution (0.4 μg/ml). The peptide-preabsorbed antibody was reacted with a blot filter and processed as described for the experimental group.

Measurement of osmolality. For measurement of osmolality, 15 μl of serum and urine were sampled and measured with a freezing-point osmometer (One-Ten, Fiske, Norwood, MA).

Calculation and statistical analysis. AQP2 and AQP3 mRNA levels determined by QRT-PCR were normalized by the values for β-actin mRNA. Data were analyzed by the Mann-Whitney U-test, and P values were determined.

RESULTS

Expression of AQP1, AQP2, AQP3, and AQP4 in the kidney of STR/N polydipsic mice. The RT-PCR technique was employed to confirm the expression of various AQPs in the kidney of 44-wk-old STR/N polydipsic mice compared with that in the same tissue of normal ICR mice (Fig. 1). The four major AQPs (AQP1, AQP2, AQP3, and AQP4), known to be expressed in the kidney, were also expressed in the kidney of polydipsic

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mice. In male and female mice, there was no difference in the band intensities of cDNA fragments for AQP1 and AQP4 between STR/N polydipsic and normal ICR mice (Fig. 1, A and E). However, apparent differences in the band intensities of cDNA fragments for AQP2 were seen between polydipsic and normal mice; i.e., the cDNA bands for male STR/N mice were less intense than those for male ICR mice (Fig. 1B). No difference between STR/N and ICR was observed in female mice. By decreasing the cycle number of PCR, however, there appeared to be a difference in the AQP2 mRNA expression level between STR/N and ICR female mice as well (Fig. 1C), suggesting that 25, not 35, cycles of PCR are a presaturation condition and appropriate for the present experiment. The levels of β-actin expression were the same for all samples, indicating no degradation of sample RNAs. Because RT-PCR is only a semiquantitative technique, QRT-PCR was conducted to pursue this difference further. Similarly, although only the level of male AQP3 appeared lower in STR/N than in ICR mice by electrophoresis data (Fig. 1D), the QRT-PCR experiment detected the difference of AQP3 expression between STR/N and ICR in male and female mice (see Fig. 3D).

QRT-PCR of AQP2 and AQP3 mRNAs. The RNA competitor for mouse AQP2 was synthesized as described above, and a primer set that can specifically hybridize to RNA competitor and target AQP2 mRNA was prepared. In the QRT-PCR assay, different amounts of competitor RNA were mixed with a fixed amount of target RNA, and the RT-PCR was carried out. Two cDNA fragments appeared: one from target mRNA and the other from competitor RNA (top and bottom bands in Fig. 2A). The intensity of the top band decreased, while that of the bottom band increased, when the amount of competitor RNA was increased (Fig. 2), indicating that the assay system was effective for quantitative determination of mouse AQP2 mRNA. The amount of target mRNA was determined as shown in Fig. 2B. Similarly, the mouse AQP3 and β-actin mRNA levels could also be determined by the same procedure (data not shown), and AQP2 and AQP3 mRNA levels were normalized by the β-actin mRNA level.

Alteration in water intake and kidney AQP2 and AQP3 mRNA levels in polydipsic STR/N mice. At 11 mo (44 wk) of age, STR/N mice have developed strong polydipsia, and in this study they drank 5.5 (males) or 5.2 (females) times more water...
Fig. 3. Water intake, body weight, and kidney AQP2 and AQP3 mRNA levels in 44-wk-old male and female polydipsic STR/N and normal ICR mice. Values are means ± SE for 4 mice. Statistical significance was determined by Mann-Whitney U-test. A: water intake. *P < 0.005; **P < 0.002 vs. respective ICR mice. B: body weight. *P < 0.05; **P < 0.01 vs. respective ICR mice. No statistical difference was noted between male and female ICR and STR/N mice. C: kidney AQP2 mRNA levels. *P < 0.02; **P < 0.005 vs. male and female ICR mice. §P < 0.01 vs. male ICR mice. D: kidney AQP3 mRNA levels. *P < 0.05 vs. respective ICR mice. §P < 0.05 vs. male ICR mice.

Fig. 2. Determination of AQP2 mRNA by QRT-PCR in the kidney of normal ICR mice. A: 0.1 μg of RNA from a normal male ICR mouse was mixed with competitor RNA, and RT-PCR was carried out. Reaction mixture was subjected to electrophoresis. B: data in A were analyzed by NIH Image software, and percentages are plotted. Amount of mRNA for AQP2 was determined from the point where the 2 lines cross.
than control ICR mice when they were given water ad libitum (Fig. 3A). The AQP3 level was 40–45% lower in STR/N than in ICR male and female mice (Fig. 3D). We found a big difference in the kidney AQP2 mRNA level between polydipsic and normal ICR mice: the expression level for kidney AQP2 mRNA of polydipsic mice was 10% and 5% of that in control ICR for males and females, respectively (Fig. 3C). The differences in AQP2 mRNA and water intake between the two strains were greater than the difference in their body weight; the body weight of the polydipsic STR/N mice was only slightly less (~25%) than that of the normal ICR mice (Fig. 3B).

In the STR/N mouse, polydipsia does not appear initially but gradually develops from ~8 wk of age (12). Thus it is important to analyze the AQP2 mRNA expression level, along with the water intake, in infant STR/N mice.

**Developmental changes in AQP2 mRNA expression in the kidney of STR/N polydipsic and normal ICR mice.** Starting from 3 wk of age, the AQP2 mRNA expression and water intake were measured for STR/N and ICR mice (Figs. 4 and 5). From 3 to 44 wk of age, no significant changes were observed in the water intake by ICR mice. A slight increase in water intake was observed in STR/N mice at 10 wk (36% increase compared with 10-wk-old ICR, \( P < 0.02, n = 14 \)). At 10 wk of age, AQP2 expression had decreased to approximately one-fifth of that in 3-wk-old STR/N mice. The level further decreased at 44 wk and became about one-tenth of that in 3-wk-old STR/N mice. In ICR mice, although an apparent decrease in AQP2 expression was seen at 10 wk, the difference between 3 and 10 wk of age was not statistically significant (\( P = 0.197, n = 9 \)). Nor was the level between STR/N and ICR at 3 wk of age significantly different (\( P = 0.476, n = 7 \)).

We investigated the above data (those of STR/N mice) more precisely after normalizing them with the data for ICR mice (Fig. 5): each value obtained for STR/N mice was divided by the mean value obtained for ICR mice (Fig. 5). The water intake increased as indicated previously, and the body weight changed very little during development. The data in Fig. 5 clearly show that the AQP2 mRNA in 3-wk-old polydipsic STR/N mice was expressed at ~80% of the level of normal ICR mice and that this relative level decreased to 20% and 10% at 10 and 44 wk, respectively. These findings suggest that the AQP2 expression level in STR/N mice was normal at 3 wk of age but became decreased at 10 wk when water intake began to increase. In a separate experiment, we analyzed the sequence of AQP2 cDNAs prepared from kidney RNAs. No mutation was found in the sequence of AQP2 cDNAs from polydipsic STR/N mice (data not shown), indicating that the AQP2 structure in polydipsic mice is normal and, therefore, is expected to function normally.

**Expression levels of AQP2 protein in the kidney of STR/N polydipsic and ICR normal mice.** The expression of AQP2 protein was examined to confirm whether the difference in AQP2 mRNA is reflected in its protein expression level (Fig. 6). In the kidney membrane fraction from STR/N as well as ICR mice, glycosylated and nonglycosylated AQP2 bands were stained (Fig. 6, inset). These bands disappeared when the antiserum was preabsorbed with its immunogen peptide (10). Expression levels of AQP2 protein specifically recognizes the AQP2 protein. At 4 wk and 10 mo of age, the levels of glycosylated and nonglycosylated AQP2 were lower in the STR/N than the ICR mice. In the STR/N
strain, the level significantly decreased when the mice aged. These results concurred with the changes in mRNA during growth and between different strains shown in Fig. 4.

Effects of water restriction on AQP2 expression and osmolality of serum and urine in STR/N polydipsic and ICR normal mice. In an animal model of vasopressin escape, sustained administration of vasopressin and compulsive water supply result in progressive hypotonic polyuria (2, 18). In this model, hydration decreases the AQP2 expression level (2). Because the decrease of AQP2 in polydipsic STR/N mice was caused by increased water intake, we hypothesized that such a decrease could be recovered by restriction of the water supply. We therefore examined whether the decreased AQP2 level in the STR/N polydipsic mice is reversed by restriction of water supply (Fig. 7). By restriction of the water supply to a normal level (7 ml/mouse·day⁻¹ for 48 h), the AQP2 expression level increased (246 in polydipsic mice). Therefore, the angiotensin II receptor binding exists in the genes for AVP and its receptors in STR/N mice. Tribollet et al. (16) found that angiotensin II receptor binding was abundant in the brainstem region, particularly in the nucleus of the solitary tract in STR/N mice. They speculated that such an increased binding in the nucleus of the solitary tract may have indirectly stimulated AVP expression in the hypothalamic paraventricular nucleus. Indeed, injections of saralasin, an angiotensin II antagonist, as well as captopril, an angiotensin I-converting enzyme inhibitor, reduced the water intake in STR/N polydipsic mice but not in controls (9). Taken together, therefore, the angiotensin II-AVP system in the central nervous system appears to be altered in the STR/N mice, perhaps resulting in induction of thirst and increase in serum AVP levels, although the precise mechanism remains unknown.

On the other hand, if the kidney AVP receptor level is reduced in the STR/N mice, the AQP2 level in this tissue would also be expected to be reduced, even though their serum mRNA in the supraoptic and paraventricular nuclei in STR/N mice was increased at ≥20 wk of age, although it was low at 8 wk of age. Also Yambe et al. confirmed that no mutation exists in the genes for AVP and its receptors in STR/N mice. Tribollet et al. (16) found that angiotensin II receptor binding was abundant in the brainstem region, particularly in the nucleus of the solitary tract in STR/N mice. They speculated that such an increased binding in the nucleus of the solitary tract may have indirectly stimulated AVP expression in the hypothalamic paraventricular nucleus. Indeed, injections of saralasin, an angiotensin II antagonist, as well as captopril, an angiotensin I-converting enzyme inhibitor, reduced the water intake in STR/N polydipsic mice but not in controls (9). Taken together, therefore, the angiotensin II-AVP system in the central nervous system appears to be altered in the STR/N mice, perhaps resulting in induction of thirst and increase in serum AVP levels, although the precise mechanism remains unknown.

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<th>Osmolality, mosmol/kg H₂O</th>
<th>Serum</th>
<th>Urine</th>
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<tr>
<td>ICR Control</td>
<td>372±17.5</td>
<td>1,799±252</td>
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<tr>
<td>RWS</td>
<td>370±14.2</td>
<td>1,664±245†</td>
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<td>STR/N Control</td>
<td>398±15.0</td>
<td>264±15.7</td>
</tr>
<tr>
<td>RWS</td>
<td>385±11.1</td>
<td>868±148*</td>
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Values are means ± SE; n=4. RWS, restricted water supply. *Significantly different from ICR control group, P<0.05. †Not significantly different from ICR control. There was no statistical difference among values for serum osmolality.

DISCUSSION

The mechanisms by which polydipsia develops in the STR/N strain have been unclear. The primary cause of polydipsia is attributed to an abnormality in the central nervous system. There is an apparent contradiction in polydipsic STR/N mice: they drink a large amount of water, yet the serum AVP level is normal or even increased (2, 18). Yambe et al. (19) and Nishi et al. (12) reported that the synthesis of AVP
AVP remains at normal or higher levels. Tian et al. (15) reported that the downregulation of the kidney AVP receptor and AQP2 expression parallels age-associated defects in urine concentration. Also, Yambe et al. (19) reported renal susceptibility to AVP in 8-wk-old, but not 20-wk-old, STR/N mice. Thus the possible nonsusceptibility of the kidney to AVP and related cellular systems in relation to AQP2 expression needs to be examined.

In STR/N mice, although the serum AVP level would be expected to be decreased because of hyperhydration and subsequent water retention, the level of AVP mRNA in the hypothalamus was elevated (12) and the AVP concentration in urine was increased (unpublished observations), suggesting a high serum AVP level in these mice. Despite the excess intake of water, the blood osmolality is normal or even slightly higher in STR/N mice (6, 14) (Table 1), probably because of the strong polyuria. Such an increase may be another reason that the AVP mRNA level does not decrease in these mice. A physiological condition similar to that of polydipsic mice can be set up experimentally (2, 18). Ecelbarger et al. (2) reported that water loading under infusion of 1-deamino-[8-D-arginine]vasopressin decreased the expression of AQP2 in the rat kidney collecting duct cells while infusion of 1-deamino-[8-D-arginine]-vasopressin alone without hyperhydration did not change the expression level of AQP2. The decrease in AQP2 expression was accompanied by the increased urine excretion and the decreased serum osmolality, suggesting the existence of a vasopressin-independent mechanism in the regulation of AQP2 expression (2). In turn, decreased AQP2 expression in STR/N mice may be due to an intrinsic polydipsia. This is supported by an experimental observation that restriction of water supply increased the expression of AQP2 (Fig. 7). Although the mechanism by which hyperhydration causes the downregulation of AQP2 is not clear, the urine increase caused by AQP2 reduction would have resulted in imbalance of body fluid/electrolytes, leading to an increase in water intake. This would further decrease the level of AQP2 expression. The two experimental models (rats with compulsive water drinking under AVP infusion and STR/N polydipsic mice) are very similar, in that the AQP2 expression level was decreased, although their pathophysiology may be not completely the same; i.e., the kidney AQP3 level was high in the former model (2), whereas it was a bit low in the latter (Fig. 3D; although the AQP3 difference in female mice was not clear in Fig. 1D, QRT-PCR, a more reliable quantitative technique, revealed such a difference between STR/N and ICR among the female mice).

The results of the present study explain, for the first time, how serum osmolality in STR/N mice is kept close to normal levels. Expressions of the protein and mRNA for AQP2 were severely reduced in the kidney of polydipsic mice. The reduction in the AQP2 level was prominent at 10 wk of age, when water intake was increased only by 36%. It is uncertain whether this small increase was sufficient for the reduction in the kidney AQP2 mRNA level via a vasopressin-independent mechanism or by some putative humoral factor(s). If the mechanism discussed above (nonsusceptibility of the kidney to AVP) is involved in this reduction in AQP2 mRNA, it is speculated that expression of the AVP receptor is altered by high serum AVP and hyperhydration.

The present data imply that the reduction in AQP2 mRNA expression extensively increased urine excretion and, thus, reduced water retention. It is still probable, however, that the reduction or downregulation of the AQP2 mRNA level was caused by a putative humoral factor(s) that affects the transcription of this gene. There are a number of responsive elements in the promoter of the AQP2 gene, including a cAMP-responsive element and AP1 and AP2 sites (17). Thus two issues need to be pursued: 1) the possible existence of humoral a factor(s) that regulates AQP2 expression and 2) the downregulation of the AVP receptor in the kidney of STR/N polydipsic mice.

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