Aquaporin-2 downregulation in kidney medulla of aging rats is posttranscriptional and is abolished by water deprivation

S. Combet,1,2 S. Gouraud,1 R. Gobin,1 V. Berthonaud,1 G. Geelen,3 B. Corman,1,4 and J.-M. Verbavatz1
1CEA, Institut de Biologie et Technologies de Saclay and CNRS URA 2096, Gif-sur-Yvette and LRA17V University Paris-Sud 11, Orsay; 2Laboratoire Léon-Brillouin, UMR 12 CEA/CNRS, CEA-Saclay, Gif-sur-Yvette; 3Laboratoire de Physiologie, UFR de Médecine Grange Blanche, Université Claude-Bernard Lyon I, and Exploration Fonctionnelle Endocrinienne et Métabolique, CBN, Hôpital de la Croix Rousse, Lyon; and 4Successful Aging Database, Boulogne-Billancourt, France

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Aging kidney is associated in humans and rodents with polyuria and reduced urine concentrating ability. In senescent female WAG/Rij rats, this defect is independent of arginine-vasopressin (AVP)/V2 receptor/cAMP pathway. It has been attributed to underexpression and mistargeting of aquaporin-2 (AQP2) water channel in the inner medullary collecting duct (IMCD). We showed previously that dDAVP administration could partially correct this defect. Since AQP2 can also be regulated by AVP-independent pathways in water deprivation (WD), we investigated AQP2 and phosphorylated AQP2 (p-AQP2) regulation in thirsted adult (10 mo old) and senescent (30 mo old) female WAG/Rij rats. Following 2-day WD, urine flow rate decreased and urine osmolality increased in both groups. However, in agreement with significantly lower cortico-papillary osmotic gradient with aging, urine osmolality remained lower in senescent animals. WD induced sixfold increase of plasma AVP in all animals which, interestingly, did not result in higher papillary cAMP level. Following WD, AQP2 and p-AQP2 expression increased hugely in 10- and 30-mo-old rats and their mistargeting in old animals was corrected. Moreover, the age-related difference in AQP2 regulation was abolished after WD. To further investigate the mechanism of AQP2 underexpression with aging, AQP2 mRNA was quantified by real-time RT-PCR. In the outer medulla, preservation of AQP2 protein expression was achieved through increased AQP2 mRNA level in senescent rats. In the IMCD, no change in AQP2 mRNA was detected with aging but AQP2 protein expression was markedly lower in 30-mo-old animals. In conclusion, there is a posttranscriptional downregulation of AQP2 with aging, which is abolished by WD.

Aquaporins; urine concentration; AVP; cAMP; cGMP; phosphorylated AQP2

URINE CONCENTRATION IN KIDNEY requires water reabsorption mediated by aquaporin (AQP) water channels. In the collecting duct, this process is controlled by the antidiuretic hormone arginine-vasopressin (AVP) that binds to V2 receptors and induces cytosolic cyclic AMP (cAMP) accumulation, phosphorylation of AQP2 by protein kinase A (PKA) at Ser 256, and insertion of AQP2 into the apical plasma membrane of principal cells (27). Both water deprivation (WD) and AVP administration result in long-term upregulation of AQP2 expression in kidney collecting duct (44).

In humans and rodents, urine concentrating ability declines with age, resulting in polyuria and lower urine osmolality (14, 27, 30). In some strains of rats, this defect has been attributed to loss of nephrons or impaired AVP secretion (45, 50). In contrast, senescent female WAG/Rij rats exhibit impaired urinary concentration with no kidney disease, no decrease in AVP plasma level, no defect in V2 receptor mRNA expression, and no change in the medullary cAMP content (17, 25, 35). In this strain, reduced kidney concentrating capacity with aging has been attributed to 1) underexpression of AQP2 and impaired targeting of both AQP2 and phosphorylated AQP2 (p-AQP2) within the cytoplasm of principal cells (11, 12, 46), resulting in decreased water reabsorption in the inner medulla; and 2) lower cortico-papillary osmotic gradient resulting from papillary hypotonicity due, at least in part, to low urea content and underexpression of urea transporters in the inner medulla (11, 12, 47).

We previously suggested that, despite proper AVP signaling, the threshold for AQP2 upregulation by AVP is higher in senescent rats than in adults. Indeed, administration of the V2 receptor agonist dDAVP to old animals is sufficient to bring AQP2 expression back to control values (12). Furthermore, long-term food restriction is also associated with a higher plasma AVP level, which prevents the polyuria of senescent rats (11). Nevertheless, dDAVP stimulation does not completely correct urine concentration in senescent animals, suggesting that molecular mechanisms other than the AVP signaling pathway might be involved in the age-related concentrating defect.

Since WD can induce regulation pathways other than the AVP-dependent signaling cascade (28), we examined the effect of a 2-day WD, in young and old female WAG/Rij rats, on AQP2 and p-AQP2 expression and trafficking in the papilla, in relation with water balance, urine and papillary osmolalities, plasma AVP concentration, as well as cAMP and cGMP papillary contents.

Furthermore, we investigated by real-time RT-PCR the downregulation of AQP2 mRNA in aging. Our results demonstrate that AQP2 downregulation in aging is posttranscriptional but that senescent rats remain fully sensitive to AQP2 and p-AQP2 upregulation by WD.
MATERIALS AND METHODS

Animals. Female WAG/Rij rats were born, raised, and maintained in the animal care facility of CEA/Saclay (Gif-sur-Yvette, France) in a 12:12-h light-dark cycle, at 50% humidity and 21°C. The suitability of the WAG/Rij rats for the study of kidney aging is related to the absence of chronic progressive nephropathy or pituitary tumors with age (2, 13). The present experiments were performed in adult (10 mo old) and senescent (30 mo old) rats. Protocols were approved by the ethical committee on animal research of Paris-Sud.

WD. Rats were habituated in metabolic cages for 2 days, followed by 2 days of control daily food intake determination and urinary collection under mineral oil to minimize evaporation. Urine osmolality was determined with a Roebling Automatik osmometer. Drinking water was then removed for 2 days from all rats.

Papillary osmolality and urea concentration. Following the 2-day WD protocol, animals were decapitated and kidneys were rapidly removed. Total white papilla was excised on ice from each kidney, weighed, and thoroughly homogenized with a glass potter after addition of 600 μl of distilled water. The average homogenate osmolality was measured with a Roebling Automatik osmometer and urea concentration was measured using a specific kit (BioMérieux, Lyon, France). Average papilla osmolality and urea concentration were calculated assuming that 80% of papilla weight is water (1).

Plasma AVP concentration and osmolality. Animals were killed by decapitation at the end of the 2-day WD period and trunk blood was rapidly collected and centrifuged at 4°C. Plasma AVP concentration was measured by RIA with antiserum K9-IV (24) (gift of Dr. L. C. Keil, NASA Ames Research Center, Moffett Field, CA) and 125I-labeled iodo-AVP (17, 24). The minimum sensitivity of the assay was 0.25 pg/assay. For each animal, determination of plasma AVP concentration was performed in triplicate with independent standard curves. Plasma osmolality was measured on the same samples with a Fiske One-Ten osmometer.

Papilla cAMP and cGMP contents. Animals were killed by decapitation and the kidneys were quickly excised and chilled in ice-cold PBS. The white papilla was excised from both kidneys of each rat, pooled, weighed, and homogenized manually with a glass potter in 600 μl of a 5% formic acid-ethanol solution. The samples were evaporated overnight and resuspended in an appropriate volume of enzyme immunoassay buffer. The cAMP and cGMP contents of the samples were determined in triplicate by enzyme immunoassay (Cayman Chemical) according to Pradelles et al. (34). The results were expressed as picomoles per gram of wet papilla. To check the effectiveness of the method, papillary cAMP and cGMP contents were measured in normal adult rats intraperitoneally injected with a single dose of 10 μg dDAVP and killed 30 min later.

Western blot analysis. The whole inner medulla from each kidney was dissected on ice and was homogenized manually with a glass potter in 500 μl of PBS containing protease inhibitors (Complete Mini EDTA-free, Roche). Homogenate protein concentration was determined by the Bradford method (Bio-Rad). Samples were then solubilized in Laemmli buffer and heated at 65°C for 10 min. Equal protein amounts (5 μg/lane) were separated by 12% SDS-PAGE and transferred to PVDF membranes. Blots were blocked with PBS supplemented with 5% nonfat dry milk and then incubated with the primary antibody (1 h at room temperature). AQP2 was detected with an affinity-purified polyclonal antibody raised against the COOH terminus of peptide sequence of rat AQP2 (11, 12, 35), p-AQP2 was revealed with a specific antibody directed against AQP2 phosphorylated at the PKA site, Ser 256, and previously characterized (8, 11) (gift of Dr. S. Nielsen). Finally, the blots were incubated for 45 min with a peroxidase-conjugated goat anti-rabbit antibodies (Promega), and then washed 3 × 10 min, and mounted in 50% glycerol solution containing 2% n-propyl-gallate before observation under a fluorescence microscope (Olympus van OX AH-2). Experiments were carried out in parallel and micrographs were taken the same day for all conditions. Image contrast was enhanced in each condition to facilitate the identification of subcellular protein localization. Thus, staining intensities are not quantitative.

AQP2 mRNA preparation and real-time RT-PCR. Rats were decapitated and each kidney was rapidly removed and dissected on ice. The inner stripe of the outer medulla and the white papilla were excised from each kidney, minced, and fast-frozen in the liquid nitrogen. Total mRNA was isolated with TRIZol (Invitrogen), with subsequent removal of residual contaminants by use of the RNeasy Total RNA Isolation Kit (Qiagen). Primers for AQP2 and GADPH were designed as published by Roxas et al. (39). Synthesis of cDNA from total RNA was carried out in a 12-μl reaction volume containing 1 μg total mRNA, 0.8 mM dNTPs, and 0.17 μM antisense AQP2 and GAPDH primers, followed by incubation at 65°C for 5 min. The reaction was cycled for 5 min and 4 μl of a 5× first-strand buffer (Invitrogen) and 2 μl of 0.1 M DTT mixture were added. After incubating the reaction at 42°C for 2 min, 1 μl of Superscript II Reverse Transcriptase (Invitrogen) was added and the mixture was incubated for 50 min at 42°C. Finally, the reaction was stopped by heating the samples to 70°C for 15 min and putting them in ice, before a treatment with 1 μl RNase H (20 min at 37°C). The RT-PCRs were carried out in 96-well plates in a 50-μl reaction volume containing 25 μl of IQ SYBR Green Supermix (Bio-Rad), 10 ng cDNA (10 μl of 1/100 diluted samples), and 0.2 μM each forward and reverse primers. The thermocycle was 94°C for 1 min for denaturation, followed by 40 cycles of amplification (94°C for 10 s and 60°C for 1 min). Each sample was treated at least three times to ensure the reproducibility of the results. Data acquisition and analyses were performed using Bio-Rad software. Expression was normalized to GAPDH as an endogenous reference, assuming that GAPDH expression is not modified in aging kidney. For both outer and inner medullas, results obtained in left and right kidneys were pooled for each rat.

Statistics. Results were expressed as means ± SE. Differences between control and thirsted rats at both ages were analyzed by one-way ANOVA. Differences were considered significant for P < 0.05.

RESULTS

Water balance and urine osmolality. The age-related polyuria was confirmed in senescent 30-mo-old female WAG-Rij rats, which exhibited significantly higher urine flow rate and reduced urinary osmolality, compared with younger animals (Table 1). The protocol of WD caused a dramatic fall of urinary flow rate and a doubling of urine osmolality in both age groups (Table 1). Consistently with a lower average papilla osmolality, urine concentration remained significantly lower in senescent than in adult rats following WD. Urea concentration plays a significant role in the papillary osmolality defect with
aging since it is markedly lower in senescent animals (Table 1) (47). Average papillary urea concentration was increased by WD but remained also significantly lower in water-deprived senescent rats compared with adult ones. However, the fraction of urea to papillary osmolality, which was significantly lower with aging (25 and 17% in 10- and 30-mo-old rats, respectively), increased to comparable levels after WD in both age groups (34 and 31% in 10- and 30-mo-old rats, respectively). Statistical analysis revealed that the final urine concentration in all the animals was best correlated to the average urea concentration in the papilla rather than to average papillary osmolality or to papillary urea fraction.

**AVP and plasma osmolality concentration.** As expected, aging had no effect on plasma AVP level in the basal state (Table 2). We found that WD induced a sixfold increase in plasma AVP in both groups (Table 2). In thirsted 30-mo-old rats, plasma osmolality was significantly increased (Table 1), demonstrating that WD resulted in dehydration in senescent animals.

**Papilla cAMP and cGMP contents.** The cAMP response of adult rats injected 30 min before death with dDAVP, a specific agonist of V₂ receptor, was tested by enzyme immunoassay. As expected, a significant 60% increase in papillary cAMP was measured in dDAVP-injected animals (254 ± 64 vs. 326 ± 32 pmol/g wet tissue in un.injected animals, P < 0.002). As shown in Table 2 and previously (11, 35), aging did not modify cAMP content level in the inner medulla. Surprisingly, despite a marked increase of plasma AVP, papillary cAMP was not significantly higher in water-deprived animals (Table 2). This suggests that response to AVP results in a transitory intracellular cAMP increase but not in significant long-term cAMP stimulation. In addition, cGMP content level was identical in 10- and 30-mo-old rats in control condition and was not significantly different following WD (Table 2) or acute dDAVP injection (data not shown).

**AQP2 and p-AQP2** protein expression. Figure 1 shows AQP2 and p-AQP2 protein expression in the inner medulla by Western blotting. Wells were loaded with equal amounts of protein and no significant difference was seen between samples on Coomassie-stained blots. Following WD, both AQP2 and p-AQP2 expression hugely increased in the inner medulla of adult (Fig. 1A) and senescent rats (Fig. 1B) and finally resulted in comparable, elevated levels of AQP2 and p-AQP2 in 10- and 30-mo-old rats (Fig. 1C). Semiquantitative analysis of the blots demonstrated that WD resulted in dramatic and highly significant increase in AQP2 (Fig. 1D) and p-AQP2 (Fig. 1E) expression in both age groups. In baseline condition, AQP2 expression level was significantly different between 10- and 30-mo-old rats. Following WD, AQP2 protein level was no longer significantly different with aging (Fig. 1D). The regulation of p-AQP2 exhibited exactly the same behavior as that of AQP2 (Fig. 1E).

**AQP2 subcellular localization.** AQP2 localization in inner medullary collecting duct (IMCD) cells was performed by indirect immunofluorescence. In control adults, AQP2 showed punctuate intracellular staining throughout IMCD cells (Fig. 2a), including the basolateral side (arrowhead). Following WD, most staining for AQP2 was on the apical side of IMCD cells (Fig. 2b). In senescent rats, AQP2 staining was intracellular (Fig. 2c) and variable between tubules: in some tubules, AQP2 was hardly detectable (Fig. 2c, *). After WD, AQP2 in senescent rats was evenly distributed among IMCD cells and tubules and was markedly redistributed along the apical membrane (Fig. 2d).

**p-AQP2 subcellular localization.** Staining for AQP2 phosphorylated at Ser 256 (p-AQP2), selectively recognized by a specific antibody, exhibited an apical localization in adult rats (Fig. 3a). Following WD, p-AQP2 remained localized in the apical region of cells (Fig. 3b). In senescent animals, p-AQP2 staining was weaker and heterogenous (Fig. 3c). In this age group, p-AQP2 exhibited punctuate staining in the apical region of IMCD cells (Fig. 3c, inset). After WD, p-AQP2 in 30-mo-old rats increased and showed a continuous staining along the apical region of the cells, like in 10-mo-old animals (Fig. 3d).

### Table 1. Body weight, daily food and water intakes, urinary flow rate, as well as urine, papilla, and plasma osmolalities and papillary urea concentration in 10- and 30-mo-old female WAG/Rij rats, control or after 2-day WD

<table>
<thead>
<tr>
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<th>Control</th>
<th>WD</th>
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<tbody>
<tr>
<td>Body Weight, g (n = 15)</td>
<td>195±4</td>
<td>220±5*</td>
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<tr>
<td>Food Intake, g/24 h (n = 15)</td>
<td>11.1±0.4</td>
<td>12.2±0.5</td>
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<tr>
<td>Water Intake, ml/24 h (n = 15)</td>
<td>14.8±0.8</td>
<td>24±1*</td>
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<tr>
<td>Urine Flow Rate, ml/24 h (n = 15)</td>
<td>5.7±0.5</td>
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<tr>
<td>Urine Osmolality, mosmol/kg H₂O (n = 15)</td>
<td>1,870±78</td>
<td>1,120±136*</td>
</tr>
<tr>
<td>Average Papilla Osmolality, mosmol/kg H₂O (n = 6)</td>
<td>1,084±37*</td>
<td>1,084±37*</td>
</tr>
<tr>
<td>Average Papillary Urea Concentration, mmol/l (n = 6)</td>
<td>257±22</td>
<td>257±22</td>
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<tr>
<td>Plasma Osmolality, mosmol/kg H₂O (n = 6)</td>
<td>283±3</td>
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Values are means ± SE; n, number of animals. *P < 0.05 vs. 10-mo-old rats in the same group–control or after water deprivation (WD). †P < 0.05 vs. control rats at the same age.

### Table 2. AVP plasma concentration and cAMP and cGMP papillary contents in 10- and 30-mo-old WAG/Rij rats, control or after 2-day WD

<table>
<thead>
<tr>
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<th>Control</th>
<th>WD</th>
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<tr>
<td>Plasma AVP Concentration, pg/ml (n = 6)</td>
<td>1.4±0.2</td>
<td>1.7±0.2</td>
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<tr>
<td>Papillary cAMP Content, pmol/g papilla (n = 6)</td>
<td>291±70</td>
<td>288±24</td>
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<tr>
<td>Papillary cGMP Content, pmol/g papilla (n = 6)</td>
<td>7.2±0.9</td>
<td>9.0±2.3</td>
</tr>
<tr>
<td>Average Papilla Osmolality, mosmol/kg H₂O (n = 6)</td>
<td>10.9±0.6*</td>
<td>10.9±1*</td>
</tr>
<tr>
<td>Average Papillary Urea Concentration, mmol/l (n = 6)</td>
<td>317±24</td>
<td>355±36</td>
</tr>
<tr>
<td>Plasma Osmolality, mosmol/kg H₂O (n = 6)</td>
<td>5.7±0.7</td>
<td>5.1±0.3</td>
</tr>
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</table>

Values are means ± SE; n, number of animals. *P < 0.05 vs. control rats at the same age. Differences were not statistically significant vs. 10-mo-old rats in the same group (control or after WD), as well as for cAMP and cGMP papillary contents in all conditions.
Altogether, these data show that, in response to WD, AQP2 and p-AQP2 proteins were upregulated and suggest that AQP2 was translocated to the plasma membrane of IMCD cells in water-deprived 10- and 30-mo-old rats. Finally, a 2-day WD resulted in a complete correction of expression and localization of both AQP2 and p-AQP2 in senescent animals.

**AQP2 mRNA expression.** To investigate the molecular mechanism of AQP2 protein downregulation with aging in the IMCD, AQP2 mRNA was quantified by real-time RT-PCR in adult and senescent animals. As shown in Fig. 4, the AQP2 transcript did not significantly decrease with aging in kidney inner medulla (ratio of mRNA content in 30- over 10-mo-old rats = 0.87, P > 0.05). Interestingly, AQP2 mRNA was significantly increased in the outer medulla of old rats (ratio = 1.41, P = 0.04), where AQP2 is less downregulated by aging than in the IMCD (35). Thus, the impaired AQP2 protein expression of senescent rats appears as a posttranscriptional defect, which is not restricted to the inner medulla, but which is compensated for by higher AQP2 mRNA levels in the outer medulla.

**DISCUSSION**

The reduced concentrating ability of aging kidney in rats has been associated with underexpression and mistrafficking of AQP2 in IMCD principal cells (11, 35, 46). In senescent female WAG/Rij rats, this defect is independent of AVP secretion (17, 25, 35) and the AVP signaling pathway (12, 35). Instead, previous studies suggested that the threshold for AQP2 regulation by AVP is increased with aging (11, 12). Since AQP2 expression and localization can also be regulated by AVP-independent pathways, as observed in dehydrated rats supplemented with V2-receptor antagonist (28), we investigated the responsiveness of old female WAG/Rij rats to WD.

A 2-day WD elicited a twofold concentration of urine osmolality and a marked fall in urine output in 10- and 30-mo-old animals. However, after WD, senescent female WAG/Rij rats exhibited a higher urine flow rate and a reduced urinary osmolality than adults. Consequently, WD resulted in dehydration in old rats, as shown by their higher plasma osmolalities. This is consistent with the impaired response to
WD with aging reported in Fischer 344 rats (3) and humans (38). Nevertheless, WD induced a dramatic and highly significant upregulation of the expression of both AQP2 and its phosphorylated form (p-AQP2) in 10- and 30-mo-old rats. AQP2 upregulation by WD is in agreement with previous studies (29, 31, 46) but was not seen in old male F344BNF1 rats, which did not increase their urine osmolality either (7, 43). Strain differences in the aging process and related diseases, such as pituitary tumors or chronic progressive nephropathy, with an impaired AVP secretion, probably explain this difference.

Our data show that trafficking of AQP2 was corrected by WD in 30-mo-old animals. At the baseline condition, AQP2 was distributed throughout inner medullary collecting duct (IMCD) cells, including the basolateral side (arrowhead). In senescent rats, AQP2 staining was intracellular and variable between tubules: in some tubules, AQP2 staining was extremely low (c, *). After WD, AQP2 was markedly redistributed along the apical membrane of IMCD cells in both 10- and 30-mo-old animals (b and d, respectively). Bar = 20 μm.

Along with AQP2 upregulation, WD induced a sixfold increase of plasma AVP in 10- and 30-mo-old female WAG/Rij rats, confirming that the AVP secretion capacity remains intact with aging in these animals in response to WD (17). Surprisingly, papillary cAMP was not increased by WD, whereas acute dDAVP injection showed a significant cAMP increase. Contradictory results have been reported for cAMP...
After WD. In dehydrated Wistar rats, no change in papillary cAMP content was observed by Christensen (9), whereas an 80% increase in papillary cAMP has been reported following WD in Sprague-Dawley rats (4). Our results suggest that inner medullary cAMP accumulation is a transitory response to AVP, which is not sustained in WD, despite elevated AVP. As previously reported (12), chronic dDAVP administration did not elicit the dramatic AQP2 upregulation observed after WD. Indeed, this suggests that mechanisms independent of the AQP-cAMP pathway may be involved. A cGMP-dependent regulation pathway has been reported in AQP2 phosphorylation and trafficking (5). However, this process is not involved here since cGMP remained constant in all conditions. Interstitial toxicity has also been suggested to regulate AQP2, through transcription activation (16, 21, 42). Indeed, Umenishi et al. (48) observed that hyperosmolality enhances the apical insertion of nonglycosylated AQP2 via a cAMP- and PKA-independent regulation pathway. In our studies, low AQP2 expression in senescent animals is associated to a reduced papillary osmolality. WD induced a tremendous increase in AQP2 protein expression in both age groups, despite lower papillary osmolality in old rats compared with adults. However, urea contribution to papillary osmolality was increased by WD to comparable levels between adult and senescent rats (34 and 31%, respectively). These observations suggest that urea fraction in papillary osmolality, but not total osmolality, may be involved in AQP2 upregulation during WD.

To investigate further the mechanism of AQP2 misregulation with aging, we quantified, by real-time RT-PCR, changes in AQP2 mRNA levels. In agreement with another study, made by microarray and RT-PCR but in the whole kidney (36), AQP2 mRNA was not significantly different between young and senescent rats in the IMCD. However, in the outer medulla, where AQP2 protein expression is only slightly downregulated with aging (35), AQP2 mRNA was significantly higher in senescent animals than in controls. These results suggest either a defect in translation or in the stability of the AQP2 protein with aging in the kidney medulla of WAG/Rij rats. Uncoupling between AQP2 mRNA and protein is unusual: in most cases, the AQP2 protein is directly modulated by AQP2 mRNA levels. To our knowledge, only one other study reports that polyuria in hypercalcemic rats is accompanied in the IMCD by a decreased expression of AQP2 protein, but not mRNA (40). Such a dissociation between transcript and protein levels has already been reported for other proteins (19, 23). In the context of aging, one study reported recently a differential expression of the fragile X retardation protein (FMRP) and its transcript in mice brain during aging (41). In human kidney, Eikmans et al. (15) found increased mRNA level with aging for collagen IV, whereas the protein expression was unchanged. Our results and these examples underscore the importance of proteomic approaches (18, 26, 33) in addition to transcriptomic screening for kidney physiology studies.

Thus, our results show that the molecular origin of the age-associated defect in AQP2 regulation is posttranscriptional. The possible role of mRNA translational rate and/or protein degradation in the regulation of protein expression has been discussed recently (6, 32). Coller and Parker (10) mentioned a general and unknown process by which the vast majority of mRNA can enter a translationally inactive state. Another study suggested a competition between the assembly of the translational machinery and the assembly of the general repression or decay machinery, including “mRNA decapping,” which would promote repression and degradation into the mRNA-processing bodies, the so-called “P bodies” (32). The regulation of degradation pathways is still poorly understood for the AQP2 protein. In immortalized mouse collecting duct principal cells (mpkCCDc14), Hasler et al. (20) reported the involvement of both proteasome and lysosomes in AQP2 degradation. More recently, they confirmed that a posttranscriptional regulation of AQP2 protein may be mediated by AQP2 protein degradation, rather than altered protein synthesis (22). AQP2 may also be a substrate for calpain-mediated proteolysis (37), as suggested in hypercalcemic rats (40), for which the decreased AQP2 protein expression could be mediated via a possible calcium-activated calpain protease activity (49). Finally, a putative role of urea, but not NaCl, on AQP2 protein stability has been suggested (48). Accordingly, in the present study, AQP2 protein expression is correlated with the urea fraction in total papillary osmolality.

In conclusion, we show that senescent female WAG/Rij rats were able to increase their urinary concentrating ability in response to WD. However, after WD, urinary osmolality remains significantly lower with aging, in correlation with lower urea concentration in the papilla. Expression of AQP2 and p-AQP2, which is remarkably low in old animals, was hugely increased by WD. This resulted in similar AQP2 and p-AQP2 expression levels between age groups after WD. AQP2 mistargeting in senescent rats was corrected by WD, through improved phosphorylation of the protein, as shown by the apical localization of p-AQP2. In addition, we report that AQP2 protein downregulation with aging does not result from decreased AQP2 mRNA. In the outer medulla, AQP2 transcripts actually increase in senescent animals. These observations demonstrate that the downregulation of AQP2 in aging is posttranscriptional. Nevertheless, a physiological osmotic challenge, such as WD, can restore proper AQP2 expression and intracellular regulation.

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Present address of S. Gouraud: Dept. of Physiology, Wakayama Medical University, School of Medicine, 811-1 Kimiidera, Wakayama City 641-8509, Japan.

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