Aging and fluid homeostasis in rats

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Rowland, Neil E., Annie Morien, Mircea Garcea, and Melvin J. Fregly. Aging and fluid homeostasis in rats. Am. J. Physiol. 273 (Regulatory Integrative Comp. Physiol. 42): R1441–R1450, 1997.—The capacity of aging rats to defend body fluid homeostasis in response to a variety of dipsogenic and natriurexigenic stimuli was assessed. Male and female rats of both the Fischer 344 (FR) and Sprague-Dawley (SD) strains were used and tested at target ages of 5, 10, 15, and 20 mo in both longitudinal and cross-sectional studies. There were no consistent age-related declines in water intake in response to water deprivation or acute administration of hypertonic NaCl; angiotensin (ANG) I, II, III; or isoproterenol. Likewise, there were no major impairments in either urinary excretion of the hypertonic NaCl load or excretion of water or hypotonic NaCl loads, although the latter were excreted more slowly in the older cohorts. The preference/aversion functions for NaCl solutions differed between SD and FR rats, but did not change with age except in male FR rats that lost their aversion to dilute NaCl at 20 mo of age. Intake of hypertonic NaCl solution after acute sodium depletion (furosemide treatment) showed a partial decline with age, and the older rats sustained larger estimated sodium deficits after a 6-h repletion period. A more complete age-related decline was observed in the intake of hypotonic NaCl stimulated by chronic dietary administration of a kininase II inhibitor (ramipril). Male rats of 15–20 mo of age showed no ramipril-induced sodium appetite. Brain ANG II receptor density, determined by autoradiography, declined by almost 50% in the paraventricular nucleus at 20 mo of age and declined slightly in the organum vasculosum laminae terminalis but did not decline in either the supraoptic nucleus or subfornical organ. Thus the major deficits in fluid intake in aging rats are related to salt appetite; the mechanism was not identified definitively.

angiotensin II; thirst; sodium appetite; dehydration; kininase II inhibitors; paraventricular nucleus; circumventricular organs; Fischer 344 rat; diurnal rhythm

Disorders of fluid balance account for a substantial fraction of hospital admissions in the elderly. The problems include edema and hyponatremia, sequelae of the known decline in renal excretory capacity, as well as dehydration. Several laboratory studies have shown that dehydration is associated with obtunded thirst sensation in the elderly compared with young controls and a commensurately inadequate intake in the face of systemic dehydration (17, 21, 24–26). Other studies found no age-related decline in water or fluid intake (6, 7, 38).

To address these discrepancies, as well as to study underlying mechanisms, we have undertaken a comprehensive study of fluid intake and excretion in aging rats. The physiological and neurobiological controls of fluid intake are reasonably well known in this species. In particular, water intake has at least two mechanisms, osmometric and volumetric, that can be engaged either alone or in combination (14). Sodium appetite is also crucial for restoration of fluid volume (30). Thus in the present studies we present physiological data on fluid excretion and behavioral data from tests of thirst and sodium appetite. To determine the potential brain responsiveness to angiotensin (ANG)-related stimuli, we also measured the abundance of ANG II receptors in discrete brain regions.

Rats of the Fischer 344 (FR) stock are used frequently in aging research (13), but we have shown previously that these rats differ from outbred stock such as Sprague-Dawley (SD) in some aspects of fluid intake (4, 31). These include an aversion to isotonic NaCl solution when presented as a choice with water and reduced drinking to administration of ANG III. Thus we deemed it necessary to study both the FR and SD stocks, the latter being commonly used in studies of fluid intake. This work has appeared in abstract form (28). We have also published a report on studies with kininase II inhibitors (29) using additional rats not included in this paper.

METHODS

Animals and housing. At the start of the study, 32 male and 32 female rats each of the SD and FR stocks were purchased from Harlan Industries (Indianapolis, IN) at 3 mo of age. They were randomly divided into four equal cohorts consisting of eight rats of each stock and gender. At target ages of 5, 10, 15, and 20 mo, one of these cohorts was administered a battery of tests lasting 8 wk and then killed (at 7, 12, and 17 mo of age). Immediately thereafter, a replacement cohort (8 of each stock and gender, ~3 mo of age) was purchased. The replacement cohorts remained untested until the remaining original cohort was 20 mo of age, and then all were tested simultaneously.

Thus our design had two cross-sectional parts. One tested different cohorts of rats at target ages, but all rats were from the same initial population. The other was a simultaneous test of rats of the target ages, but each age cohort was purchased at different times. The first of these is not strictly a longitudinal study in the sense that the same animals were tested at each age, but it will be referred to as longitudinal to distinguish it from the second design, which is truly cross-sectional.

All rats were housed individually in suspended stainless steel cages (7 × 7 × 10 in.) above pine shavings. Purina Rodent Chow 5001 pellets and tap water were available at all times unless stated. The vivarium was conventional and dedicated to this aging colony. A reversed 12:12-h light-dark cycle (lights off 1000) was in effect so that the testing could be conveniently performed at the start of the night cycle. Ambient temperature was 23 ± 2°C. The rats were handled periodically throughout the study so that they remained docile.

Procedures: General. The test battery can be divided into three parts. In the first, we examined urinary excretion and water intake in response to a variety of stimuli, generally with 3–4 days between each test. In the second, NaCl

† Deceased 13 January 1996.
preference and appetite were tested. In the third and terminal part, brain or tissue measures were performed.

Procedures: Urinary excretion and water intake. In the first two tests, the rats were given intraperitoneal injections (3% body wt and warmed to 37°C) of either water or 0.075 M NaCl. The rats were placed in clean cages on a metabolic rack and urine volume was measured hourly for 6 h. An aliquot of the 6-h urine was saved for determination of Na⁺ and K⁺ concentrations by flame photometry.

The next test measured urine output during and water intake after 24-h water deprivation. Cages were again placed on a metabolic rack, and urine was collected over the 24-h period during which food was present. The rats were also weighed at the start and end of the 24 h. At the end of this time, urine volume was noted and an aliquot was saved for Na⁺ and K⁺ analysis. The food was then removed and a calibrated drinking tube (a burette with a metal sipper stopper) was presented at the front of the cage. Water intake was recorded after 1 h.

The next test measured water intake and urinary excretion in response to acute injection of hypertonic NaCl. For this and all subsequent drinking tests, food was removed during the test. Cages were again transferred to a metabolic rack. The rats were injected with 2 M NaCl (1 ml/kg, 0.2% lidocaine to minimize any pain). Water intake was recorded after 0.5, 1, and 2 h, urine volume was recorded after 2 h, and an aliquot was taken for Na⁺ assay.

The next four tests measured water intake and urine output as above, except that urine samples were not assayed. The dipsogenic agents were isoproterenol (25 µg/kg sc; expressed as the HCI salt of the racemate purchased from the Sigma Chemical, St. Louis, MO) and ANG I (250 µg/kg), II (200 µg/kg), and III (900 µg/kg). ANG peptides were the Ile5 forms from Sigma and injected subcutaneously. The dose of ANG III is higher in part because this peptide apparently sticks to plastic ware; these doses were chosen on the basis of previous studies (4) to be in the mid-to-high range of behavioral efficacy.

Procedures: NaCl preference and appetite. The preference test consisted of a two-bottle choice between distilled water and an ascending series of NaCl concentrations: 0.05, 0.15, and 0.45 M. Each was presented for 4 days, alternating the side of presentation daily. The food present during this test was Purina Chow in powdered form, available in glass jars secured inside the cages with wire springs.

Immediately after the last day of the preference test, the food was changed to chow to which the orally active kininase II inhibitor (15) ramipril maleate (0.1 g/kg) had been mixed thoroughly. Intakes of 0.45 M NaCl and water were recorded daily for 6 days.

At the end of the experimental series to be described below, and in only male SD rats, one-half at each age were again given 0.05% ramipril chow and the other one-half remained on regular food. As before, 0.45 M NaCl and water were available. On day 5 after the final intake measure, rats were sedated by inhalation of methoxyflurane and a blood sample (0.3 ml) was taken by heart puncture and placed in chilled, EDTA-treated tubes. Plasma was then separated and stored at −20°C. Plasm was then separated and stored frozen for subsequent analysis of plasma renin activity (PRA) using a commercially available ANG I radioimmunoassay kit (DuPont).

After the main ramipril study, rats were then allowed 1 wk to recover. They were then placed in clean cages on a metabolic rack and were acutely treated with furosemide (two injections, 2 h apart, 1 ml/kg of a 10 mg/ml solution of Lasix). They were fed pelleted sodium-free diet (Hartroft diet; Teklad) and distilled water for the next 24 h. Urine was collected after 6 h for Na⁺ assay. At 1100 the next day, rats were presented with 0.05 M NaCl in addition to water and sodium-free food. Intake of NaCl was recorded hourly for 6 h and again after 24 h. Tests of NaCl appetite commonly use hypertonic, nonpreferred concentrations, such as in the ramipril study above. However, using such a solution, furosemide-treated young rats drink two to three times more than their sodium deficit within 1–2 h. In contrast, repletion of the sodium deficit is more gradual and matched to the deficit when hypotonic NaCl is presented (32) and we chose this paradigm as potentially more sensitive for screening possible age-related declines in acute sodium appetite.

Procedures: Brain and tissue measures. At the end of the cross-sectional study, all of the rats were killed. They were first anesthetized with pentobarbital sodium (100 mg/kg) and perfused intracardially with chilled phosphate-buffered saline. The brains were removed and frozen at −80°C for subsequent autoradiographic determination of ANG II receptor density (27). The heart and kidneys were inspected grossly and weighed.

The brains were cut coronally into 20-µm slices from just rostral to the lamina terminalis through the posterior hypothalamus using a Hacker Instruments cryostat. Alternate sections were thaw-mounted onto gelatin-coated slides. Slides were then preincubated at room temperature for 30 min in a buffer containing 150 mM NaCl, 5 mM EDTA, 0.1 mM bacitracin, and 50 mM buffered sodium phosphate (pH 7.1). The slides were then placed into one of the following three solutions: for determination of total binding, buffer containing 300 µM [125I]-labeled [Sar1,Ile8] ANG II; for determination of nonspecific binding, 300 µM [125I]-[Sar1,Ile8] ANG II plus 1 µM unlabeled ANG II; for determination of AT-1 displacable binding, 300 µM [125I]-[Sar1,Ile8] ANG II plus 0.5 µM losartan potassium. The [125I] ligand and microscale standards were generously supplied by Dr. Robert Speth, Radiodionization Center, Pullman, WA. After a 1-h incubation, slides were dipped rapidly in three changes of distilled water, followed by five changes of buffer (1 min each) and three more rinses with distilled water.

The slides were air-dried and exposed to BetaMax Hyperfilm (Amersham) for 5 days. Films were developed for 5 min using D-19 (Kodak), washed and fixed, and allowed to dry. The autoradiograms were quantified using a computer-assisted densitometry system (MCID). Cresyl violet-stained sections were superimposed on the video image to help delineate anatomical boundaries (23). An average of six to eight density readings were taken in each area and automatically converted to femtomoles per milligram tissue using a best-fit third polynomial equation based on the radioactivity of the standards.

Data analysis and presentation. Data were analyzed using analysis of variance (ANOVA) programs (SAS PC version). Longitudinal and cross-sectional studies were analyzed separately. In general, results from both studies were essentially the same and so constitute a replication. Thus, for brevity, only results from the cross-sectional study will be presented. We expected some differences between SD and FR rats and between genders, so in most cases we present the analyses of one-way ANOVAs with age as the factor plus Duncan’s post hoc tests (P < 0.05) for significance of differences.

RESULTS

General health and survivorship. Most animals remained in good health through 18 mo of age, with only a few losses from either known or unknown causes. Rats that appeared in poor health were not tested. After 18
mo, attrition due to unidentified factors increased, especially among male SD rats. Even in these, >50% survived to 22 mo (the end of testing), which corresponds quite closely to survivorship in barrier colonies. We had foreseen these losses, so additional rats had been purchased initially to ensure adequate numbers remained in the 20-mo-old cohort. At the other end of the survivorship range only two female FR rats, euthanized due to mammary tumors, died before 22 mo of age.

Mean body weight is a good index of health of a colony. These data are shown in Table 1. Most groups gained weight slowly during the 2-mo battery of tests at each age (Table 1). Male SD at 15 and 20 mo were the exception to this rule; they lost 3–4% body weight. All drugs were injected on the basis of body weight, so absolute doses can be derived from these weight charts. Furthermore, absolute intakes can be transformed into intake per unit body weight using these numbers.

The body weights and organ weights at death in the cross-sectional cohorts are shown in Table 2. There were no age-related changes in organ weights relative to body weight. Heart, lungs, and kidneys appeared grossly normal, except for a few instances of enlarged or abnormal (brown) kidneys in the oldest groups. The most common pathological symptom of aging was the observation (made when brains were removed) of tumors or hemorrhage in the basal hypothalamus or anterior pituitary. These were of variable severity and were observed in over one-half of the 20-mo-old SD rats and in ~25% of the FR rats.

Excretion of water and 0.075 M NaCl loads. For both the water and salt load studies, three-way ANOVA revealed significant main effects of age on volume excreted (both absolute and relative to body weight) at 6 h and in the fraction (by volume) excreted at 3 vs. 6 h. These variables also showed strain and gender main effects and two-way interactions with age. To separate these for presentation and statistical analysis, each strain and sex are graphed and one-way ANOVAs (age) are presented separately in Fig. 1.

In male SD rats, the volume of water excreted per unit body weight in the 6 h after the water load did not differ across ages (F<sub>3,20</sub> = 0.89), but the older rats were slower in excreting the load (Fig. 1, bottom left) (F<sub>3,20</sub> = 0.75, P < 0.05). The fraction of the 6-h total excreted after 3 h was significantly lower in the 20-mo-old compared with either 5- or 10-mo-old cohorts. The data were similar for the NaCl load, with a lower fractional 3-h excretion (F<sub>3,20</sub> = 5.63, P < 0.01) in 20-mo-old compared with either 5- or 15-mo-old cohorts (Fig. 1, bottom right). The decline in excretion of the water load seemed to be more severe than for the saline load.

In female SD rats, the volume excreted in the 6 h after the water load, relative to body weight, was higher at 20 mo than at any earlier age (F<sub>3,20</sub> = 5.25, P < 0.01). The fraction excreted at 3 h was higher at 5 mo than at any later age (F<sub>3,20</sub> = 4.53, P < 0.05). After the NaCl load, the fractional volume excreted after 3 h was lower in the 20-mo-old cohort than at any other age (F<sub>3,20</sub> = 8.24, P < 0.001).

In male FR rats, the relative volume excreted in 6 h after the water load was lower at 15 and 20 mo than either 5 or 10 mo (F<sub>3,20</sub> = 6.35, P < 0.01), and the fraction excreted after 3 h showed an age-related decline (F<sub>3,20</sub> = 12.57, P < 0.001), with all ages lower than the 5-mo-old group and 20 mo < 10 mo. Similar declines in relative volume and fraction were not observed after the NaCl load.

In female FR rats, the 3-h fraction excreted after the water load was lower at 10 and 20 mo than at 5 mo (F<sub>3,20</sub> = 4.98, P < 0.01). The fraction excreted 3 h after the NaCl load was lower at 20 mo than at 5 and 10 mo (F<sub>3,20</sub> = 11.77, P < 0.001).

The urinary electrolyte data showed no age, gender, or strain differences and so will not be presented in detail. Summarily, after the water loads, urinary sodium concentration was low (means < 10 meq/l) and the range of group mean potassium concentrations was 21–61 meq/l. After the NaCl loads, the ranges of group mean urinary sodium and potassium concentrations were 19–51 and 55–99 meq/l, respectively.

Water deprivation. A three-way ANOVA of the data from the cross-sectional study showed urinary volume (both in ml and ml/kg wt) during 24-h water deprivation varied with age and strain, but there were no two-way interactions. The data for absolute volume (Fig. 2, left) plotted by strain and age show that the mean urine volume was highest in each of the 20-mo-
old groups (all $F_{3,20} > 5.41, P < 0.01$), and this was also true for output in milligrams per kilogram body weight (data not shown). Although differences between strains and genders are evident in Fig. 2, these are largely attributable to differences in body weight (Table 1). The urinary concentrations of Na$^+$ and K$^+$ decreased with age, but the total Na$^+$ or K$^+$ loss did not show consistent age-related trends (Table 3). Body weight loss during the 24-h deprivation did not show consistent age-related trends (Table 3).

Water intake (in ml) after deprivation showed no significant age-related changes (Fig. 2, right), although strain and sex differences were significant, reflecting mainly the differences in body weight. The present studies did not determine fluid balance directly, but comparison of the data in the left and right panels of Fig. 2 indicates that the volume of water consumed in the 1-h test was generally similar to the volume of urine lost in the previous 24 h, with the exception of the 15- and 20-mo-old SD and FR male groups whose intake was less than the fluid loss.

Hypertonic NaCl injection. The 2-h water intakes showed a significant age-related increase in FR females ($F_{3,20} = 7.27, P < 0.01$), but not in the other three groups ($F_{3,20} < 1.50$) (Fig. 3). However, intake relative to body weight did not change significantly with age in any group. Intake per unit body weight was also lower in FR than corresponding SD groups. The 2-h urinary sodium excretion showed significant age effects, with the 20-mo-old group excreting more Na$^+$ relative to body weight (and thus a greater fraction of the 4 meq/kg NaCl load) than younger ages of both strains and both genders (data not shown). Even in the oldest groups, only a fraction of the load (1.0–1.4 meq/kg) was excreted within 2 h.

Isoproterenol and ANG I, II, and III. In male SD rats, absolute water intake after injection of isoproterenol was lower at 15 and 20 mo than at 5 mo of age. The older rats were also heavier (Table 1), so the intakes expressed relative to body weight declined even more (data not shown). A similar decline in absolute and relative intakes was observed after injection of ANG.
Table 3. Urinary electrolytes and weight loss during 24-h water deprivation

<table>
<thead>
<tr>
<th>Measure</th>
<th>Age, mo</th>
<th>SD Male</th>
<th>SD Female</th>
<th>FR Male</th>
<th>FR Female</th>
</tr>
</thead>
<tbody>
<tr>
<td>[Na⁺]_{urine}, meq/l</td>
<td>5</td>
<td>301 ± 11</td>
<td>254 ± 12</td>
<td>271 ± 28</td>
<td>220 ± 15</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>177 ± 22</td>
<td>110 ± 7*</td>
<td>195 ± 14*</td>
<td>232 ± 23</td>
</tr>
<tr>
<td></td>
<td>15</td>
<td>162 ± 26*</td>
<td>141 ± 10*</td>
<td>240 ± 17</td>
<td>214 ± 2</td>
</tr>
<tr>
<td>[K⁺]_{urine}, meq/l</td>
<td>5</td>
<td>482 ± 19</td>
<td>424 ± 24</td>
<td>481 ± 41</td>
<td>503 ± 18</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>309 ± 24*</td>
<td>250 ± 11*</td>
<td>438 ± 22</td>
<td>517 ± 22</td>
</tr>
<tr>
<td></td>
<td>15</td>
<td>273 ± 38*</td>
<td>256 ± 15*</td>
<td>449 ± 24</td>
<td>493 ± 13</td>
</tr>
<tr>
<td>[Na⁺]_{loss}, meq/24 h</td>
<td>5</td>
<td>2.04 ± 0.22</td>
<td>1.33 ± 0.14</td>
<td>1.10 ± 0.13</td>
<td>0.45 ± 0.06</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>1.70 ± 0.31</td>
<td>0.87 ± 0.11*</td>
<td>1.04 ± 0.12</td>
<td>0.21 ± 0.09</td>
</tr>
<tr>
<td></td>
<td>15</td>
<td>1.63 ± 0.15</td>
<td>1.11 ± 0.09*</td>
<td>1.66 ± 0.18*</td>
<td>0.87 ± 0.08*</td>
</tr>
<tr>
<td>Body wt, g</td>
<td>5</td>
<td>7.8 ± 0.4</td>
<td>7.2 ± 0.5</td>
<td>6.1 ± 0.2</td>
<td>7.1 ± 0.4</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>6.1 ± 0.3</td>
<td>8.1 ± 0.4</td>
<td>4.5 ± 0.2*</td>
<td>5.2 ± 0.4</td>
</tr>
<tr>
<td></td>
<td>15</td>
<td>6.2 ± 0.6</td>
<td>7.5 ± 0.4</td>
<td>4.7 ± 0.4*</td>
<td>5.7 ± 0.7</td>
</tr>
<tr>
<td>Body wt loss, %</td>
<td>5</td>
<td>7.4 ± 0.9</td>
<td>8.1 ± 0.6</td>
<td>5.9 ± 0.6</td>
<td>5.8 ± 0.8</td>
</tr>
<tr>
<td>in 24 h</td>
<td>10</td>
<td>6.1 ± 0.2</td>
<td>7.2 ± 0.5</td>
<td>5.9 ± 0.6</td>
<td>5.8 ± 0.8</td>
</tr>
<tr>
<td></td>
<td>15</td>
<td>6.2 ± 0.6</td>
<td>7.5 ± 0.4</td>
<td>4.7 ± 0.4*</td>
<td>5.7 ± 0.7</td>
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<tr>
<td></td>
<td>20</td>
<td>7.4 ± 0.9</td>
<td>8.1 ± 0.6</td>
<td>5.9 ± 0.6</td>
<td>5.8 ± 0.8</td>
</tr>
</tbody>
</table>

Values are means ± SE (n = 6). Brackets indicate concn. *P < 0.05 vs. corresponding 5-mo-old group.

III. However, intakes after either ANG I or ANG II did not show any age-related changes. Urine outputs (not shown) were always lower than water intakes, with no age-related changes, so rats were in positive water balance after each test (Fig 3).

In female SD rats, there were significant declines in absolute water intakes after isoproterenol, ANG I, and ANG III, but only in the 20-mo-old cohort. When expressed relative to body weight, the declines relative to 5 mo were significant after 15 mo. ANG II drinking did not show any age-related changes. Absolute intakes in SD females were generally lower than in SD males, but in proportion to their lower body weights.

In male FR rats, absolute water intakes did not change with age. When expressed relative to body weight, isoproterenol-induced intake was reduced at 10 and 20 mo relative to 5 mo, ANG I-induced intake was reduced at 20 mo relative to 5 mo, and ANG III-induced intake was reduced at 10, 15, and 20 mo relative to 5 mo. Again, ANG II-induced intake did not change with age.

In female FR rats, no age-related changes in either absolute or relative intakes were present after any of the dipeptidases.

Spontaneous preference/aversion for NaCl solution. FR rats showed the expected avoidance of low concentrations of NaCl solution compared with the preference in SD rats. Male and female SD rats showed net preferences for the 0.05 and 0.15 M NaCl solutions over water and aversions for 0.45 M, with no age-related changes, except for an aversion to 0.15 M by the 20-mo-old SD males (P < 0.05 vs. all other ages). Male and female FR rats showed net aversions for all three concentrations of NaCl in choice with water, except for the 20-mo-old groups that showed greater intakes of NaCl than other ages, (P < 0.05). This was most evident in males, in which the 0.05 M solution was slightly preferred over water (Fig. 4).

The daily baseline intakes of water alone were not measured in this experiment. However, the data obtained in the present study, especially in the phases in which water was offered in a choice with 0.45 M NaCl, which was consumed in minimal amounts, provide an indication of basal intakes. The total intakes from all three phases are shown in Table 4. Intakes increased with age, especially in males, but at no time reached levels that would be considered hyperdipsic. When expressed as intake per unit body weight (see Table 1), the total intakes (in the 0.45 M phase) of both SD and FR males increased by ~25% between 5 and 20 mo of age, whereas females showed increases of ~10%.

Ramipril-induced sodium appetite. The mean intakes of 0.45 M NaCl at the end of the baseline period ranged from 0 to 3.6 ml/day, corresponding to preferences of 0 to 11%. With the exception of FR females, addition of ramipril to the diet stimulated NaCl intake.
in the 5- and 10-mo-old cohorts, but had little effect in the 15- and 20-mo-old cohorts (Fig. 5, top). In the SD females, the age-related decline in sodium appetite was less marked than in male SD and FR and was not statistically significant.

In the subsequent study in which PRA was measured, the behavioral results (SD males only) were as above (mean intakes of 5- and 20-mo-old ramipril groups were 15.2 and 5.0 ml 0.45 M NaCl/day, respectively, with no subtraction of baseline intake). PRA was elevated (P < 0.05) in ramipril-treated groups. Values did not differ with age: 7.9 ± 0.8 at 37°C in 15- and 20-mo-olds and 9.0 ± 1.0 ng ANG I·ml plasma⁻¹·h⁻¹ in 5- and 10-mo-old rats. Untreated control values were 1.0 ng ANG I·ml plasma⁻¹·h⁻¹.

Table 4. Daily total fluid intake during choice paradigm

<table>
<thead>
<tr>
<th>NaCl Conc</th>
<th>Age, mo</th>
<th>SD Male</th>
<th>SD Female</th>
<th>FR Male</th>
<th>FR Female</th>
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<tr>
<td>0.05 M</td>
<td>5</td>
<td>49.7 ± 5.1</td>
<td>57.7 ± 0.7</td>
<td>24.4 ± 0.9</td>
<td>21.4 ± 0.9</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>58.2 ± 5.7</td>
<td>80.2 ± 6.4</td>
<td>23.3 ± 1.3</td>
<td>24.8 ± 0.6</td>
</tr>
<tr>
<td></td>
<td>15</td>
<td>64.8 ± 8.2</td>
<td>75.9 ± 6.4</td>
<td>28.0 ± 0.8</td>
<td>28.7 ± 1.1</td>
</tr>
<tr>
<td></td>
<td>20</td>
<td>84.9 ± 6.9</td>
<td>84.6 ± 6.9</td>
<td>48.9 ± 7.5</td>
<td>31.1 ± 4.1</td>
</tr>
<tr>
<td>0.15 M</td>
<td>5</td>
<td>60.9 ± 7.3</td>
<td>72.2 ± 9.5</td>
<td>24.2 ± 0.8</td>
<td>18.5 ± 1.0</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>71.9 ± 7.8</td>
<td>99.9 ± 5.9</td>
<td>24.2 ± 1.0</td>
<td>27.7 ± 1.6</td>
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<tr>
<td></td>
<td>15</td>
<td>73.2 ± 7.9</td>
<td>92.2 ± 6.2</td>
<td>28.0 ± 0.9</td>
<td>29.3 ± 2.0</td>
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<tr>
<td></td>
<td>20</td>
<td>83.6 ± 9.7</td>
<td>109.2 ± 8.0</td>
<td>51.3 ± 6.7</td>
<td>32.8 ± 3.4</td>
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<tr>
<td>0.45 M</td>
<td>5</td>
<td>41.6 ± 4.5</td>
<td>50.6 ± 10.5</td>
<td>22.0 ± 1.3</td>
<td>16.9 ± 1.1</td>
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<tr>
<td></td>
<td>10</td>
<td>50.5 ± 10.5</td>
<td>63.6 ± 3.2</td>
<td>22.5 ± 0.5</td>
<td>23.7 ± 0.9</td>
</tr>
<tr>
<td></td>
<td>15</td>
<td>58.1 ± 7.2</td>
<td>66.1 ± 3.1</td>
<td>27.7 ± 1.1</td>
<td>25.9 ± 1.4</td>
</tr>
<tr>
<td></td>
<td>20</td>
<td>60.7 ± 5.5</td>
<td>68.9 ± 4.2</td>
<td>33.7 ± 1.4</td>
<td>29.3 ± 2.7</td>
</tr>
</tbody>
</table>

Values are means ± SE (n = 6/group) in ml/24 h (water + NaCl of shown concn.).

Fig. 5. Top: mean increase in daily intake of 0.45 M NaCl during treatment with ramipril (average of last 2 days) compared with predrug baseline intakes (shown in Fig. 4) as a function of age. Highly significant age-related declines are evident in each group. Ramipril did not stimulate NaCl appetite above baseline after 10 mo of age. Bottom: mean 3-h intakes of 0.05 M NaCl after depletion of sodium (furosemide and sodium-free diet) as a function of age. An age-related decline in intake (ml) was significant in only the male FR group.
Acute sodium depletion. In the first 6 h after furose- 
mide, the urinary sodium loss was consistently higher 
in SD than FR rats (Fig. 5, bottom; Table 5), even when 
expressed relative to body weight. The 20-mo-old 
cohorts of male SD and male and female FR excreted 
significantly more sodium than the corresponding 5-mo-
old cohorts, but, when expressed relative to body weight, 
this remained significant only for male SD (20 mo) 
significantly more sodium than the corresponding 5-mo-
olds of male SD and male and female FR excreted 
that in the subfornical organ did not. Specific (ANG II 
receptor density) binding in organum vasculosum laminae 
transversi, the urinary sodium loss was consistently higher 
during the rest of the 24-h depletion period; in other 
studies in young rats, we have found this to be <20% of 
the 6-h total.

The intake of 0.05 M NaCl in milliliters (Fig. 5) was 
slightly higher in females than males of the same 
strain, was higher in SD than FR, and showed signifi-
cant age-related declines in FR males (at 10, 15, and 20 
mo) and in SD males (at 20 mo) relative to the 5-mo-old 
groups. All rats showed a sodium appetite: concurrent 
water intake was very low in FR rats (between 79 and 
100%, median 89%, of the fluid consumed was 0.05 M 
NaCl), but was higher in SD rats (range 58–100%; 
median 74%). The 6-h intakes (not shown) were qualita-
tively similar to the 3-h intakes, but were 30–60% 
higher.

Sodium balance was not measured directly, but an 
approximation can be computed as 6-h sodium intake 
minus prior furosemide-induced (6 h) urine sodium loss 
(Table 5). After 6 h of access to 0.05 M NaCl, female rats 
(FR and SD combined) had consumed all but ~0.1 meq 
of the sodium lost in the first 6 h after furosemide. This 
deficit was higher (~0.5 meq) in 20-mo-old females. In 
contrast, the 6-h intake in males typically left them 1 
meq short of the 6-h loss, and, in 15- and 20-mo-old SD 
males, the mean deficit was 2 meq. That is, their intake 
had replaced <30% of the loss.

ANG II receptor density. ANOVA main effects, show-
ing an age-related decline, were found for AT-1 (losar-
tan displaced) binding in organum vasculosum laminae 
transversi and paraventricular nucleus (PVN; P < 
0.05). The decline in binding in the median preoptic 
nucleus approached significance (P < 0.06), whereas 
that in the subfornical organ did not. Specific (ANG II 
displaced) binding showed an age-related decline in 
PVN only (P < 0.05). Main effects of gender and rat 
strain were not significant, so these have been com-
bined for presentation in Fig. 6. Post hoc tests showed 
that binding declined in only the oldest cohorts. The

## Table 5. Urinary sodium loss in 6 h after furosemide injection and estimated sodium balance 6 h after

<table>
<thead>
<tr>
<th>Age, mo</th>
<th>SD Male</th>
<th>SD Female</th>
<th>FR Male</th>
<th>FR Female</th>
</tr>
</thead>
<tbody>
<tr>
<td>5</td>
<td>2.04 ± 0.11</td>
<td>1.46 ± 0.10</td>
<td>1.00 ± 0.05</td>
<td>0.71 ± 0.06</td>
</tr>
<tr>
<td>10</td>
<td>2.04 ± 0.14</td>
<td>1.43 ± 0.14</td>
<td>1.43 ± 0.10</td>
<td>0.81 ± 0.04</td>
</tr>
<tr>
<td>15</td>
<td>2.77 ± 0.42*</td>
<td>1.71 ± 0.21</td>
<td>1.35 ± 0.05</td>
<td>0.91 ± 0.07</td>
</tr>
<tr>
<td>20</td>
<td>2.80 ± 0.31*</td>
<td>1.60 ± 0.25</td>
<td>1.71 ± 0.27*</td>
<td>1.13 ± 0.07*</td>
</tr>
<tr>
<td>5</td>
<td>-0.83 ± 0.21</td>
<td>0.08 ± 0.17</td>
<td>-0.19 ± 0.12</td>
<td>-0.11 ± 0.09</td>
</tr>
<tr>
<td>10</td>
<td>-0.99 ± 0.28</td>
<td>-0.08 ± 0.23</td>
<td>-0.78 ± 0.10*</td>
<td>-0.26 ± 0.08</td>
</tr>
<tr>
<td>15</td>
<td>-2.00 ± 0.22*</td>
<td>-0.70 ± 0.18*</td>
<td>-1.26 ± 0.29*</td>
<td>-0.53 ± 0.10*</td>
</tr>
<tr>
<td>20</td>
<td>-2.00 ± 0.38*</td>
<td>-0.05 ± 0.14*</td>
<td>-0.70 ± 0.18*</td>
<td>-1.26 ± 0.29*</td>
</tr>
</tbody>
</table>

Values are means ± SE (n = 6). Estimated sodium balance is 6 h test intake of 0.05 M NaCl minus the previous day. *P < 0.05 vs. corresponding 5-mo-old group.

**DISCUSSION**

The principal purpose of this study was to examine 
whether there are age-related declines in fluid intake 
in response to one or more challenges to body fluid 
homeostasis. This was tested in both male and female 
SD and FR rats. There were no systematic declines in 
water intake after either water deprivation or acute 
administration of hypertonic NaCl; ANG I, II, III; or 
isoproterenol. Furthermore, renal excretion of water 
and NaCl loads was modestly slowed with age and so 
does not seem to be related to the drinking response.

These findings agree with clinical studies that have 
found no age difference in prospective or actual intakes 
of fluids after various dehydrating challenges in hu-
mens (6, 38). Because our stimuli were clearly supra-
threshold it is possible there is impaired sensitivity, for 
example at the level of osmoreceptors for thirst and/or 
vasopressin secretion (17). In very old rats (28–30 mo), 
a deficit in plasma vasopressin secretion to an osmotic 
load was related to impaired posterior pituitary stores 
of vasopressin (37). There was no apparent defect in 
transduction of the osmotic signal, because the induct-
on of Fos-immunoreactivity (Fos-ir) in the supraoptic 
nucleus (SON) remained normal. Given our finding of 
reduced ANG II receptors in PVN, but not SON at 22 
mo of age, additional studies should be performed to 
examine whether signal transduction in the PVN may 
decline selectively after 20 mo of age. We should also
note that, due to constraints inherent to the design of 
this study, we were unable to account for possible 
effects of prior tests on either behavioral or physiologi-
cal outcomes (such as ANG binding). Each rat received 
this sequence of tests, so any effects that we have 
assessed to age could potentially be an interaction 
between age and cumulative prior testing.

The most consistent and dramatic age-related de-
cline in fluid intake was the almost complete loss of 
sodium appetite elicited by ramipril at 15 and 20 mo 
of age (Fig. 5), findings that replicate and extend preli-
nary data presented elsewhere (29). Kininase II inhibi-
tors such as ramipril are hypothesized to produce 
sodium appetite via an “ANG I spillover” mechanism.
involving a paradoxical increase in ANG II in the circumventricular regions of the brain (9–11, 30, 31, 33). It is interesting in this regard that there was a decline in ANG II binding in the OVLT, a region that has been implicated in salt appetite (14, 20). A less dramatic age-related decline in NaCl appetite occurred in response to sodium depletion with furosemide (Fig. 5), especially in males who remained in an apparently substantial negative sodium balance after 6 h of access to 0.05 M NaCl: the old males excreted more sodium and consumed less NaCl than young males. Furosemide-induced sodium appetite is believed to require the synergistic action of ANG II and adrenocortical hormones (30). However, in addition to the different physiological stimuli used (ramipril and furosemide), the concentration of NaCl and the duration of the test differed between the studies. We reported (32) no difference in the volumes of dilute (0.03 M) and concentrated (0.3 M) NaCl consumed by young SD rats in the first hour after sodium depletion (with furosemide) and that intake of 0.3 M NaCl greatly exceeds the urinary losses. Thus we used hypotonic NaCl and an extended (6 h) test in the present work to use a paradigm in which intake might be closely coupled to losses and so be sensitive to possible age-related changes. It is possible that the changes we did find with 0.05 M might have been even larger if we had used 0.45 M and been more comparable to the essentially complete loss of ramipril-induced NaCl appetite.

The preference/aversion data also replicate the known aversion in male (but not female) FR rats had dissipated by 20 mo of age. One interpretation of this finding is that the intensity or quality of the taste of dilute NaCl may diminish with age in this group. We think this unlikely, however, because there are only minimal age-related declines in salt taste perception in humans (39) as well as in gustatory nerve responses in FR rats (19). In unpublished pilot work in FR rats, we found a total preference (but not hyperdipsia) for 0.15 M NaCl developed in two rats but, at autopsy, we found these rats had tumors near the basal hypothalamus. Several of the oldest rats in the present study also had small tumors, but there was no evidence of extreme NaCl preference or hyperdipsia that might have indicated a diabetes insipidus-like condition, nor were there obvious changes in renal excretion (or other behaviors) in rats with these postmortem indications compared with those lacking these conditions. An increase in total fluid intake has been reported in SD rats 22–28 mo of age (22), and our data (Table 4) partially confirm this observation. For the reasons noted, we do not believe that gross neuropathology can account for these effects. Age-related declines in water intake in response to hypertonic NaCl; ANG I, II, III; isoproterenol, or after 24-h water deprivation were not observed. We noted in the introduction that studies of thirst in healthy aging humans yielded mixed results, some showing declines and others no change. Our intake data generally favor the latter position. We reported several measures of urinary excretion (and performed some tests not reported) and found no marked age-related impairments, except for slowed output of fluid loads. It has been...
reported (36) that water intake induced by peripheral injection of ANG II was lower in old compared with young male Brown Norway rats; however, closer inspection of the data show this effect is due entirely to the higher body weight of the older rats. That is, as in our study (Fig. 4), the intake (in ml) was conserved while the relative intake (ml/kg body wt) decreased. This raises the issue of the more appropriate way to express intakes. The objective of administering the drug on a body weight basis is to achieve a consistent plasma (or tissue) level. Because the receptors involved in the dipsogenic action of peripherally administered ANG II are located exclusively in the SFO (14, 20, 34) and there is no age-related loss of ANG II receptors in this organ (Fig. 6), there is no reason to suppose that the ANG II “on” signal differs with age. We did not assess the possibility that the “off” or satiety signal(s) associated with ingestion could change with age, and it is not clear that such signals would be proportional to body weight.

We have previously reported a decline in water intake elicited by combined peripheral administration of a kininase II inhibitor and bradykinin in aged rats (29). Some aspects of this thirst seem to be related to ANG II (12) and it appears that reduced release of renal renin may underlie this behavioral deficit (29). In the present study, we confirm using ramipril (Fig. 5) the data previously obtained (29) on sodium appetite using enalapril, but we failed to confirm an associated reduction in circulating renin. The reason for this discrepancy is not clear. In this context, it is interesting that isoproterenol-induced water intake was not consistently reduced with age (Fig. 4). This agent is thought to cause drinking by both renin-related and independent (probably baroreceptor) mechanisms. We did not ascertain whether renin release to isoproterenol was obfuscated in the older groups but, if it were the case, this may indicate an increased importance of baroreceptor inputs in the aging cohorts. A decline in renal function or renin release has been noted in aging humans and rats (1, 5, 8, 16, 18).

**Perspectives**

With the steadily increasing active elderly population, especially in warmer climates, it is important to understand to what extent deficits in transduction of dehydrational signals and consequently in sensations of thirst could pose a potential health hazard. The present studies suggest there is no major neurobiological reason for defective transduction in aging. Whether there may be a systemic insensitivity, and/or a problem translating the transduced signal into thirst sensations in humans, is controversial (6, 17, 24–26, 38) and cannot be resolved by our studies.

There is one issue that was not explicitly studied, but was considered in the design of our studies. It is well known that the day/night rhythmicity of fluid intake, as well as many other biological parameters, becomes less evident with age (2). We designed our acute behavioral studies to be at the beginning of the night, when spontaneous ingestion is highest, perhaps to maximize the behavioral capacities of the aging rats.

Thus, at other times of day, these rats might have been less responsive. The PVN is one of the major regions receiving inputs from the suprachiasmatic nucleus (SCN), the principal endogenous “clock” in mammals, and so it is interesting to speculate that the decline in ANG II receptors in PVN but not SON may be secondary to changes in clock functions. Indeed, an age-related decline in melatonin binding in the SCN was correlated with an age-related decline in nocturnal drinking (40). A loss in the well-known rhythm of corticotropin-releasing hormone mRNA has been found in the PVN as early as “middle age” in rodents (3), a time at which ramipril-induced sodium appetite disappeared. Furthermore, sodium appetite induced by kininase II inhibitors is primarily nocturnal (35). Clearly, further work will be needed to examine the impact of diurnal rhythmicity in PVN functions on diurnal capacities for regulatory behaviors.

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