Correction of age-related polyuria by dDAVP: molecular analysis of aquaporins and urea transporters

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Combet, Sophie, Nancy Geffroy, Véronique Berthonaud, Bernhard Dick, Laurent Teillet, Jean-Marc Verbautz, Bruno Corman, and Marie-Marcelle Trinh-Trang-Tan. Correction of age-related polyuria by DDAVP: molecular analysis of aquaporins and urea transporters. Am J Physiol Renal Physiol 284: F199–F208, 2003. First published August 13, 2002; 10.1152/ajprenal.00167.2002.—Senescent female WAG/Rij rats exhibit polyuria without obvious renal disease or defects in vasopressin plasma level or V2 receptor mRNA expression. Normalization of urine flow rate by 1-desamino-8-D-arginine vasopressin (dDAVP) was investigated in these animals. Long-term dDAVP infusion into 30-mo-old rats reduced urine flow rate and increased urinary osmolality to levels comparable to those in control 10-mo-old rats. The maximal urinary osmolality in aging rat kidney was, however, lower than that in adult kidney, despite supramaximal administration of dDAVP. This improvement involved increased inner medullary osmolality and urea sequestration. This may result from upregulation of UT-A1, the vasopressin-regulated urea transporter, in initial inner medullary collecting duct (IMCD), but not in terminal IMCD, where UT-A1 remained low. Expression of UT-A2, which contributes to medullary urea recycling, was greatly increased. Regulation of IMCD aquaporin (AQP)-2 (AQP2) expression by dDAVP differed between adult and senescent rats: the low AQP2 abundance in senescent rats was normalized by dDAVP infusion, which also improved targeting of the channel; in adult rats, AQP2 expression was unaltered, suggesting that IMCD AQP2 expression is not regulated by dDAVP directly. Increased AQP3 expression in senescent rats may also be involved in improved urine-concentrating capacity owing to higher basolateral water and urea reabsorption capacity.

papilla osmolality; nitric oxide synthase; glucocorticoids; 1-desamino-8-D-arginine vasopressin

AGING IS COMMONLY ASSOCIATED with polyuria, corresponding to lower urinary osmolality in humans and rodents (4, 5, 13, 26). In some strains of rats, this has been related to a defect in vasopressin (AVP) secretion and/or severe renal diseases (48). However, in other strains, such as WAG/Rij rats, AVP plasma level and V2 receptor mRNA, the number of nephrons, and single glomerular filtration rates remain constant in the course of aging, suggesting altered renal responsiveness to AVP with age (10, 17, 32, 40). This could result from altered V2 receptor function, as suggested by a 30% decrease in AVP-binding capacity of papillary membrane suspensions (32). Yet, cAMP accumulation in senescent rats was unaltered by this decrease (32), which, rather, suggests that the defect occurs downstream of cAMP synthesis.

Previous experiments have shown that aquaporin-2 and -3 (AQP2 and AQP3) abundance in the inner medulla (IM) was reduced by 80% and 50%, respectively, in senescent (30-mo-old) female WAG/Rij rats compared with adult (10-mo-old) animals (8, 32). Moreover, AQP2 was not located in apical plasma membranes but was diffusely distributed within the intracellular compartment of collecting duct cells. In addition, the osmolality of the inner medulla was reduced, resulting in an impaired corticopapillary gradient. This defect could involve sodium and urea accumulation. A reduced AVP-dependent reabsorption of sodium by the thick ascending limb of Henle’s loop has been reported in senescent mice (12). Defective sequestration of urea in the medulla in senescent rats is a consequence of abated expression of UT-A1, the AVP-regulated urea transporter in medullary collecting ducts, and UT-B, the urea transporter in descending vasa recta irrigating the IM, as recently reported (8). UT-A2, the urea transporter of descending thin limbs, was not altered with age (8).

A reduction in age-related polyuria by administration of exogenous AVP has been reported in 16- to 18-mo-old Wistar/Tw rats (24). In humans with polyuria, administration of 1-desamino-8-D-arginine vaso-
pressin (dDAVP), a specific V2 receptor agonist, has been proposed as a treatment (14, 26, 27). In the present study, we tried to elucidate the mechanisms underlying the response of aging kidney to dDAVP with respect to polyuria, urine osmolality, intrarenal corticopapillary gradient, aquaporins, and urea transporter expression. The time course of urine output and osmolality was measured in adult and senescent female WAG/Rij rats treated for 6 days with dDAVP. Papillary osmolality, urea content, and expression of UT-A1, UT-A2, UT-B, AQP2, and AQP3 were measured by Western blotting. Because AQP2 targeting to plasma membranes is regulated by the AVP-mediated cAMP pathway (18), AQP2 localization was examined by immunofluorescence. Another pathway for AQP2 membrane targeting, involving nitric oxide (NO)-stimulated cGMP, has been recently reported (7). Therefore, medullary contents of NO synthase (NOS) isoforms were determined. Finally, the plasma levels of corticosterone and 11-dehydrocorticosterone were measured, inasmuch as glucocorticoids might regulate expression of AQP2 and UT-A1 (29, 35).

MATERIALS AND METHODS

Animals and DDAVP administration. Female WAG/Rij rats, born and raised in the animal care facility of the Commissariat à l’Energie Atomique, Saclay, were used in this study. The suitability of this strain for aging kidney studies has been demonstrated previously (4). Briefly, in these rats, there is a low incidence of pituitary tumor, no glomerulosclerosis or loss of nephrons, and constant blood pressure and mortality, and 21°C. They were fed ad libitum a commercial growing diet (DO4, Usine d’Alimentation Rationnelle, Villemoisson, France). The present experiments were performed in adult (10-mo-old) and senescent (30-mo-old) animals.

dDAVP, a synthetic V2 receptor-specific agonist, was administered using osmotic minipumps (Alzet, Palo Alto, CA) inserted intraperitoneally during transient anesthesia by halothane. The first series of rats received the hormone at 200 ng/day for 6 days. The second series of rats was treated with dDAVP at 1,000 ng/day to ascertain that the lower dose was supramaximal in this rat strain at 10 and 30 mo of age. Saline-containing minipumps were implanted into control rats.

Metabolic cage collections. All rats were placed in metabolic cages for a 2-day habituation period. Thereafter, water and food intake and urinary volume were measured daily during the 48-h pretreatment period and the following 6 days of dDAVP administration. At the end of the experiment, the animals were decapitated and blood was immediately collected. Urine was collected under mineral oil to avoid evaporation, and urine osmolality was determined with a Roebling Automatik osmometer.

Measurement of papillary osmolality and urea content. After the animals were decapitated, kidneys were rapidly removed and refrigerated on ice. The white papilla was excised from each kidney, weighed, and thoroughly homogenized with glass potter after addition of 600 μl of distilled water. Homogenate osmolality was measured according to the freezing-point procedure using a Roebling Automatik osmometer, and urea concentration was measured using a specific kit (BioMérieux, Lyon, France). Papilla osmolality and urea content were calculated with the assumption that 80% of papilla wet weight is water (1).

Antibodies. AQP2 and AQP3 were detected with polyclonal antibodies raised against the carboxy terminus of the peptide sequence of rat AQP2 and rat AQP3 (3, 34). Three NOS isoforms were detected using mouse monoclonal antibodies raised against human endothelial and neuronal isoforms (eNOS and nNOS, respectively) and mouse inducible isofrom (iNOS; Transduction Laboratories, Lexington, KY). Specificity of these three antibodies against their respective NOS isoforms has been demonstrated previously using positive controls (9). UT-A1 and UT-A2 were revealed with a purified rabbit polyclonal antibody raised against the carboxy-terminal peptide sequence common to rat UT-A1 and UT-A2 (Alpha Diagnostics International, San Antonio, TX). UT-B was detected by a purified rabbit polyclonal antibody against the carboxy terminus of rat UT-B. This antibody has been characterized previously (44).

Western blot analysis. For AQP and NOS expression studies, the whole IM was dissected on ice from each kidney and homogenized manually with a glass potter in 500 μl of PBS containing protease inhibitors (Complete EDTA-free mini protease inhibitor cocktail tablets, Roche Diagnostics, Mannheim, Germany). Because urea transporters are not homogeneously expressed in the kidney inner stripe (IS) of the outer medulla and in the IM (31, 38), the IS was divided into upper and lower halves and the IM was divided into base (upper third) and tip (two lower thirds). These tissue fragments were thoroughly homogenized (Ultra-Turrax) in 0.5 ml of ice-cold lysis buffer (250 mmol/l sucrose and 10 mmol/l trisethanolamine, pH 7.6) containing protease inhibitors. Homogenate protein concentration was determined by the Bradford method (Bio-Rad, Hercules, CA). Samples were then solubilized in Laemmli buffer and heated at 65°C for 10 min for aquaporins and urea transporters and at 90°C for 2 min for NOS. Equal protein amounts (5, 10, and 15 μg/lane for aquaporins, NOS, and urea transporters, respectively) were separated by SDS-PAGE (12% for aquaporins, 7.5% for NOS, and 10% for urea transporters), and immunoblotting was performed as previously described (8). Polyvinylidene difluoride membranes were blocked for 30 min at room temperature with PBS supplemented with 5% nonfat dry milk and then incubated with the primary antibody for 1 h at room temperature. The membranes were then washed and incubated for 45 min with a peroxidase-conjugated anti-rabbit IgG polyclonal antibody for aquaporins and urea transporters or with a peroxidase-conjugated anti-mouse IgG monoclonal antibody for NOS before visualization on Hyperfilm-ECL (Amersham) by chemiluminescence (ECL Plus, NEN, Boston, MA). Equal protein loading was verified by Coomassie blue staining of the membranes at the end of the experiment. After the films were scanned, semiquantitative densitometry was performed using Molecular Analyst software (Bio-Rad).

Immunofluorescence. For indirect immunofluorescence, animals were anesthetized and kidneys were fixed by perfusion at 120 mmHg pressure through the abdominal aorta with a solution of PBS-4% paraformaldehyde at 37°C. Kidneys were sliced, postfixed overnight in PBS-4% paraformaldehyde, and washed extensively with PBS. Fixed kidney slices were infiltrated overnight in 30% sucrose and frozen in liquid nitrogen, and 5-μm sections were collected on glass slides (SuperFrost Plus). Sections were preincubated in PBS containing 1% BSA (PBS-BSA) for 5 min, incubated for 1 h at room temperature in PBS-BSA containing affinity-purified antibodies against AQP2 (1:20), and washed three times for 10 min each in PBS. Sections were then incubated for 45 min.
at room temperature in FITC-conjugated goat anti-rabbit antibodies (Promega; 10 μg/ml), washed three times for 10 min each, and mounted in 50% glycerol solution containing 2% n-propyl-gallate before observation under a fluorescence microscope (Van OX, Olympus).

**Measurement of plasma corticosterone and 11-dehydrocorticosterone concentration.** Five 10-mo-old and five 30-mo-old rats were decapitated, and blood was immediately collected in heparinized tubes. After 100 ng of 11α-tetrahydrocortisol were added to 1 ml of plasma as an internal standard, the sample was extracted with 10 ml of dichloromethane on a rotator. After centrifugation at 3,000 g for 5 min and separation of the phases, the organic layer (containing the unconjugated steroids) was evaporated under a stream of nitrogen at room temperature. The sample was derivatized to form the methyloxime-trimethylsilyl ethers. The excess of derivatization reagent was removed by gel filtration on a Lipidex-5000 column. As an internal standard, 11α-tetrahydrocortisol (100 ng) was added to the water phase (containing the conjugated steroids). Plasma proteins were precipitated with 5 ml of methanol. After evaporation of the solvent, the sample was reconstituted in 0.1 M acetate buffer and hydrolyzed with 12.5 μl of powdered Helix pomatia (Sigma) and 12.5 μl of β-glucuronidase-arylsulfatase (Roche) at 55°C for 3 h. The hydrolysis mixture was extracted with a Sep-Pak C18 column, and the resulting free steroids were derivatized to form the methyloxime-trimethylsilyl ethers. The excess of derivatization reagent was removed by gel filtration on a Lipidex-5000 column. Samples were analyzed by gas chromatography-mass spectrometry using a gas chromatograph (model 6890, Hewlett-Packard) equipped with a mass-selective detector (model 5973, Hewlett-Packard) by selective ion monitoring. One characteristic ion was chosen for each compound being measured. Masses 548 and 474 were chosen for corticosterone and 11-dehydrocorticosterone, respectively. Calibration lines were established over the range 5–250 ng/ml. Correlation coefficients were >0.98. Coefficients of variation for intra- and interday analysis were <15%.

**Statistics.** Values are means ± SE. Differences were analyzed by ANOVA in 10- and 30-mo-old dDAVP-treated and control animals. Differences were considered significant at *P* < 0.05.

**RESULTS**

**Water balance and urine osmolality.** In control conditions, senescent rats exhibited a marked polyuria with low urine osmolality and drank more (Table 1). Food intake was similar between the senescent and adult rats.

The urine-concentrating activity induced by dDAVP at 200 ng/day reached a plateau within 24 h after minipump implantation in 10- and 30-mo-old rats (Fig. 1). A higher dose (1,000 ng/day) did not raise urine osmolality further. Therefore, results for both dDAVP doses were pooled. Nonetheless, even at the higher dose of dDAVP, urine osmolality measured on day 6 of treatment was significantly lower in senescent than in adult rats (Table 1). The maximal urine osmolality of treated senescent rats was close to that of control adult rats. dDAVP treatment did not affect food intake or body weight (Table 1). Kidneys of control 30-mo-old rats were larger than those of 10-mo-old rats. Kidneys were hypertrophied by dDAVP administration at both ages but to a lesser extent in senescent (+15%) than in adult (+25%) rats (Table 2).

**Papillary osmolality and urea content.** The osmolality of the whole papilla was significantly lower in 30- than in 10-mo-old rats (Table 2). It was increased roughly in the same proportion as urine osmolality by dDAVP infusion (29% in 10-mo-old rats and 65% in 30-mo-old rats; Tables 1 and 2). Papilla urea concentration of senescent rats was half that of adult rats (Table 2). dDAVP had a greater effect on papillary urea accumulation in senescent than in adult rats: papilla urea concentration increased by 215% in senescent rats and by only 30% in adult rats (Table 2), resulting in comparable papillary urea concentration after dDAVP treatment. The percentage of urea among total osmoles was similar in adult and senescent animals treated with dDAVP (Table 2).

**AQP2 and AQP3 expression.** As previously reported in aging rats (8, 32), expression of aquaporins in the IM was reduced: greatly for AQP2 and moderately for AQP3 (data not shown). Changes in aquaporin expression, induced by dDAVP, were somewhat different for AQP2 and AQP3. AQP2 was significantly upregulated by chronic dDAVP administration in senescent, but not adult, rats (Fig. 2). As a result, AQP2 expression was identical in dDAVP-treated 10- and 30-mo-old rats (Fig. 3). On the other hand, AQP3 expression was increased by dDAVP in adult and senescent animals (Fig. 2), with a greater increment in older than in younger rats, also resulting in equivalent AQP3 abundance (Fig. 3).

**AQP2 subcellular localization.** In principal cells of inner medullary collecting duct (IMCD), AQP2 labeling was mainly localized in the apical region in adult kidney, whereas it was diffuse in the cytoplasm in

Table 1. Body weight, food and water intakes, urinary flow rate, and osmolality in control 10- and 30-mo-old female WAG/Rij rats and animals treated with dDAVP by osmotic minipumps for 6 days

<table>
<thead>
<tr>
<th></th>
<th>n</th>
<th>Body Wt, g</th>
<th>Food Intake, g/24 h</th>
<th>Water Intake, ml/24 h</th>
<th>Urine Flow Rate, ml/24 h</th>
<th>Urine Osmolality, mosmol/kg H2O</th>
</tr>
</thead>
<tbody>
<tr>
<td>10 mo old Control</td>
<td>21</td>
<td>198 ± 3</td>
<td>10.9 ± 0.3</td>
<td>15.7 ± 0.4</td>
<td>4.9 ± 0.3</td>
<td>1,868 ± 71</td>
</tr>
<tr>
<td>dDAVP</td>
<td>8</td>
<td>195 ± 4</td>
<td>10.0 ± 0.4</td>
<td>13.2 ± 0.6*</td>
<td>2.6 ± 0.3*</td>
<td>2,228 ± 113*</td>
</tr>
<tr>
<td>30 mo old Control</td>
<td>23</td>
<td>224 ± 5†</td>
<td>11.2 ± 0.4</td>
<td>24.0 ± 1.0†</td>
<td>14.0 ± 2.0†</td>
<td>1,115 ± 105†</td>
</tr>
<tr>
<td>dDAVP</td>
<td>8</td>
<td>230 ± 5†</td>
<td>10.6 ± 0.5</td>
<td>14.7 ± 0.8*</td>
<td>5.9 ± 0.4*†</td>
<td>1,837 ± 94†</td>
</tr>
</tbody>
</table>

Values are means ± SE; n, number of animals. dDAVP, 1-desamino-8-d-arginine vasopressin. *P < 0.05 vs. control rats at the same age. †P < 0.05 vs. 10-mo-old rats in the same group.
aging kidney (Fig. 4, a and c). In adult rats, there was no obvious change in apical labeling after dDAVP administration (Fig. 4b). In senescent rats, however, dDAVP treatment resulted in improved targeting of AQP2 to the apical region (Fig. 4d).

Urea transporter expression. In the tip of the IM, where UT-A1 is normally located, UT-A1 expression was abolished by the AVP analog in 10-mo-old rats (Fig. 5B). In 30-mo-old rats, no change was detectable because of the already very low protein expression in untreated rats (Fig. 5B). However, UT-A1 protein abundance in the base of the IM was significantly increased by dDAVP in 10- and 30-mo-old rats (Fig. 5A).

Table 2. Kidney and papilla weights, osmolality, and urea concentration of the papilla in control 10- and 30-mo-old female WAG/Rij rats and animals treated with dDAVP by osmotic minipumps for 6 days

<table>
<thead>
<tr>
<th>Group</th>
<th>Kidney Wt, g</th>
<th>Papilla Wt, mg</th>
<th>Papillary Osmolality, mosmol/kgH2O</th>
<th>Papillary Urea Conc, mmol/l</th>
<th>Papillary Urea, % of total osmoles</th>
</tr>
</thead>
<tbody>
<tr>
<td>10 mo old</td>
<td></td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>0.60 ± 0.02</td>
<td>30 ± 2</td>
<td>1,038 ± 41</td>
<td>257 ± 22</td>
<td>25 ± 2</td>
</tr>
<tr>
<td>dDAVP</td>
<td>0.75 ± 0.03*</td>
<td>35 ± 2</td>
<td>1,339 ± 76*</td>
<td>430 ± 26*</td>
<td>32 ± 1*</td>
</tr>
<tr>
<td>30 mo old</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>0.73 ± 0.02†</td>
<td>39 ± 2†</td>
<td>736 ± 40†</td>
<td>126 ± 16†</td>
<td>17 ± 3†</td>
</tr>
<tr>
<td>dDAVP</td>
<td>0.84 ± 0.01†</td>
<td>40 ± 1</td>
<td>1,208 ± 42†</td>
<td>396 ± 7†</td>
<td>33 ± 1†</td>
</tr>
</tbody>
</table>

Values are means ± SE of 6 animals in each group. dDAVP was administered at 200 ng/24 h. *P < 0.05 vs. control rats at the same age. †P < 0.05 vs. 10-mo-old rats in the same group.
The pattern of UT-A2 changes after dDAVP treatment was similar in 10- and 30-mo-old rats (Fig. 6). In 10-mo-old rats, UT-A2 was overexpressed by long-term administration of dDAVP. The increment was greater in the upper (Fig. 6A) than in the lower (Fig. 6B) half of the IS. In 30-mo-old rats, an increase was also visible but did not reach statistical significance.

UT-B expression was not affected by dDAVP in the upper or lower IS (data not shown). On the other hand, dDAVP tended to reduce UT-B abundance in the base and the tip of the IM (Fig. 7). However, this decline reached statistical significance only in the base of 10-mo-old rats and the tip of 30-mo-old rats.

Because urine concentration remained lower in senescent than in adult rats before and after dDAVP treatment, the abundance of the three urea transporter proteins was compared between 10- and 30-mo-old animals in both conditions (Fig. 8). In untreated rats, UT-A1 abundance was very low in 30-mo-old rats, as reported elsewhere (8). However, after dDAVP treatment, there was twice as much UT-A1 in the base of the IM in senescent rats as in adult rats. After dDAVP treatment, the amount of UT-A1 in the tip of the IM was so low in senescent and adult rats that a potentially lower expression was undetectable in older animals. In dDAVP-treated senescent rats, expression of UT-A2 was lower in upper half of the IS, whereas there...
was no age-related change in UT-A2 expression in untreated rats (Fig. 8). There was less UT-B in the base and more UT-B in the tip of the IM in senescent than in adult rats with or without dDAVP (Fig. 8).

**NOS expression in papilla.** nNOS was not detected in adult or senescent rats (not shown). eNOS abundance was equivalent in adult and senescent rats (Fig. 9). iNOS was not detected in renal papilla in untreated rats. Expressions of eNOS and iNOS were unaffected by administration of dDAVP in adult or senescent rats (Fig. 9).

**Corticosterone and 11-dehydrocorticosterone plasma level.** The distribution of individual measurements in each group of rats overlapped (Fig. 10). Nonetheless, the mean plasma level of corticosterone was twofold higher in 30- than in 10-mo-old rats (Fig. 10). 11-Dehydrocorticosterone plasma concentration was also twofold higher in senescent animals (Fig. 10).

**DISCUSSION**

Polyuria is common in aging rodents (5, 6). We previously showed that, in senescent female WAG/Rij rats devoid of kidney disease, this impaired urine-concentrating capacity does not result from abnormal AVP plasma levels or cAMP accumulation in the IM but, rather, is related to defective expression of aquaporins and urea transporters (8, 32). Nevertheless, the present study shows that in 30-mo-old rats the ability to respond to a chronic supramaximal dose of dDAVP is intact, because their urine osmolality increased by 65%, whereas the increase was only 20% in adult rats. Despite this large effect, the urine osmolality of dDAVP-treated senescent rats remained slightly lower than that of dDAVP-treated adult animals. The deficit can be explained by several differences in the patterns of AVP-induced changes in papilla osmolality, urea concentration, aquaporins, and urea transporters.

The low urine osmolality of senescent rats is consistent with low papillary osmolality and urea concentration found in this study. The proportion of urea among total osmoles in the papilla is also significantly lower in senescent rats. This can be related to blunted expression of UT-A1 in senescent rats, as reported previously (8) and in the present study. This age-related defect in UT-A1 expression might be related to an increase in corticosterone and 11-dehydrocorticosterone plasma levels, found in this study, between 10 and 30 mo. Indeed, glucocorticoids are known to increase urinary urea excretion (22), probably through a decrease in the amount of UT-A1 in rat IM (21) and in urea permeability of rat terminal medullary collecting ducts (29). Saito et al. (35) reported an upregulation of AQP2 gene expression in glucocorticoid-deficient rats. Conversely, the high plasma level of corticosterone in senescent

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**Fig. 6.** Western blot analysis of effect of dDAVP administration on abundance of UT-A2 in inner stripe of outer medulla in 10- and 30-mo-old rats. Top: immunoblots of upper half (A) and lower half (B) of inner stripe. Each lane was loaded with 15 μg of protein and probed with a purified polyclonal antibody against UT-A2 (0.25 μg/ml). Bottom: density quantification data. Values (means ± SE) are expressed relative to respective controls at the same age. *P < 0.05 or less by 1-way ANOVA.

**Fig. 7.** Western blot analysis of effect of dDAVP administration on abundance of UT-B in inner medulla of 10- and 30-mo-old rats. Top: immunoblots of base (A) and tip (B) of inner medulla. Each lane was loaded with 15 μg of protein and probed with a purified polyclonal antibody against rat UT-B (0.25 μg/ml). Bottom: density quantification data (sum of 29- and 48-kDa forms). Values (means ± SE) are expressed relative to respective controls at the same age. *P < 0.05 or less by 1-way ANOVA.
rats may also be responsible for the low level of AQP2 expression in aging kidney, but a direct effect of glucocorticoids cannot be demonstrated. Because of impaired expression of AQP2 and UT-A1 with age, a longer delay or a higher dose of exogenous dDAVP may be expected for a full kidney response in senescent rats. This apparently does not apply to the present experimental model. As shown in Fig. 1, maximal urine concentration was reached within 24 h after the beginning of dDAVP infusion in senescent and young rats. Moreover, a very large dose of hormone did not further increase urine osmolality.

It is believed that long-term dDAVP infusion causes an increase in AQP2 expression (11, 15, 20, 41). This is consistent with our findings in senescent rats. However, we found no increase in AQP2 expression in dDAVP-treated adult rats. The effects of AVP on AQP2 expression may depend on baseline urine-concentrating activity: in Brattleboro rats lacking endogenous AVP, which exhibit a markedly reduced urine osmolality and expression of AQP2, AVP infusion for 5 days greatly increases urine concentration and AQP2 abundance in whole IM (11) and in cortical collecting ducts and IMCD (20). Similar increases were found here in polyuric senescent rats. However, our study shows that AVP does not affect AQP2 abundance in the IM of adult WAG/Rij rats with normal endogenous AVP plasma level. This may be related to their high baseline AQP2 expression compared with Brattleboro or senescent rats. The increased AQP2 expression reported by Ecelbarger et al. (15) in Sprague-Dawley rats infused for 4 days with dDAVP concerned the whole kidney and could result from an increase in AQP2 in cortical or outer medullary collecting ducts, but not in IMCD, or from a lower baseline expression of AQP2 in this strain than in WAG/Rij rats.

As previously reported, AQP2 in polyuric senescent rats, in addition to being poorly expressed, is not properly targeted to collecting duct apical plasma membranes.

Fig. 8. UT-A1 (A), UT-A2 (B), and UT-B (C) abundance in medulla subzones in untreated (control) and dDAVP-treated 10- and 30-mo-old rats. Values (means ± SE) are expressed relative to respective untreated or treated 10-mo-old rats. *P < 0.05 or less by 1-way ANOVA.

Fig. 9. Immunoblotting analysis of endothelial and inducible nitric oxide synthase (eNOS and iNOS, respectively) expression in renal inner medulla of untreated (control) and dDAVP-treated 10- and 30-mo-old rats. Each lane was loaded with 10 μg of protein and probed with a monoclonal antibody against eNOS or iNOS (0.12 μg/ml). In the case of eNOS, a single band at 140 kDa corresponding to eNOS was observed in all papilla extracts from 10- and 30-mo-old rats. There was no difference in eNOS expression between 10- and 30-mo-old rats or after dDAVP treatment. In the case of iNOS, 130-kDa band corresponding to iNOS was undetectable, regardless of experimental condition.

Fig. 10. Plasma concentration of corticosterone and 11-dehydrocorticosterone in 10-mo-old (open symbols) and 30-mo-old rats (filled symbols). Measurements were performed on 5 rats of each age.
The present study shows that higher AVP levels can restore proper membrane targeting of AQP2 in senescent rats, which is necessary for better urine concentration. Bouley et al. (7) recently showed that NO can stimulate cGMP-dependent AQP2 membrane insertion. Inasmuch as AVP could stimulate NO production in rat IMCD (28), immunoreactive eNOS and iNOS were quantified in renal medulla in adult and senescent rats infused with dDAVP. Neither eNOS nor iNOS expression was affected by age or dDAVP in this study. They are, therefore, unlikely to be involved in an age-related difference in AQP2 expression and targeting.

AQP3 and AQP4 are the basolateral water channels of collecting duct principal cells. Senescent rats exhibit a moderate, 50% decrease in AQP3 expression compared with adult rats (32). AQP3 abundance was reported to increase all along the collecting duct after long-term dDAVP infusion in Brattleboro rats (41). The present study reveals that AQP3 is also significantly upregulated by chronic dDAVP infusion in rats with normal endogenous AVP. This occurs in adult and senescent rats, with a much higher increase in senescent (3.5-fold increase) than in younger (1.5-fold increase) rats. In adult rats, this increase occurs without change in AQP2 expression, thus representing an additional situation where expression of AQP2 and AQP3 does not vary in parallel (30, 37). It suggests that the signals controlling expression of the two channels are not identical, as proposed previously (25). In our study, IMCD AQP3 expression is regulated by dDAVP in adult and senescent rats. In contrast, IMCD AQP2 expression is not regulated by dDAVP in adult rats. Instead, it appears to be more closely related to papillary osmolality: AQP2 expression is low only when papillary osmolality is low (untreated senescent rats), despite normal AVP plasma levels (8). AQP2 expression is high when papillary osmolality is high, whether in the absence (untreated adult rats) or presence (treated rats) of dDAVP.

The role of urea transporters in the urine-concentrating process has been well documented (2, 23, 36). They are involved in the medullary accumulation of urea, which contributes to the medulla hypertonicity required for water conservation. As indicated in Table 2, dDAVP treatment induced a medullary accumulation of urea of much greater magnitude than the accumulation of nonurea solutes in adult and senescent rats, as observed years ago (46). At first glance, this contrasts with the underexpression of UT-A1 in the tip of the IM containing terminal IMCDs in the presence of dDAVP. Nevertheless, UT-A1 downregulation at the tip of the IM is likely compensated for by an upregulation at the base of the IM containing initial IMCDs. Two previous observations in AVP-deficient Brattleboro rats are consistent with this pattern of changes: 1) dDAVP upregulated UT-A1 mRNA in the base but not in the tip of the IM (33), and 2) in situ hybridization and immunocytochemistry revealed a decrease in UT-A1 in the tip and an increase in the base of the IM in chronically dDAVP-treated Brattleboro rats at the mRNA and protein levels (39). A dDAVP-induced axial shift in the IM has been found in Sprague-Dawley rats as well (45). It is likely that, under dDAVP treatment, the IM urea is supplied in the earlier part of medullary collecting ducts. Phosphorylation of UT-A1 can also improve urea transport. Indeed, it has been recently demonstrated that the AVP-induced urea permeability mediated by UT-A1 does not result from increased UT-A1 protein abundance or recruitment in the apical membrane of medullary collecting ducts (18) but from the phosphorylation of membrane-preinserted proteins (49). Alternatively, the blunted expression of UT-A1 could be compensated for by UT-A3, a spliced variant of the UT-A gene that has the same subcellular and axial distribution as UT-A1 in intracellular membranes and in the apical region of the terminal portion of rat IMCD (42). Unfortunately, UT-A3 regulation by vasopressin has not been studied. Finally, the increase in AQP3 expression by dDAVP may represent an adaptation of the basolateral transport of urea in view of the possible urea permeability of AQP3 (16, 19).

UT-A2 is markedly increased all along the thin descending limbs by long-term dDAVP treatment in adult rats in the upper and lower part of the IS of the outer medulla. Such an effect has been observed by Northern blotting (33) and immunocytochemistry (47). DDAVP-treated senescent rats also undergo UT-A2 upregulation along thin descending limbs but to a lesser extent. This weaker augmentation of UT-A2 in senescent rats than in younger rats could result from lower outer medullary osmolality after dDAVP infusion, because UT-A2 is believed to be regulated by hypertonicity, rather than by AVP (43).

We previously showed that chronic dDAVP infusion into Brattleboro AVP-deficient rats downregulates UT-B mRNA abundance in the base and the tip of the IM (33). This is consistent with our finding of depressed UT-B protein expression by dDAVP in normal rats. Senescent rats also respond to dDAVP by a decrease in UT-B protein expression, mainly in the tip of the IM. Such a downregulation of UT-B protein has also been recently reported in adult Sprague-Dawley rats (44).

In conclusion, the present study shows that senescent rats treated with dDAVP exhibit urine osmolality and flow rates equivalent to those of untreated adult rats. This rescue is likely related to the upregulation of AQP2 and AQP3 expression and AQP2 membrane targeting. dDAVP infusion in senescent rats also increases papillary urea accumulation, owing to UT-A1 and UT-A2 upregulation.

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