Aging-related impairment of urine-concentrating mechanisms correlates with dysregulation of adrenocortical angiotensin type 1 receptors in male Fischer rats

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Ji H, Zheng W, Wu X, Speth RC, Verbalis JG, Stein LM, Yosten GL, Samson WK, Sandberg K. Aging-related impairment of urine-concentrating mechanisms correlates with dysregulation of adrenocortical angiotensin type 1 receptors in male Fischer rats. Am J Physiol Regul Integr Comp Physiol 310: R513–R521, 2016. First published December 23, 2015; doi:10.1152/ajpregu.00131.2015.—To investigate age-associated impairments in fluid homeostasis, 4-mo (young) and 32-mo (old) Fischer 344/BN male rats were studied before and after a dietary sodium load. Transferring young rats from a low-sodium (LS) to a high-sodium (HS) diet increased water intake and urine volume by 1.9- and 3.0-fold, respectively, while urine osmolality and plasma aldosterone decreased by 33 and 98%. Concomitantly, adrenocortical angiotensin type 1 receptor (AT1R) density decreased by 35%, and AT1aR mRNA decreased by 39%; no changes were observed in AT1bR mRNA. In contrast, the increase in water intake (1.4-fold) was lower in the old rats, and there was no effect of the HS diet on urine volume or urine osmolality. AT1R densities were 29% less in the old rats before transferring to the HS diet, and AT1R densities were not reduced as rapidly in response to a HS diet compared with the young animals. After 6 days on the HS diet, plasma potassium was lowered by 26% in the old rats, whereas no change was detected in the young rats. Furthermore, while plasma aldosterone was substantially decreased after 2 days on the HS diet in both young and old rats, plasma aldosterone was significantly lower in the old compared with the young animals after 2 wk on the LS diet. These findings suggest that aging attenuates the responsiveness of the adrenocortical AT1R to a sodium load through impaired regulation of AT1aR mRNA, and that this dysregulation contributes to the defects in water and electrolyte homeostasis observed in aging.

angiotensin type 1 receptor and angiotensin type 2 receptor messenger ribonucleic acid; urine volume; urine osmolality; aldosterone; adrenal cortex

INDIVIDUALS 65 YR AND OLDER are one of the most rapidly growing segments of the United States population. Change in the control of sodium and water balance is a major characteristic of the normal human aging process and includes a decrease in thirst, urinary-concentrating ability, and capacity to excrete water and electrolytes (32). These age-related changes in humans are also observed in animals. Aging impairs the ability of rats to excrete a sodium load (11) and to maximally concentrate urine (12). These changes in fluid and electrolyte regulation can put the elderly at increased risk for disorders of hyponatremia (due to water retention) or hypernatremia (as a result of sodium retention), which can cause central nervous system dysfunction and also negatively impact medication effectiveness, resulting in adverse clinical events and surgical outcomes as well as other physiological functions (34, 38).

The adrenal steroid hormone aldosterone plays a key role in the homeostatic mechanisms controlling fluid and electrolyte balance (28, 40). In humans (10, 21–23) and experimental studies of animal models (9), aging is associated with decreased plasma aldosterone levels. Aging-related changes in aldosterone are magnified under conditions that stimulate aldosterone secretion, indicating that not only is plasma aldosterone reduced in the old, the aldosterone responsiveness to appropriate stimuli is diminished. Sitting upright increases plasma aldosterone in both young adult and old individuals, but the magnitude of this increase is smaller in the elderly (35, 55, 63). Likewise, when sodium intake is restricted or plasma volume is reduced, plasma aldosterone levels rise to a greater degree in young adult compared with old individuals (14, 66).

Aldosterone is synthesized in adrenal glomerulosa cells within the adrenal cortex, and secretion of this hormone is regulated by sodium, potassium, adrenocorticotropic hormone, and angiotensin II (ANG II). One likely contributor to the aging-associated decrease in plasma aldosterone is an attenuation in adrenal responsiveness to ANG II since ANG II is the major controller of aldosterone production when dietary sodium is altered (30). ANG II infusion in young adult 8–10 mo of age and old (28–32 mo) Long-Evans rats increased plasma aldosterone; however, the response to ANG II was significantly smaller in the old rats compared with the young adult rats (51). These findings are not restricted to rodents, since ANG II-induced aldosterone production was lower in adrenal glomerulosa cell suspensions from old cows compared with those from young cows (50).

Aldosterone release from the adrenal cortex is primarily mediated by activation of the angiotensin type 1 receptor (AT1R). Many studies in young adult animals have shown that the adrenal AT1R plays a key role in maintaining electrolyte balance in response to changes in dietary sodium. A high-sodium (HS) diet downregulates adrenal AT1R expression and aldosterone release, whereas a low-sodium (LS) diet has the
reverse effects (2, 3). What is not well known is how aging alters the adrenal AT\(_1\)R response to dietary sodium manipulation. This study investigated the regulation of adrenocortical AT\(_1\)R protein and mRNA during the adaptation response to a sodium load as a function of age to increase our understanding of the mechanisms influencing age-associated impairments in fluid homeostasis. To maximize the sodium load, we maintained the rats on a LS diet for 2 wk before transferring them to a HS diet. We chose the Fischer 344/BN rat to avoid the confounds of age- and sodium-induced hypertension since these animals remain normotensive throughout their lifespan (7) and their blood pressure increases only marginally on a HS diet (16).

MATERIALS AND METHODS

Animals. Male Fischer 344BN rats at 4 mo (young) and 32 mo (old) of age were purchased from the National Institutes of Aging and individually housed in a temperature-controlled animal facility. All rats were maintained on a LS (0.13% NaCl) diet for 2 wk. Subsequently, all rats were then transferred to a HS (4% NaCl) diet for up to 6 days (Teklad, Madison, WI). The animals were given tap water ad libitum under controlled conditions (12:12-h light-dark schedule at 24°C). Body weight was measured daily. Animals were placed in metabolic cages for determination of daily water and food intake and collection of urine. Under isoflurane anesthesia, the adrenal was removed, trunk blood was collected by cardiac puncture, and the animals were killed by exsanguination. The Georgetown University Animal Care and Use Committee approved all procedures.

Urinary and plasma analysis. Urine osmolality was measured by freezing-point depression (model 3900 osmometer; Advanced Instruments, Norwood, MA). Plasma was collected from heparinized trunk blood and plasma sodium and potassium were determined by an EasyLyte Na/K Analyzer (MEDICA, Bedford, MA). Plasma aldosterone was measured by RIA (Coat-a-count, Siemens, Los Angeles, CA). Plasma vasopressin (AVP) content was measured by radioimmunoassay after extraction, as previously described (56, 65).

AT\(_1\)R radioligand binding. Membranes were prepared from the adrenal cortex as described previously (25, 67). Membranes (5–10 \(\mu\)g protein/tube) were incubated for 1–2 h at room temperature with increasing concentrations of the ANG II antagonist. \(^{125}\)I-labeled [Sar\(^1\),Ile\(^8\)]ANG II in the presence of 1 \(\mu\)M PD-123319, an AT\(_2\)R antagonist, to ensure only AT\(_1\)R expression was measured (67). \(^{125}\)I-labeled [Sar\(^1\),Ile\(^8\)]ANG II was prepared as previously described (61). Binding reactions were terminated by rapid filtration through a Brandel cell harvester. Specific AT\(_1\)R binding was determined by the total amount of radiolgand bound minus nonspecific binding (defined as the amount bound in the presence of 200 nM ANG II, i.e., 100 \(\times\) \(K_d\) for ANG II). Data points were obtained in triplicate. \(K_d\) and \(B_{\text{max}}\) values were determined using the one-site saturation binding nonlinear regression analysis program, PRISM (GraphPad Software).

Real-time PCR. Total RNA from adrenal cortex was extracted using TR1xol reagent (Life Technologies). First-strand cDNA was made from total RNA using the iScript cDNA synthesis kit (Bio-Rad, Hercules, CA) with Moloney murine leukemia virus RNase H\(^+\) reverse transcriptase, oligo(dT), and random hexamers. Quantitation of specific mRNAs and 18S rRNA (for control) was performed by reverse transcriptase, oligo(dT), and random hexamers. Quantitation of specific mRNAs and 18S rRNA (for control) was performed by real-time PCR using the AB1 Prism 7700 Sequence Detection System (Applied Biosystems, Foster City, CA) (67). The PCR reaction mixture consisted of RNase-free water, TaqMan Universal PCR Master Mix (Perkin Elmer Applied Biosystems), and 300 nM specific primers and 10 \(\mu\)M probe [for AT\(_{1A}\)R primers and probe: forward (F) primers 3'-untranslated region (UTR)-47F, 5'-GCA GCC TCT GCC GAC GAG ACC ATT-3', reverse (R) primer 3'-UTR-142R, 5'-CAA GAC GGC TTT GCT TGG TTA-3', and probe 3'-UTR-70T, 6-FAM-AGT GTT CAA CCT GCA ATC TTC GGA- TAMRA]; for AT\(_{1B}\)R primers and probe: forward primers 3'-UTR-38F, 5'-AGC AGA AGC CAG AGG ACC ATT-3', reverse primer 3'-UTR-142R, 5'-CAC TGA GTG CTT TCT CTT CA-3', and probe 3'-UTR-89T, 6-FAM-AGT GTT CAA CCT GCA ATC TTC GGA-TAMRA] and cDNA samples. PCR conditions were optimized for the two probes (232T and 89T) and sets of primers (47F and 191R and 38F and 142R) using control cDNAs. The specificity of these primers was confirmed using the AT\(_1\)R and AT\(_{1B}\)R in pCR3 (Invitrogen, Grand Island, NY); we did not detect any amplified products using AT\(_{1A}\)R specific primers in the AT\(_{1A}\)R-expressing cells and vice versa. The expression of 18S rRNA, AT\(_{1A}\)R, and AT\(_{1B}\)R mRNA in each sample was quantitated using the specific primers specified above. PCR reactions without reverse transcription were included to control for contamination by genomic DNA. The standard curves for 18S rRNA, AT\(_{1A}\)R, and AT\(_{1B}\)R mRNA were made from a series of 10 dilutions (5\(^5\), 5\(^4\), 5\(^3\), 5\(^2\), 5\(^1\), and 5\(^0\)) for each cDNA. The tissue levels of these cDNAs were calculated based on the standard curves.

Statistics. Data are expressed as means \(\pm\) SE and in some cases as the ratio of the parameters measured under HS and LS dietary conditions at day 0. Statistical significance of the differences between groups was assessed by Student’s t-test and two-way ANOVA. Differences were considered significant at \(P < 0.05\).

RESULTS

Body weight. Young male rats were approximately one-half the body weight of old rats (Fig. 1A). Switching from a LS to a HS diet reduced body weight in the old but not the young rats (\(P < 0.0001\), young vs. old by 2-way ANOVA) (Fig. 1, A and B). When the data were normalized to body weight on day 0 of the HS diet, the body weight of the old rats dropped by 12% on day 2 and remained reduced on day 6 (Fig. 1B).

Water and food intake. Young rats rapidly increased their water intake by 1.9-fold on day 2 after switching to a HS diet and remained increased on day 6 (Fig. 2, A and B). Water...
intake on the LS diet was 51% less in the old rats and remained lower after switching to a HS diet compared with the young rats \((P < 0.0001, \text{young vs. old by 2-way ANOVA})\) (Fig. 2A). In response to the HS diet, the old rats also increased their water intake although in a slower manner compared with the young rats; water intake increased by 1.4-fold on day 2 and by 1.9-fold on day 6 (Fig. 2B).

Young rats responded to the HS diet by decreasing their food intake by ~30% on days 2 and 6 (Fig. 2, C and D). Old rats decreased their food intake to a larger extent than young rats: 52% less food on day 2 of HS and 36% less food on day 6 \((P < 0.01, \text{young vs. old by 2-way ANOVA})\). This accounts for the loss of body weight in old rats in contrast to the minimal changes in body weight observed in the young rats.

There was no difference in the NaCl intake per kilogram body weight per day between the young and old rats on the LS diet (Fig. 3). The consumption of NaCl per kilogram of body weight increased dramatically in both age groups with the HS diet on days 1-2 and 3-6, but the young rats increased their consumption of NaCl more than the old rats \((P < 0.01\) by 2-way ANOVA). The young rats continued to increase their NaCl consumption on days 3-6 relative to days 1-2 on the HS diet \((P < 0.01\), but the old rats did not significantly increase their NaCl consumption from days 1-2 to days 3-6.

\textbf{Urine volume and osmolality.} In young rats, urine volume increased by threefold 2 days after being transferred from a LS to a HS diet and remained elevated on day 6 (Fig. 4., A and B). Urine volume in the old rats was similar to the young rats on day 0 of the HS diet; however, urine volume did not increase after switching to the HS diet \((P < 0.0001, \text{young vs. old by 2-way ANOVA})\) (Fig. 4A).

Urine osmolality in 4-mo-old rats decreased by 33% 2 days after being transferred from a LS to a HS diet (Fig. 4, C and D). Four days later, the urine osmolality was indistinguishable from day 0 in the young rats (Fig. 4, C and D). No significant differences in urine osmolality were detected between young and old rats maintained on a LS diet for 2 wk (Fig. 4, C and D). In contrast to the young animals, urine osmolality in the old rats was not attenuated in response to switching to a HS diet (Fig. 4D).

\textbf{Plasma sodium and potassium.} There were no differences in plasma sodium between the young and old rats (Fig. 5A) or in plasma potassium (Fig. 5C) between young and old rats maintained on the LS diet. There were also no detectable differences in the magnitude of the plasma sodium increase in response to the HS diet in young (1.2-fold) and old (1.2-fold) rats on day 6 (Fig. 5B).

Transferring to a HS diet lowered plasma potassium in young and old rats at 2 days (Fig. 5C). On day 6, plasma potassium returned to levels observed before the sodium load in the young rats. In contrast, plasma potassium remained lower than before the sodium load in the old rats \((P < 0.002, \text{young vs. old by 2-way ANOVA})\) (Fig. 5D).

\textbf{Plasma aldosterone.} In the young rats, plasma aldosterone decreased by 98% 2 days after being transferred from a LS to

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**Fig. 3.** Effect of age on dietary sodium intake in response to a HS diet. Dietary sodium intake was normalized to body weight, and the results are expressed as means ± SE on days 0, 2, and 6 after the onset of a HS diet; \(* P < 0.05\) vs. day 0 within the same age groups; \#P < 0.05 vs. young rats on the same day; \(n = 6\) group.

**Fig. 2.** Effect of age on water and food intake in response to a HS diet. Water \((A \text{ and } B)\) or food intake \((C \text{ and } D)\) was normalized to body weight, and the results are expressed as means ± SE \((A \text{ and } C)\) or HS-to-LS ratio \((B \text{ and } D)\) on days 0, 2, and 6 after the onset of a HS diet; \(* P < 0.05\) vs. day 0 within the same age groups; \#P < 0.05 vs. young rats on the same day; \(n = 6\) group. BW, body weight.
a HS diet (Fig. 6, A and B). Six days later, plasma aldosterone remained reduced to the same extent. Although the amount of the reduction in plasma aldosterone after switching to HS was similar in the old rats (87% by day 2 and 84% by day 6), plasma aldosterone was 45% less in the old rats compared with the young rats before switching to the HS diet.

**Plasma AVP.** In young rats, plasma AVP levels increased after 6 days on HS, but the difference was not statistically significant (LS: 3.0 ± 0.8 pg/ml, n = 4; HS: 3.9 ± 0.4, n = 4). Similarly, there was no significant difference in plasma AVP levels in old rats on LS (7.2 ± 1.4, n = 4) vs. HS (6.5 ± 1.1, n = 3). Plasma AVP levels were significantly higher in old vs. young animals on either the LS or HS diets (P < 0.05).

**Adrenocortical AT1R density.** Radioligand binding assays on adrenocortical membranes using 125I labeled [Sar1, Ile8]ANG II revealed that the density of AT1R in the adrenal cortex of young rats was decreased by 35 and 43%, respectively, on days 2 and 6 after switching to the HS diet (Fig. 7, A and B). AT1R densities were 29% less in the old rats before switching to the HS diet, and AT1R densities were not reduced as rapidly in response to a HS diet compared with the young animals (P < 0.001, young vs. old by 2-way ANOVA). In fact, a significant

![Fig. 4. Effect of age on urine volume and osmolality in response to a HS diet. Urine volume (A and B) or urine osmolality (C and D) was normalized to body weight, and the results are expressed as means ± SE (A and C) or HS-to-LS ratio (B and D) on days 0, 2, and 6 after the onset of a HS diet; *P < 0.05 vs. day 0 within the same age group; #P < 0.05 vs. young rats on the same day; n = 7–8/group.](image)

![Fig. 5. Effect of age on plasma sodium and potassium in response to a HS diet. Plasma sodium (A and B) or plasma potassium (C and D) is expressed as means ± SE (A and C) or HS-to-LS ratio (B and D) on days 0, 2, and 6 after the onset of a HS diet; *P < 0.05 vs. day 0 within the same age groups; #P < 0.05 vs. young rats on the same day; n = 7–8/group.](image)
adolescence was not observed until day 6 in the old rats (Fig. 7). Two-way ANOVA showed no significant differences in binding affinity as a function of age or days on the HS diet, although a trend to a greater reduction in NaCl intake (mg/kg body wt) in the old rats relative to the young rats (Fig. 3).

Our findings support previous studies in male Fischer 344 rats subjected to dehydration. Maximum urine electrolyte concentration after 40 h of dehydration was significantly lower in old, 23-mo rats compared with 4-mo rats (12). Furthermore, the fraction of infused sodium excreted during and after expansion with isotonic saline was attenuated in old (22–24 mo) rats compared with the young (4–6 mo) rats (11). Similar findings of an impaired ability to excrete sodium with volume expansion are observed in humans. Elderly men placed on a sodium-restricted diet had lower urine osmolality than young men (36).

**Discussion**

The main finding of this study is that aging impaired the adrenal AT1R response to a dietary sodium load in male Fischer rats. Adrenal AT1R densities and AT1bR mRNA were 29 and 48% less, respectively, in old rats before the sodium load, and AT1R densities were not reduced as rapidly in response to a HS diet compared with the young animals. These age-associated effects on adrenal AT1R densities and AT1bR mRNA levels correlated with reduced water intake and plasma aldosterone with little change in urine volume, urinary osmolality, or plasma AVP.

In response to an increase in dietary sodium, urine volume increases in an effort to rid the body of excess sodium; however, this ability to increase urine volume is impaired in the elderly (35). These findings in humans are also observed in experimental models of aging. Previous studies have shown that an intracarotid injection of hypertonic sodium chloride resulted in a blunted antidiuretic response in the old rat (20 mo) compared with the young (<6 mo) male (19). Our study in male Fischer 344/BN rats extends these findings by demonstrating that, in response to a dietary sodium load, compared with the young rats, the old animals exhibited a diminished ability to rapidly increase water intake (Fig. 2, A and B) and urine volume (Fig. 4, A and B), which resulted in a decreased ability to rapidly lower urine osmolality (Fig. 4, C and D) and maintain plasma potassium homeostasis (Fig. 4, C and D). This diminished ability to handle a dietary sodium load may have contributed to the greater reduction in NaCl intake (mg/kg body wt) in the old rats relative to the young rats (Fig. 3).

Plasma aldosterone is expressed as means ± SE (A) or HS-to-LS ratio (B) on days 0, 2, and 6 after the onset of a HS diet; *P < 0.05 vs. day 0 within the same age groups; #P < 0.05 vs. young rats on the same day; n = 7–8/group.
Previous studies have shown that aging alters aldosterone metabolism. While aging had little effect on plasma aldosterone on an unrestricted sodium diet, men and women greater than 50 yr of age had markedly lower plasma aldosterone levels on a LS diet compared with those who were 20–30 yr old (23). Furthermore, urinary aldosterone excretion in 70- to 90-yr-old men was significantly less than found in 18- to 28-yr-old men (21). Urinary excretion of other adrenal mineralocorticoids was shown to be inversely correlated with increasing age from 20 to 70 (64). Consistent with this clinical research, we found that plasma aldosterone in the old rats maintained for 2 wk on a LS diet was nearly one-half the level found in the young rats (Fig. 6).

Not only did we observe lower levels of plasma aldosterone in the old rats on a sodium-restricted diet, we also found the density of adrenocortical AT1R was markedly lower in the old rats compared with the young animals (Fig. 7). We previously demonstrated that a positive correlation exists between the modulation of adrenal AT1R density and plasma aldosterone; a 30% reduction in adrenocortical AT1R density induced by 17β-estradiol in ovariecotomized female rats was associated with significant reductions in plasma aldosterone (53). Taken together, our findings suggest the age-associated decline in plasma aldosterone is due to reduced adrenocortical AT1R densities. This observation supports previous studies showing that the adrenal zona glomerulosa in humans (42, 45), rats (9, 20), and cows (50) undergoes an age-dependent impairment in its aldosterone secretory response to ANG II. While our study was conducted solely in male rats, we expect adrenocortical AT1R densities would also be decreased in old female Fischer 344/BN rats since aging (22–24 mo) in female Long-Evans rats was associated with diminished aldosterone secretion in response to ANG II (51).

In contrast to our findings in the adrenal cortex, a previous study in Fisher 344 rats reported higher immunoreactive AT1R protein expression in the adrenal medulla in 24-mo-old rats compared with young adult animals (17). However, this previous study measured immunoreactive AT1R protein expression by Western blot rather than receptor density by radioligand binding, and several studies have challenged the specificity of available AT1R antibodies (13, 24). Thus, it is possible that immunoreactive AT1R protein expression does not correlate with receptor density due to antibody nonspecificity, posttranslational regulation, and/or because of discordant regulation of the AT1R in the adrenal cortex and adrenal medulla. Moreover, it is important to note that the AT1R that mediates aldosterone secretion is located in the zona glomerulosa cells and not the adrenal medulla. The adrenal cortex is not the only tissue reported to exhibit an age-related decline in AT1R density. AT1R densities determined by autoradiography were found to be diminished by >50% in the paraventricular nucleus and by ~35% in the organum vasculosum laminae terminalis in 20-mo Fischer 344 rats compared with 5- to 15-mo rats (54).

ANG II (via its actions on AT1Rs in the adrenal cortex) is the major regulator of plasma aldosterone during altered sodium intake. Early studies showed that adrenal AT1Rs are upregulated by ANG II and sodium restriction (4–6). Thus, the age-related reduction in adrenocortical AT1Rs is likely a response to decreased plasma ANG II as a function of aging. Although we had insufficient sample material to measure plasma ANG II in this study, reports in male rats have shown that there is an age-related decrease in the rate-limiting step in ANG II formation, i.e., plasma renin activity (37) as well as serum angiotensin-converting enzyme activity (41). Furthermore, plasma ANG II and plasma renin activity are both lower in the elderly compared with young individuals (44). Studies have also shown that the dipsogenic response to ANG II administered subcutaneously was more robust in rats at 3 mo of age compared with 12, 20, or 24 mo of age (60).

Aging is associated with reduced plasma potassium levels (47) and lower fractional excretion of potassium in men and women greater than 50 yr of age (41, 42). Studies have shown that plasma ANG II formation, i.e., plasma renin activity (37) as well as serum angiotensin-converting enzyme activity (41). Furthermore, plasma ANG II and plasma renin activity are both lower in the elderly compared with young individuals (44). Studies have also shown that the dipsogenic response to ANG II administered subcutaneously was more robust in rats at 3 mo of age compared with 12, 20, or 24 mo of age (60).
women (43). The elderly are at greater risk for impaired potassium homeostasis than young adults (48, 49). The AT_{1}R plays a critical role in maintaining plasma potassium levels. In this study, we found that the HS diet substantially reduced adrenal AT_{1}R densities after switching from the LS to the HS diet in both young and old rats; however, young rats were able to reduce the density of adrenocortical AT_{1}Rs within 48 h while old rats took at least 6 days to achieve the same magnitude effect (Fig. 7). These data suggest that aging impairs the ability to rapidly downregulate the AT_{1}R in response to an increase in dietary sodium. Therefore, impairment in adrenocortical AT_{1}R regulation could contribute to the reduced ability of the elderly to respond appropriately to a sodium load. Furthermore, the attenuation of rapid AT_{1}R regulation was coincident with the impairment in urine-concentrating ability and the finding that plasma potassium fell in response to increased dietary sodium in old but not young rats (Fig. 5, C and D). Taken together, these findings suggest that the slower ability of adrenal AT_{1}Rs to downregulate in response to sudden increases in dietary sodium results in a more sluggish water intake and urine-concentrating response and subsequent impaired potassium homeostasis.

There are two subtypes of the AT_{1}R (AT_{1a}R and AT_{1b}R) in rats and mice. These receptor subtypes share 95% amino acid homology (57), and there are no currently available pharmacological agents that can effectively differentiate between their protein expression; however, the mRNA levels of these receptor subtypes can be distinguished (33). Studies have shown that the AT_{1a}R mRNA is widely expressed throughout rodent tissues and, in general, is far more abundant than the AT_{1b}R mRNA except in a few tissues, including the adrenal cortex where PCR amplification (27, 59) and in situ hybridization (26) showed the majority (80%) of adrenal AT_{1}R is of the AT_{1b}R subtype. Therefore, we expect the adrenal AT_{1b}R plays a greater role in the response of the adrenal AT_{1}R to a sodium load than the AT_{1a}R subtype; however, we cannot rule out that the minority population of adrenal AT_{1a}Rs also contributed to the age-associated dysregulation of adrenal AT_{1a}Rs.

Old rats express 48% less AT_{1b}R mRNA in the adrenal cortex than the young rats under conditions of sodium restriction. Therefore, the lower density of adrenocortical AT_{1}Rs is likely the result of an age-related reduction in the transcription of the AT_{1b}R mRNA, since lower mRNA levels could lead to less mRNA translation into AT_{1b}R protein. As a consequence, fewer AT_{1b}Rs would be available in the old rat adrenal cortex to stimulate aldosterone secretion in response to ANG II. This age effect on plasma aldosterone would be magnified under LS conditions, since ANG II levels would be elevated and ANG II-induced aldosterone secretion would be increased compared with high dietary sodium conditions in which ANG II levels are suppressed and aldosterone secretion is maximally inhibited (4). In contrast, the AT_{1a}R subtype is not likely to contribute to age effects on adrenal AT_{1}R function, including aldosterone secretion, since no differences were observed in the mRNA expression of the AT_{1a}R transcript in the adrenal cortex between young and old rats on a LS diet. This differential regulation of the two subtypes is not surprising given that these two receptor subtypes have been shown to be coordinately regulated by dietary sodium in other tissues, including the brains of adult rats (58) and mice (15), suggesting that tissue-specific receptor subtype regulation occurs.

Our finding that the HS more rapidly downregulates the AT_{1b}R transcript in young compared with old rats and strongly correlates with changes in AT_{1}R densities in the young and old animals suggests that impairment in the transcriptional regulation of the AT_{1b}R contributes to the age-associated defect in AT_{1}R regulation in response to increased dietary sodium. Decreased mRNA expression levels could also reflect increased receptor mRNA turnover since receptor expression is known to be regulated at the level of mRNA stability. For example, ANG II downregulates AT_{1}R densities in vascular smooth muscle cells by decreasing mRNA stability (31).

Aging blunts thirst and alters AVP responsiveness to physiologically relevant stimuli (8). Although originally controversial, it has now been demonstrated in numerous studies that, in rodents and humans, plasma AVP increases with aging, and indeed aging has been characterized as a state of relative AVP resistance (8, 39). Our results (Fig. 2, A and B) support prior findings and demonstrate not only an age-associated decrease in water intake, but also a blunted drinking response to HS diet. Additionally, our results indicate that AVP levels, while elevated in old animals compared with young animals, do not change in response to a dietary sodium load. Although much more work is needed, in particular a detailed examination of the time course of potential changes in plasma AVP as animals adjust to the increased salt intake, our preliminary data suggest that, in the case of the young rats, renal mechanisms accommodate the increased salt load so that there is no stimulus for additional AVP secretion. In old animals, on the other hand, it is possible that AVP secretion is already maximally elevated, and the switch to HS fails to alter the already activated state. It would be interesting as well to determine at the cellular level whether production of AVP and the other neurohypophysial hormone, oxytocin, changes with HS in young vs. old rats. Oxytocin is known to exert anorexigenic actions in rodents (46), and central release of oxytocin has been demonstrated in rats following osmotic stimuli (29, 62). If oxytocin released centrally in response to HS in our animals is the reason for the maintained suppression of food intake in the old rats as in the young animals, then, unlike AVP, it would not appear that aging is an oxytocin-resistant state. There is evidence that, unlike AVP, plasma levels of oxytocin do not significantly differ between 3- and 32-mo-old rats (18).

We did not measure blood pressure in this study; however, the male Fischer 344/BN rat is not a model of dietary sodium-induced hypertension. Chugh et al. (16) showed in young (2 mo) and old (20 mo) male Fischer 344/BN rats that a HS (8% NaCl) diet for 4 wk increased systolic blood pressure by <5 mmHg. Therefore, it is unlikely that the age-associated effects were due to hypertension.

**Perspectives and Significance**

In conclusion, the age-related decline in AT_{1b}Rs in the adrenal cortex contributes to reduced water intake and plasma aldosterone levels during conditions of sodium restriction and dysregulation of AT_{1}R-mediated responses to sodium loading, including water intake, urine-concentrating ability, and potassium homeostasis. These findings suggest that dysregulation of adrenocortical AT_{1}Rs in response to dietary sodium manipulation contributes to the defects in water and electrolyte homeostasis observed in the old Fischer male rat. These findings
may have clinical implications for dietary sodium consumption by the elderly and suggest this population could be more susceptible to the adverse consequences of a HS diet.

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DISCLOSURES
No conflicts of interest, financial or otherwise, are declared by the authors.

AUTHOR CONTRIBUTIONS

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