Downregulation of renal vasopressin V2 receptor and aquaporin-2 expression parallels age-associated defects in urine concentration

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Submitted 13 November 2003; accepted in final form 7 June 2004

Tian, Ying, Ryota Serino, and Joseph G. Verbalis. Downregulation of renal vasopressin V2 receptor and aquaporin-2 expression parallels age-associated defects in urine concentration. Am J Physiol Renal Physiol 287: F797–F805, 2004. First published June 22, 2004; 10.1152/ajprenal.00403.2003.—Renal concentrating ability is known to be impaired with aging. The antidiuretic hormone AVP plays an important role in renal water excretion by regulating the membrane insertion and abundance of the water channel aquaporin-2 (AQP2); this effect is primarily mediated via the V2 subtype of the AVP receptor (V2R). This study evaluated the hypothesis that decreased renal sensitivity to AVP, with subsequent altered renal AQP2 expression, contributes to the reduced renal concentrating ability with aging. Our results show that under baseline conditions, urine osmolality is significantly lower in aged Fischer 344 and Brown-Norway F1 hybrid (F344BN) rats despite equivalent plasma AVP concentrations as in young rats. Levels of kidney V2R mRNA expression and AQP2 abundances were also significantly decreased in aged F344BN rats, as was AQP2 immunostaining in collecting duct cells. In response to moderate water restriction, urine osmolality increased by significantly lesser amounts in aged F344BN rats compared with young rats despite similar increases in plasma AVP levels. Moderate water restriction induced equivalent relative increases in renal AQP2 abundances in all age groups but resulted in significantly lower abundances in total kidney AQP2 protein in aged compared with young F344BN rats. These results therefore demonstrate a functional impairment of renal concentrating ability in aged F344BN rats that is not due to impaired secretion of AVP but rather appears to be related to impaired responsiveness of the kidney to AVP that is secondary, at least in part, to a downregulation of renal V2R expression and AQP2 abundance.

aging; aquaporins; kidney

AN AGE-RELATED DECLINE IN urinary concentrating ability has been documented in experimental animals as well as in elderly humans (2, 3, 13, 16, 17, 20, 30, 31). Both the increase in urine concentration and the decrease in urine volume that are characteristically observed after water deprivation are reduced in aged compared with young humans. For example, maximum urine osmolality was found to be significantly lower in elderly subjects (aged 60–79) than in young subjects (aged 20–39) following a 24-h period of water deprivation (882 ± 49 vs. 1,109 ± 22 mosmol/kgH2O, respectively, P < 0.01) (37). Normally, such decreases in urine concentrating ability are easily compensated for by increased fluid intake stimulated by neural thirst pathways. However, the elderly also have a decreased sensation of thirst in response to dehydration, and as a result they drink less water following periods of dehydration (34). These combined neural and kidney impairments make elderly humans much more susceptible to developing life-threatening degrees of hyperosmolality and hypovolemia in response to periods of dehydration that are rarely problematical in younger humans with intact thirst and renal concentrating mechanisms.

Several hypotheses have been proposed to explain this decrease in maximal renal concentrating ability in aging, including decreased numbers of functional nephrons, reduced renal glomerular filtration rate, defects in pituitary release of AVP, and impaired response of the renal collecting duct cells to AVP (1, 11, 19). However, the results from studies evaluating these various hypotheses have been somewhat conflicting. For example, a decreased number of functional nephrons with age and hyperfiltration of the remaining glomeruli has been considered to be a potential explanation for the decreased renal concentration ability in aging, but it does not explain the increased diuresis of aged WAG/Rij rats with a constant number of nephrons and single nephron filtration rates (9, 10). Similarly, decreased AVP secretion, resulting in reduced collecting duct osmotic permeability and increased water excretion, is supported by some studies that showed decreased pituitary and plasma AVP concentrations in aging rats but not by others (36, 54). Some of these conflicting results may reflect the variety of different species, strains, and ages of animals used in aging studies from different laboratories (3, 9, 10, 12, 34, 51, 52, 53). Nonetheless, most studies are consistent with a reduced renal cellular response to AVP, leading to decreased water permeability of the collecting duct, as an important contributor to age-associated impairments in renal concentrating ability (5, 6, 19). Studies showing reduced AVP-dependent reabsorption of sodium by the thick ascending limb of Henle’s loop in senescent mice (14) and decreased expression of the AVP-stimulated urea transporter UT-A1 in aged female WAG/Rij rats (7, 8, 48) further support an altered renal responsiveness to AVP with aging.

AVP plays a critical role in the regulation of renal water excretion. AVP increases water permeability in the collecting duct of the kidney by regulating membrane insertion and abundance of the water channel aquaporin-2 (AQP2). This effect of AVP is mediated via the vasopressin V2 receptor (V2R), which is expressed in the collecting ducts of the kidney. In the present studies, we evaluated the hypothesis that downregulation of V2R expression with aging leads to a decreased renal sensitivity to AVP with subsequent decreased renal AQP2 expression, and these combined effects contribute to the
decreased urinary concentrating ability in aged rats. To test our hypothesis, we used Fischer 344 (F344) and Brown-Norway F1 hybrid (F344BN) rats, developed by the National Institute on Aging (NIA) for aging research, which live considerably longer and have less renal pathology at any given age compared with other inbred strains (26, 49). Of particular relevance to these studies, the F344BN rat has a significantly lower incidence of renal pathological processes than its parent F344 rats and other strains (26). In the present study, urinary concentrating ability, plasma AVP levels, renal V2R mRNA expression, and renal AQP2 abundances were measured in male F344BN rats of various ages under baseline conditions and in response to a moderate water restriction.

MATERIALS AND METHODS

Animals. Male F344BN rats at ages 3 mo (young), 10 mo (adult), and 24 mo (early aged) were obtained from the NIA. The rats were fed with the NIH-31/NIA fortified diet (18.74% protein as fish meal and soybean meal) while maturing at the NIA. While maintained at Georgetown University, all animals were housed in the animal care facility on a 12:12-h light-dark cycle and were fed standard laboratory rodent chow (no. 5001 diet, 23.4% protein as fish meal and soybean meal, LabDiet, St. Louis, MO) and water ad libitum.

Animal testing protocols. For studies performed under baseline conditions, rats were allowed free access to pelleted rat chow (no. 5001 diet, LabDiet) and water. For water restriction studies, rats in each age group were divided into control and water-restricted subgroups. Control rats were given water ad libitum, whereas the water-restricted rats were given only 25% of the control rats’ daily water intake. All rats were given free access to pelleted rat chow. The rats were maintained in metabolic cages to enable daily collection of urine for measurement of urine osmolality and sodium concentration. Body weight and water intakes were also measured daily. After 5 days of moderate water restriction, all rats were euthanized by decapitation. Both kidneys were rapidly removed, rinsed with ice-cold PBS buffer, and processed for further studies.

Kidney morphology. Kidneys were removed and immersion fixed with PBS-4% paraformaldehyde for 4 h and then in 75% ethanol overnight at room temperature. The tissues were then embedded in paraffin and cut into 4-μm sections in the Department of Pathology at Georgetown University Hospital. The sections were stained with hematoxylin and eosin (for general morphological examination) and periodic acid-Schiff stain (for demonstration of glycogen deposits) (50). Morphological changes were assessed by visual analysis using light microscopy.

Immunohistochemistry. Kidneys were sectioned as above. The sections were preincubated in 10% normal goat serum for 20 min at room temperature and then incubated in PBS/BSA (1%) containing primary antibody raised against AQP2 (no. 751) for 1 h. After being washed three times for 10 min in PBS, the sections were incubated in 6% H2O2 for 10 min and then incubated with a second antibody (anti-rabbit IgG, Vector, Burlingame, CA) for 1 h. The sections were washed again and then incubated in A/B solution (Vector). The stained sections were analyzed visually using light microscopy (original magnification ×400).

Plasma and urine osmolality and sodium concentration measurement. Twenty-four-hour urines were collected daily using metabolic cages. Trunk blood was collected at the time of decapitation. Urine and plasma osmolality were measured using an Advanced Instruments Osmometer (model 3900, Advanced Instrument).

Plasma AVP measurement. AVP radioimmunoassay was performed in duplicate after acetone-ether extraction of plasma using an anti-AVP antibody developed in our laboratory (43). The AVP standard curve is linear between 0.5 and 10 pg/tube, and the minimal detectable AVP concentration in extracted plasma is 0.5 pg/ml.

Renal V2R mRNA measurement. Total RNA was extracted from kidney inner medullas (IM) using TRIzol. Tissues were homogenized 2 × 10 s in 1 ml of TRIzol per renal IM using a rotary homogenizer (StedFast Stirrer, model SL 1200, Fisher Scientific). RNA purity and concentration were assessed spectrophotometrically and 10 μg of total kidney RNA were loaded in each lane. The intensity and quality of the 18s and 28s ribosomal bands were evaluated for both qualitative and quantitative differences in the RNA samples. Samples were run on 1% agarose gels containing formaldehyde. Transfer of RNA to positively charged nylon membranes (Boehringer Mannheim) was performed overnight by the capillary action of 20× SSC (3.0 M NaCl, 0.3 M sodium citrate) buffer. Labeled specific probe for the V2R was synthesized by reverse transcription of total kidney mRNA followed by PCR using a PCR DIG probe synthesis kit (Boehringer Mannheim). The sequence of V2R primers was reported previously (22). The blots were probed using standard methods (DIG wash and block buffer set and a DIG luminescent detection kit; Boehringer Mannheim) (15). Relative quantitation of the band densities from the immunoblots and Northern blots was performed by densitometry using National Institutes of Health (NIH) image-analysis software. After being stripped, the same blots were probed for actin mRNA using a digoxigenin-labeled β-actin probe (Boehringer Mannheim) to normalize equality of loading. The density of the Northern blot bands for the V2R and β-actin was measured by densitometry. The ratios of V2R to β-actin were calculated separately for each animal.

Renal AQP2 protein measurement. For preparation of inner medullary extracts, kidneys were sliced along the corticomedullary axis to separate the medulla from the cortex. The inner medullary region of the kidneys was dissected and minced in ice-cold membrane-isolation solution containing 250 mM sucrose, 10 mM triethanolamine (Sigma), 1 μg/ml leupentin (Bachem, Torrance, CA), and 0.1 mg/ml phenylmethylsulfonyl fluoride (Sigma), adjusted to pH 7.6. The IM, or whole kidney, was homogenized using a tissue homogenizer (PowerGen 125, Fisher Scientific, Pittsburgh, PA) fitted with a 10-mm microsaw tooth generator in 10 ml of ice-cold membrane-isolation solution. Protein concentration was determined using a BCA Protein Reagent kit (Pierce, Rockford, IL). Samples were diluted with the isolation solution to a protein concentration of ~2 μg/ml and solubilized at 60°C for 15 min in Laemml total sample buffer. All samples were stored at −80°C until electrophoresis. Initially, 5 μg of protein from each of the samples was loaded on 12% sodium dodecyl sulfate-polyacrylamide gels (Precast, Bio-Rad, Hercules, CA) and electrophoresed. These gels were stained with Coomassie brilliant blue (G250, Bio-Rad) to assess the quality of protein bands and the precision of the protein determinations. For immunoblotting, the electrophoresis was carried out on precast minigels of 12% polyacrylamide. The proteins were transferred from the gels electrothermally to nitrocellulose membranes. After a 30-min 5% milk block, membranes were probed overnight at 4°C with a polyclonal antibody against AQP2 (no. 751); this antibody was a kind gift from Dr. Kuepper (National Institute of Diabetes and Digestive and Kidney Diseases) and made from the same peptide described previously for L127 (33). For probing blots, antibodies were dissolved in a solution containing 150 mM NaCl, 50 mM sodium phosphate, 10 mg/dl sodium azide, 50 mg/dl Tween 20, and 0.1 g/dl BSA (pH 7.5). The secondary antibody was goat anti-rabbit IgG conjugated to horseradish peroxidase (Kirkegaard & Perry Labs, Gaithersburg, MD) and used at a concentration of 0.10 μg/ml. Sites of antibody-antigen reaction were visualized using luminol-based enhanced chemiluminescence (LumiGLO, Kirkegaard & Perry) before exposure to X-ray film (Fujiﬁlm, Fuji Medical Supplies, Stamford, CT).

Densitometry and statistical analysis. Relative intensity of the immunoblot and Northern band densities was determined by laser scanning (Scanjet 6100C) followed by analysis with NIH IMAGE software. The statistical significance of the effects of age and dehydration on protein or mRNA expression was determined either by one- or two-way ANOVA (age and treatment effects), followed by post hoc
analysis via the method of Newman-Keuls where appropriate for comparisons between individual age or treatment pairs, or by unpaired t-tests when only two groups were compared (SigmaStat software, Chicago, IL).

RESULTS

Gross pathology. No kidney tumors were noted in any rat from any age groups during the course of the experiments. Two of the 24-mo-old rats were noted to have urinary obstruction because of visualized small tumors in the bladder; these two rats were eliminated from the study.

Kidney histopathological microscopy. Histopathological microscopy was performed on kidneys from 3-, 10-, and 24-mo-old rats (n = 3 at each age; Fig. 1). Normal renal cortical and medullary morphology was observed in the 3-mo-old rats. In the 10-mo-old rats, very mild glomerulosclerosis was seen occasionally, whereas the morphology of the renal medulla appeared unchanged. At 24 mo of age, moderate glomerulosclerosis, the presence of tubular casts and dilated afferent arterioles, was seen in the renal cortex, whereas again no apparent pathological changes were observed in the renal IM. These results in conjunction with statistically equivalent kidney weights in the 10- and 24-mo-old rats (Table 1) suggest the absence of major pathological changes in the kidneys of aged F344BN rats.

Physiological parameters in F344BN rats under baseline conditions. Table 1 summarizes the physiological parameters from metabolically caged F344BN rats under baseline conditions. The 24-mo-old male F344BN rats gained only 11% more body weight than the 10-mo-old rats and therefore did not become obese with advancing age, which is a major confounding issue with the use of older Sprague-Dawley and Wistar rats for aging studies. No significant differences in kidney weights were found between the 10- and 24-mo-old rats (P > 0.05), indicating that the kidneys of F344BN rats remain free of obvious signs of age-related hypertrophy, commonly observed in other strains, after 24 mo of aging. Daily water intakes were comparable across all groups, but the ratio of water intake to body weight was significantly higher in the 3-mo-old rats compared with the 10- and 24-mo-old rats, likely indicating increased metabolic water requirements in the younger, growing animals.

Urine parameters in F344BN rats under baseline conditions. Table 1 summarizes the urine parameters from the three age groups under baseline conditions. The 24-mo-old rats had a significantly lower urine osmolality than the 10-mo-old rats (P < 0.05). The 24-h urine volume of 24-mo-old rats was slightly greater, but not significantly different, than the 3- and 10-mo-old rats. However, the ratio of water intake to urine volume was lower in the aged rats compared with the 3-mo-old rats. An age-associated loss of urinary concentrating ability, even at baseline conditions, was therefore suggested by a lower urine osmolality despite a lower ratio of water intake to urine excretion in the older rats.

Plasma AVP levels in F344BN rats under baseline conditions. Plasma AVP levels from trunk blood were not significantly different between the 3-mo-old (2.6 ± 0.2 pg/ml), 10-mo-old (2.3 ± 1.0 pg/ml), and 24-mo-old (2.6 ± 0.3 pg/ml) groups under baseline conditions (n = 6 for each group, not significant).

Renal AQP2 abundance in F344BN rats under baseline conditions. Renal AQP2 abundances in both the IM and the whole kidney were significantly decreased in the 24-mo-old rats under baseline conditions. Figure 2 shows AQP2 abun-

Table 1. Physiological and urine parameters in F344BN rats under baseline conditions

<table>
<thead>
<tr>
<th>Age</th>
<th>Body Weight, g</th>
<th>Kidney Weight, g</th>
<th>Water Intake, ml/24 h</th>
<th>Water Intake/Body Weight</th>
<th>Urine Volume, ml/24 h</th>
<th>Urine Osmolality, mosmol/kgH2O</th>
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</thead>
<tbody>
<tr>
<td>3 Mo</td>
<td>299±6</td>
<td>1.96±0.06</td>
<td>27.8±4.3</td>
<td>9.5±0.5</td>
<td>12.3±0.5</td>
<td>1,864±35</td>
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<tr>
<td>10 Mo</td>
<td>488±8*</td>
<td>2.46±0.08*</td>
<td>22.8±3.4*</td>
<td>5.0±0.2*</td>
<td>11.6±0.4</td>
<td>2,098±28*</td>
</tr>
<tr>
<td>24 Mo</td>
<td>521±10†</td>
<td>2.68±0.08*</td>
<td>22.8±3.6*</td>
<td>4.4±0.3*</td>
<td>13.1±0.4</td>
<td>1,775±31†</td>
</tr>
</tbody>
</table>

Values are means ± SE; n = 30 animals per group for body weight and water intake data; n = 9 animals per group for kidney weight and urine data. Data were analyzed by one-way ANOVA. *P < 0.05 compared with 3-mo-old rats. †P < 0.05 compared with 10-mo-old rats. ‡P < 0.05 compared with 3- and 10-mo-old rats. F344BN, Fischer 344 and Brown-Norway F1 hybrid.

Fig. 1. Renal cortical and medullary morphology in young and aged Fischer 344 and Brown-Norway F1 hybrid (F344BN) rats. Histopathological microscopy was performed on kidneys from 3-, 10-, and 24-mo-old rats. Normal renal cortical and medullary morphology was observed in 3-mo-old rats. In 10-mo-old rats, mild glomerulosclerosis was seen, but the morphology of the renal medulla appeared largely unchanged. After 24 mo of age, moderate glomerulosclerosis, the presence of tubular casts (arrow) and dilated afferent arterioles, was seen in the renal cortex, but no significant changes in the renal medulla were observed.
Levels of the 3-mo-old rats, respectively. The 29-kDa band represents the nonglycosylated form and the broad band at 35–45 kDa total protein in inner medullary homogenates from 3-, 10-, and 24-mo-old rats under baseline conditions, as well as mean levels, as determined by densitometry and expressed as a percentage of the levels of the 3-mo-old rats (Fig. 2A). AQP2 expression in the 24-mo-old rats was decreased to 60 ± 8% (IM) and 69 ± 9% (whole kidney) of the levels of the 3-mo-old rats, respectively (P < 0.05); no significant differences were found between 10- and 3-mo-old rats. Figure 2D shows a Coomassie blue-stained gel run parallel with the AQP2 gels, which shows that the differences in immunoreactive AQP2 (Fig. 2A and B) cannot be attributed to differences in protein loading onto the gel.

Immunohistochemical studies of AQP2 expression. Kidneys from 3- and 24-mo-old rats (n = 3 at each age) were stained immunohistochemically using an antibody directed against AQP2 (no. 751). Figure 3 shows that AQP2 was localized exclusively to the collecting ducts in both the IM and cortex.
Physiological parameters in F344BN rats after 5 days of moderate water restriction were analyzed using Northern blots, as described in MATERIALS AND METHODS. Shown is a summary of the V2R mRNA expression in the renal inner medulla normalized to the value of β-actin band in each lane. Statistical analysis by 1-way ANOVA confirmed V2R mRNA expression in the renal inner medulla significantly decreased in both the 10- and 24-mo-old rats (n = 6 for each group; P < 0.05 was considered significant for all tests).

Urinary concentrating ability in response to moderate dehydration. After 5 days of water restriction, the urine osmolalities in the aged rats were significantly lower compared with the adult and young rats. The curves relating urine osmolality vs. time were shifted to the right in aged 10- and 24-mo-old rats (Fig. 5A). Urine osmolality in the water-restricted 3-mo-old rats (Fig. 5A) and urine volume (Fig. 5B) after mild water restriction were given only 25% of the control rats’ daily water intake and were given free access to pelleted rat chow. After 5 days, all rats were euthanized by decapitation. Urine osmolality and volume in the water-restricted rats were expressed as a percentage of the nondehydrated rats of each age group. Values are expressed as means ± SE (n = 12; *P < 0.05 compared with 3-mo-old rats, **P < 0.05 compared with 3- and 10-mo-old rats).

Table 2. Physiological parameters in F344BN rats after 5 days of moderate water restriction

<table>
<thead>
<tr>
<th>Age</th>
<th>Body Weight, g</th>
<th>Plasma Osmolality, mmosmol/kgH2O</th>
<th>Plasma Volume, % of Control</th>
</tr>
</thead>
<tbody>
<tr>
<td>3 Mo</td>
<td>Control 300±8 (100%)*</td>
<td>292.8±1.8</td>
<td>100±1.2%</td>
</tr>
<tr>
<td></td>
<td>Dehydration 266±8 (89%)†</td>
<td>298.9±1.7†</td>
<td>95±1.6%†</td>
</tr>
<tr>
<td>10 Mo</td>
<td>Control 466±6 (90%)*</td>
<td>291.6±2.0</td>
<td>100±0.8%</td>
</tr>
<tr>
<td></td>
<td>Dehydration 417±9 (90%)†</td>
<td>294.6±2.0†</td>
<td>96±1.9%†</td>
</tr>
<tr>
<td>24 Mo</td>
<td>Control 521±6 (100%)*</td>
<td>292.6±1.5</td>
<td>100±1.1%</td>
</tr>
<tr>
<td></td>
<td>Dehydration 467±8 (90%)†</td>
<td>299.3±1.5†</td>
<td>93±1.8%†</td>
</tr>
</tbody>
</table>

Values are means ± SE; n = 16 animals per group. *Significant difference among the different ages as assessed by 2-way ANOVA (rat age and treatment effects). †Significant difference between control and dehydration as assessed by 2-way ANOVA followed by analysis of individual age pairs via Newman-Keuls test; P < 0.05 was considered significant for all tests.

Fig. 4. Age-related decreases in renal inner medullary V2 subtype of the AVP receptor (V2R) mRNA under baseline conditions. Renal inner medullary V2R mRNA from 3-, 10-, and 24-mo-old rats was analyzed using Northern blots, as described in MATERIALS AND METHODS. Shown is a summary of the V2R mRNA expression in the renal inner medulla significantly decreased in both the 10- and 24-mo-old rats (n = 6 for each group; P < 0.05 was considered significant for all tests).

Fig. 5. Age-related changes in urine osmolality (A) and urine volume (B) after mild water restriction. All rats were maintained in metabolic cages. On day 0, water-restricted rats were given only 25% of the control rats’ daily water intake and were given free access to pelleted rat chow. After 5 days, all rats were euthanized by decapitation. Urine osmolality and volume in the water-restricted rats were expressed as a percentage of the nondehydrated rats of each age group. Values are expressed as means ± SE (n = 12; *P < 0.05 compared with 3-mo-old rats, **P < 0.05 compared with 3- and 10-mo-old rats).
water-restricted rats were fed only 25% of the control rats. Control rats from each age group were fed water ad libitum, whereas the rats probed with anti-AQP2 antibody. The 29-kDa band is the nonglycosylated form and the 45-kDa bands) from immunoblots. Values represent the mean value of each sample from a different rat (10 total protein/lane).

**DISCUSSION**

Several hypotheses have been proposed to explain the mechanisms of reduced urinary concentrating ability with aging (1, 11, 19). The present studies further evaluated the hypothesis suggested by previous studies (5, 6, 19) that impaired renal responses to AVP with subsequently altered renal AQP2 expression represent a potential mechanism for age-associated defects in urinary concentrating ability. Using the F344BN rat strain, we demonstrated that urine osmolality, kidney V2R mRNA and AQP2 expression, and AQP2 staining of collecting duct cell apical membranes decreased with age under baseline conditions. More importantly, in response to water restriction for 5 days, urine osmolality increased by 215% ( whole kidney) compared with 3-mo-old rats, but only by 186±4 % in adult 10- and 24-mo-old aged rats, respectively (P<0.05), thereby confirming a reduced urinary concentrating ability in aged rats of this strain (44). Plasma AVP levels increased equivalently with moderate water restriction in rats of all ages, such that plasma AVP levels were comparable across all three age groups of rats. Despite equivalent AVP responses, moderate water restriction resulted in significantly lower abundances of whole kidney AQP2 in the 10- and 24-mo-old rats than in the 3-mo-old rats. The correlation of impaired urine concentration with a blunted total renal AQP protein in response to dehydration suggests that decreased renal AQP2 expression may contribute to the reduced urinary concentrating ability in aged F344BN rats, and this pattern despite equivalently increased plasma AVP levels suggests impaired responsiveness of the kidney to AVP in aged rats of this strain. However, the absence of significant differences in inner medullary AQP2 abundances in response to dehydration across the different age groups suggests that age-related differences in these AQP2 responses may be of even greater magnitude in other parts of the kidney.

Different rat strains have been used in previous studies of age-related polyuria, which has rendered comparisons among the various studies difficult. In this regard, the F344BN rat model has several advantages. This strain was developed by the NIA specifically for studies of kidney function with aging (41). The nephropathy characteristic of the F344 strain (27–29, 38) has not been observed in the F344BN rat. In addition, this animal has a normal growth curve, does not develop obesity with aging, and appears to have no specific tumor susceptibilities, such as the high incidence of Leydig cell tumors present in the various studies discussed. In this respect, the F344BN rat may be useful for studies of age-related changes in the AQP2 abundance and might be compared with the strain (27–29, 38) that has been used in previous studies of age-related polyuria.

The present studies examined the effects of age on the AQP2 abundance of the inner medullary collecting duct (IMCD) and the whole kidney using Western immunoblotting of homogenates from the inner medulla and the whole kidney from rats aged 3, 10, and 24 mo. Each lane was loaded with sample from a different rat (5 total protein/lane). Values represent the mean value of each sample from a different rat (10 total protein/lane).

**Fig. 6. Age-related changes in kidney AQP2 abundance after mild water restriction. A:** representative Western immunoblots of AQP2 of inner medullary homogenates from 3-, 10-, and 24-mo-old F344BN rats. Each lane was loaded with sample from a different rat (5 total protein/lane). Blots were probed with anti-AQP2 antibody. The 29-kDa band is the nonglycosylated form and a broad band at 35–45 kDa is the glycosylated form of AQP2. Control rats from each age group were fed water ad libitum, whereas the water-restricted rats were fed only 25% of the control rats’ daily water intake; all rats were given free access to food. After 5 days, all rats were euthanized by decapitation and the kidneys were rapidly removed for AQP2 protein analysis. B: representative Western immunoblots of AQP2 of whole kidney homogenates from 3-, 10-, and 24-mo-old rats. Each lane was loaded with sample from a different rat (10 total protein/lane). C: summary of densitometric analysis of AQP2 abundance from each group (sum of both 29- and 35-kDa bands) from immunoblots. Values represent the mean value of each group expressed as a percentage of the average value of the dehydrated 3-mo-old group (*P<0.05 compared with 3-mo-old rats, n=6 per group). Statistical analysis by 1-way ANOVA confirmed significant decreases in the dehydration-induced whole kidney AQP2 expression in the 10- and 24-mo-old dehydrated rats compared with 3-mo-old dehydrated rats but not in inner medulla AQP2 expression.

old rats AQP2 abundances increased by 125±6% (IM) and 147±7% (whole kidney) compared with the hydrated 10-mo-old rats; and in the 24-mo-old rats AQP2 abundances increased by 176±9% (IM) and 179±19% (whole kidney) compared with the hydrated 24-mo-old rats after 5 days of water restriction (P<0.05 for all age groups). Despite comparable relative increases in AQP2 abundances after water restriction in each age group, moderate dehydration resulted in significantly lower abundances of total kidney AQP2 protein in 10- and 24-mo-old rats compared with 3-mo-old rats: AQP2 abundances in the 10-mo-old dehydrated rats were only 88±8% (IM) and 72±5% (whole kidney) of the levels in the 3-mo-old dehydrated rats, and in the 24-mo-old dehydrated rats were only 88±7% (IM) and 63±7% (whole kidney) of the levels in the 3-mo-old dehydrated rats (Fig. 6). These differences were significant for the whole kidney (P<0.05, by 1-way ANOVA for age effect) but not for the IM extracts. Thus, despite equivalent relative increases in AQP2 after water restriction in all age groups, the absolute abundances of whole kidney AQP2 after water restriction were less in the 10- and 24-mo-old rats because of the lower basal AQP2 abundances in these groups.
in aged male F344 rats (41). Therefore, this hybrid has been used increasingly in aging related studies (24). Our experiments verify that aged F344BN rats gain only 11% more body weight than adult rats and therefore did not become obese. Even more importantly, the kidney weights of the aged rats were unchanged compared with adult rats (Table 1), and there are no major histopathological changes in the IM of 24-mo-old F344BN rats (Fig. 1), indicating that the kidneys of this strain are relatively normal anatomically. These results in combination with previous studies using this strain (5, 6) suggest that the F344BN rat represents a good animal model for studies of renal function with aging.

Most previous studies completely deprived animals of water for 2–4 days to induce dehydration (23, 32, 42, 45). However, under conditions of total water deprivation, rats decrease food intake, lose substantial amounts of body weight, and become severely hypovolemic and hypernatremic within 2–4 days (23, 32, 42, 45). The total water deprivation model therefore represents a pathological situation that renders studies of long-term physiological changes in kidney function difficult because of uncertainties concerning the potential effects of these confounding factors on physiological parameters of renal function. Furthermore, this type of extreme dehydration is not representative of disorders of water metabolism found in elderly humans; in aging humans, water intake is typically reduced but not totally eliminated. To study the effects of aging on renal function under a more physiological setting, we fed rats different amounts of water and monitored their plasma and urine parameters daily for prolonged periods. These studies resulted in the development of a protocol of restricted daily access to a fixed volume of water (25% of the normal daily water intake of weight-matched controls) that reproducibly induced a moderate physiological dehydration in rats. Our data show that this model of moderate water restriction induces lesser changes in physiological parameters after 5 days of water restriction (Table 2) compared with results obtained using total water deprivation, as reported by others (23, 32, 42, 45). In our model, rats maintained 85–90% of body weight during a 5- to 7-day period of water restriction. The plasma osmolality increased only 6, 3, and 7 mosmol/kgH2O in 3-, 10-, and 24-mo-old rats after 5 days of water restriction, respectively, compared with previous studies in which the plasma osmolality increased by 10–11 mosmol/kgH2O after only 3 days of total water deprivation (42). Plasma volume, estimated by changes in plasma protein concentrations (43), decreased by 4–7% in our model, compared with 11–15% decreases in studies using total water deprivation (45). Therefore, this model allowed us to study the effect of aging on stimulated renal concentrating ability under more physiological conditions.

Previous studies reported that AVP secretion is affected by increasing age. However, conflicting results have been reported using different strains of rats. For example, decreased AVP secretion in response to dehydration and a greater depletion of AVP content in the posterior pituitary were observed in 30-mo-old F344 rats (39, 44) but not in F344BN rats (21). Absent increases in AVP mRNA content in response to dehydration were also reported in aged rats (40). In contrast, elevated plasma AVP has been reported in aged F344BN and Wistar rats compared with young rats (18, 36). These observations indicate that AVP secretion and regulation are not identical in all species, strains, and ages of animals. The present study demonstrated that plasma AVP levels are compatible in aged and young F344BN rats under basal conditions and also in response to water restriction. These results are consistent with a previous study that demonstrated that plasma AVP concentrations were increased equivalently in both young and aged F344BN rats after 3 days of water deprivation (5).

Renal AQP2 protein content was equivalently upregulated by dehydration in all three age groups of F344BN rats. However, the absolute amount of AQP2 in whole kidney extracts of the 10- and 24-mo-old rats after dehydration was significantly lower than in the 3-mo-old rats, as depicted in Fig. 6. These results are in agreement with previous studies using this strain and support the hypothesis that impaired intrarenal AQP2 expression contributes to the defects in urinary concentrating ability with aging. Interestingly, AQP2 abundances in the IMs of the 10- and 24-mo-old dehydrated rats were not significantly different from those of the 3-mo-old dehydrated rats, which suggests that AQP2 abundances may be regulated differentially in different kidney regions. Further studies of regulation of AQP2 expression in different kidney regions in response to dehydration are needed. However, the immunohistochemical staining studies (Fig. 3) allow the possibility that decreased targeting of AQP2 to the apical membrane of collecting duct cells in the IM may account for decreased AVP-stimulated water permeability despite unchanged total IM abundances of AQP2 in response to moderate dehydration.

The present study did not replicate the results of a previous study that demonstrated that dehydration-induced increases in AQP2 protein occurred in young rats but not in aged rats (5). Discrepancies between these studies may reflect differences in the water restriction protocols and the ages of the animals used in these studies. The earlier study used 72-h total water deprivation to induce dehydration, whereas this study fed rats with 25% of the daily drinking volume of controls. Thus the 72-h total water deprivation induced a more severe dehydration than the moderate water restriction induced in this study, as discussed above. In addition, the earlier study used 28-mo-old F344BN rats as aged rats, whereas this study used 24-mo-old rats as aged rats. Although 4 mo seem a small difference, for a species with a 3-yr life span this difference is roughly comparable to 10 yr for a human living 85 yr (5). The latter point also raises the important issue of whether age-associated changes in renal concentrating ability are the result of continuous changes in the elements of the urinary concentrating system vs. discrete changes that only occur after a given age. Continuous changes are suggested by the findings that maximum urine concentration was significantly different between all three age groups (Fig. 2), as was basal V2R expression (Fig. 4). Conversely, basal AQP2 expression in the IM and whole kidney was only reduced in the 24-mo-old rats, while dehydration-induced whole kidney AQP2 was reduced equivalently in both 10- and 24-mo-old rats (Fig. 6), suggesting more discrete age-related thresholds for these phenomena.

Our finding of age-associated reductions of basal V2R mRNA expression in F344BN rats offers a potential mechanism for the impaired responsiveness of the kidney to AVP in aged rats of this strain. The V2 subtype of the AVP receptor is known to be essential for stimulating cAMP-mediated membrane insertion and abundance of AQP2 via Gsα activation of adenylate cyclase. However, in many physiological systems, including this one, there is not a linear relationship between...
receptor density and signaling due to the presence of “spare” receptors. For example, decreases in V2R mRNA and ligand-induced desensitization, but despite such desensitization AVP-treated rats are able to maintain maximal AQP2 expression and urine osmolalities even though V2R binding sites are decreased by approximately one-third (4, 25, 46). However, at some critical level of decreased V2R expression, signal transduction in the collecting duct cells is impaired with subsequent impaired maximal urinary concentration, as demonstrated by patients with nephrogenic diabetes insipidus as a result of mutations of the V2R gene. Although the exact relationship between V2R expression and downstream cellular events such as AQP2 stimulation is not known, recent studies of transgenic mice in which the V2R gene was replaced with a nonsense mutation suggest that one cause of age-associated defects in urine concentration appears to be a decreased maximal water permeability of the renal collecting duct. Because these changes in renal concentrating ability occurred in the absence of changes in circulating plasma AVP levels but in parallel with blunted expression of renal AQP2 protein and V2R mRNA, we conclude that reduced renal responsiveness to circulating AVP likely represents a significant contributing factor to the decreases in urinary concentrating ability that occur with aging in F344BN rats, consistent with the findings of previous studies using this strain (5). Our results further suggest a role for age-associated decreases in V2R expression as an underlying cause of age-associated impairments in renal concentrating ability but allow the possibility that other effects of decreased AVP V2R signaling on sodium and urea transporters in the thick ascending limb and collecting ducts may also contribute to this impairment by virtue of deleterious effects on the generation of the medullary osmotic gradients necessary for maximal urinary concentration.

ACKNOWLEDGMENTS

We thank Dr. M. A. Knepper [LKEM, National Institutes of Health (NIH), Bethesda, MD] for providing the anti-AQP2 antibody, Dr. C. Maric (George-town University) for the renal histological analysis, and Dr. K. Sandberg (Georgetown University) for critical review of this manuscript.

GRANTS

This work was supported by National Institute on Aging Grant AG-19500 (to Y. Tian) and National Institute of Diabetes and Digestive and Kidney Diseases Grant DK-38094 (to J. G. Verbalis).

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