Age-related changes in thirst, salt appetite, and arterial blood pressure in response to aldosterone-dexamethasone combination in rats

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1Department of Psychology, University of Iowa, Iowa City, Iowa; 2Department of Health and Human Physiology, University of Iowa, Iowa City, Iowa; and 3Department of Pharmacology, University of Iowa, Iowa City, Iowa; and 4François M. Abboud Cardiovascular Center, University of Iowa, Iowa City, Iowa

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Thunhorst RL, Xue B, Beltz TG, Johnson AK. Age-related changes in thirst, salt appetite, and arterial blood pressure in response to aldosterone-dexamethasone combination in rats. Am J Physiol Regul Integr Comp Physiol 308: R807–R815, 2015. First published April 1, 2015; doi:10.1152/ajpregu.00490.2014.—This work examined the effects of age on daily water and sodium ingestion and cardiovascular responses to chronic administration of the mineralocorticoid, aldosterone (ALDO) either alone or together with the glucocorticoid, dexamethasone (DEX). Young (4 mo), adult (12 mo), and aged (30 mo) male Brown Norway rats were prepared for continuous telemetry recording of blood pressure (BP) and heart rate (HR). Baseline water and sodium (i.e., 0.3 M NaCl) intake, BP, and HR were established for 10 days. Then ALDO (60 μg/day sc) was infused alone, or together with DEX (2.5 or 20 μg/day sc), for another 10 days. Compared with baseline levels, ALDO stimulated comparable increases in daily saline intake at all ages. ALDO together with the higher dose of DEX (i.e., ALDO/DEX20) increased daily saline intake more than did ALDO, but less so in aged rats. Infusion of ALDO/DEX20 increased mean arterial pressure (MAP), and decreased HR, more than did infusion of ALDO. The changes in MAP in response to both treatments depended on age. For all ages, MAP and saline intake increased simultaneously during ALDO, while MAP always increased before saline intake did during ALDO/DEX20. Contrary to our predictions, MAP did not increase more in old rats in response to either treatment. We speculate that age-related declines in cardiovascular responses to glucocorticoids contributed to the attenuated increases in sodium intake in response to glucocorticoids that were observed in older animals.

Water: thirst; aldosterone; angiotensin II; arterial blood pressure; glucocorticoid; heart rate; mineralocorticoid; sodium ingestion

Impaired thirst is the major cause of dehydration and disturbances of fluid balance that pose major health problems for the elderly. The elderly have diminished sensations of thirst (8, 37, 38, 39), do not drink as much water in response to their perceived thirst (12, 37, 39), and may feel satiated after consuming less water in response to their thirst (12) compared with young people. Elderly people also have greater difficulty in retaining both water and sodium when they are in need of them (11, 31, 39) and also have greater difficulty excreting water when it is present in excess (8) than the young. Coupled with declining cardiovascular regulation (14, 21, 26), it is clear that the elderly suffer from a host of declines in physiological and behavioral functions concerning the regulation of body fluids and blood pressure. Like the elderly, aged rats show signs of declining thirst, renal function, and cardiovascular responding. Aged rats do not drink as much water as young rats in response to deficits of either intracellular or extracellular water (3, 40, 51, 52, 55, 56, 60). Aged rats have difficulty retaining water and sodium in times of deficit and are slower than young rats to excrete excess water and sodium (1, 4, 34, 40). Aged rats have greatly reduced baroreflex control of heart rate responses (51, 52, 55). In addition, aged rats have diminished salt appetite—the seeking and ingesting of salty substances—a companion motivation to thirst. The salt appetite response to sodium depletion is profoundly impaired—and sometimes absent—in old rats (3, 40, 54, 56); moreover, the salt appetite that arises in response to excess levels of mineralocorticoid hormones is also significantly attenuated in old rats compared with younger rats (53).

Mineralocorticoid compounds such as aldosterone (ALDO) and DOCA have long been investigated for their abilities to produce hypertension (5, 13, 18–20). The mineralocorticoids promote renal sodium retention leading to volume expansion and increased arterial blood pressure (ABP) that facilitates excretion of the sodium (7, 10, 23). In such studies, animals are often provided with weakly concentrated (e.g., 1% NaCl) saline solution as the sole drinking fluid. Providing only saline solution to drink during mineralocorticoid treatment greatly exacerbates the rise in ABP due to obligatory ingestion of large volumes of sodium with water (e.g., 6, 36). The progression of hypertension in these “ALDO/salt” or “DOCA/salt” models is well described (e.g., 5, 6, 25, 27, 36, 62–64).

Mineralocorticoids are also studied for their ability to stimulate salt appetite (33, 42, 45, 50, 53, 61, 65). In behavioral studies of salt appetite, animals are offered frankly hypertonic (typically, 1.8–3.0% NaCl) saline solutions in choice with water as the drinking fluids. Animals demonstrate salt appetite in response to mineralocorticoid treatment by ingesting increasing amounts of the normally avoided, brackish salt solution. In addition, the consumption of saline solution during mineralocorticoid treatment greatly increased by the inclusion of glucocorticoid compounds, e.g., corticosterone and dexamethasone (DEX), in the treatment regimen (33, 45, 50, 61, 65). Glucocorticoids increase the salt appetite response to mineralocorticoids through a combination of systemic (50) and central (9, 33, 45, 65) effects.

Salt appetite is inhibited both by increases in blood volume (58) and ABP (15, 49). Since mineralocorticoid treatment...
increases both blood volume and ABP, there are potential inhibitory signals related to blood volume and ABP that limit sodium ingestion during mineralocorticoid treatment. In fact, one means by which glucocorticoids increase salt appetite during mineralocorticoid treatment is by promoting urinary water and sodium excretion, thereby reducing blood volume (29, 35, 57). However, the changes in ABP during mineralocorticoid treatment in behavioral studies of salt appetite—in which animals have a choice of water or concentrated saline to drink—have not been investigated. In this regard, glucocorticoids have an intrinsic ability to increase ABP (24, 29, 35, 48, 59). It is, therefore, important to ask what the relationship is between ABP and sodium ingestion during coadministration of mineralocorticoids and glucocorticoids and, further, whether the relationship holds in aged animals. For example, the impaired ability of the aging kidney to excrete water and sodium (1, 4, 34, 40) could result in exaggerated increases of ABP during mineralocorticoid treatment that may affect sodium ingestion by old rats differently compared with younger animals. In turn, the ability of glucocorticoids to increase sodium consumption during mineralocorticoid treatment has not been assessed in aged rats. Because aged animals have elevated glucocorticoid levels (43), they may respond differently to glucocorticoid treatment than younger animals. The goal of this experiment was to establish the relationships between sodium ingestion, ABP, and age using rats. In this work, we asked 1) whether ALDO stimulates salt appetite in old, as well as young rats, 2) whether DEX increases ALDO-induced salt appetite in old, as well as young rats, and 3) whether the cardiovascular consequences of administering ALDO together with DEX are similar in young and old rats. To answer these questions, we tested the effects of ALDO given alone, or in combination with DEX, on daily intakes of water and 0.3 M NaCl, mean arterial pressure (MAP), and heart rate (HR) using young, middle-aged, and aged rats. We used DEX in these studies as it is essentially a pure glucocorticoid. DEX has potent anti-inflammatory properties and is often included as a studies as it is essentially a pure glucocorticoid. DEX has been shown to increase salt appetite in old, as well as young rats, and decreases for the old rats). However, the addition of DEX (20 μg/day) is referred to as the ALDO/DEX20 treatment condition.

Methods

Animals. Male Brown Norway (BN) rats aged 3 mo (young; n = 16), 11 mo (“middle-aged” adult; n = 17), and 28–29 mo (old; n = 15) were obtained from Harlan (Indianapolis, IN) through services provided by the National Institute on Aging. They were housed individually in hanging stainless-steel cages in a temperature-controlled room (23°C) on a 12:12-h light-dark cycle with lights on at 7:00 AM. They received ad libitum access to standard Teklad rodent diet, tap water, and 0.3 M NaCl. After 3–4 wk of adaptation, the rats were transferred to individual plastic “shoebox” cages in a separate light- and temperature-controlled room (as per above) dedicated to telemetry. The experiment began after another week of adaptation with the rats at ~4, 12, and 29–30 mo of age, respectively. All procedures were approved by the University of Iowa Institutional Animal Care and Use Committee.

Drugs. Aldosterone and dexamethasone were obtained from Sigma-Aldrich (St. Louis, MO). They were dissolved in propylene glycol (Fisher Scientific, Fair Lawn, NJ) and administered subcutaneously via osmotic minipumps (Alzet model 2002) at 0.5 μl/h. Aldosterone (60 μg/day sc) was administered alone, or together with dexamethasone (2.5 or 20 μg/day sc). For combined administration, the drugs were mixed and delivered in the same minipump.

Telemetry. Rats were chronically implanted with rat blood pressure transmitters (TA11PA-C40; Data Science International, St. Paul, MN), according to our published procedures (e.g., 62–64) to directly measure ABP in individual animals. Briefly, the rats were anesthetized with isoflurane (1%). Using a ventral incision, we isolated the right femoral artery, and the catheter of a telemetry probe was inserted into the vessel. Through the same incision, a pocket was formed along the right flank. The body of the probe was inserted into the pocket and secured with silk suture. The ventral incision was closed with wound clips.

Procedures. Water and 0.3 M NaCl were provided from 100-m1 graduated cylinders with stainless-steel drinking spouts that were attached to the front of the cages. Water and saline intakes were recorded between 0900 and 1000. Daily body weights (BW) were obtained periodically. Blood pressure acquisition began 1 wk after telemetry surgery. ABP was sampled for 2 min at the top of every hour. After 10 days of baseline measures, the rats received osmotic minipumps containing ALDO (60 μg/day) or a mix of ALDO and DEX (2.5 or 20 μg/day) for another 10 days. Water and saline intakes, MAP, and HR were measured throughout.

Statistical analysis. Data were analyzed by ANOVA with test period (baseline, infusion) and days (1–10) as within-subjects factors and age (4, 12, and 29–30 mo) and treatment (ALDO, ALDO/DEX) as between-subjects factors. Planned comparisons were made with Fisher’s least significant difference tests when the global F ratio was significant. Values were significant at P < 0.05.

Results

The results from animals receiving ALDO plus the lower dose of DEX (i.e., 2.5 μg/day) were indistinguishable from those of animals receiving ALDO by itself. Therefore, the data from the animals receiving these treatments were combined to increase statistical power and simplify presentation. All of the important effects were replicated using this analysis. As the lower dose of DEX was ineffective, we refer simply to the ALDO treatment condition to facilitate discussion. Treatment involving ALDO together with the higher (i.e., 20 μg/day) dose of DEX is referred to as the ALDO/DEX20 treatment condition.

Body weights. BW differed significantly by age throughout testing (main effect, F1,42 = 87.14; P < 0.001; Fig. 1). Middle-aged rats weighed, on average, substantially (~90 g) more than young rats, while old rats weighed slightly (~30 g) more than middle-aged rats. Infusions of ALDO produced small changes in BW (i.e., increases for the young and middle-aged rats and decreases for the old rats). However, the addition of DEX (20 μg/day) to the infusions (i.e., ALDO/DEX20) produced similar, significant losses of BW (i.e., ~2% BW per day) for all ages (main effect, F1,42 = 16.04; P < 0.001). Because of the significant age differences in BW, water and saline intakes were analyzed both as absolute and as BW-adjusted values. The results for absolute and BW-adjusted measures generally paralleled one another with only a few differences.

Daily 0.3 M NaCl intake. Baseline levels of daily saline intake did not differ with age (Fig. 2). On an absolute basis, there were no significant effects involving age. Daily saline intake increased significantly in response to both treatments (F1,41 = 20.31; P < 0.001). The combination of ALDO/DEX20 increased saline intake more than did ALDO by itself. The latency to increase saline intake was significantly shorter by 1 day during ALDO/DEX20 than during ALDO (interaction effect, F9,378 = 7.18; P < 0.001). There was considerable variability in the daily saline intake of young and middle-aged
rats during the combined treatment. The ranges of daily saline intake, averaged over the 10 days of treatment, were young: 7–40, middle-aged: 1–28, and old: 7–16 ml/day. When adjusted for BW, the increases in daily saline intake during the infusion period depended on both treatment (interaction effect, $F_{1,42} = 20.36; P < 0.001$) and age (interaction effect, $F_{2,42} = 3.46; P < 0.05$). Treatment with ALDO increased daily saline intake comparably with age. Treatment with ALDO/DEX20 increased daily saline intake significantly more than did ALDO but significantly less so with age.

**Daily water intake.** There was a significant three-way interaction of test period × treatment × age for absolute levels of daily water intake [$F_{2,42} = 4.56; P < 0.05$]. During baseline, old rats had significantly higher daily water intake than the other ages (Fig. 2). During the infusion period, ALDO significantly increased daily water intake and did so for all ages, while the combination of ALDO/DEX20 did not. However, a significant interaction involving days ($F_{9,378} = 2.59; P < 0.01$) further revealed that water intake during treatment with ALDO/DEX20 significantly decreased for a few days before significantly increasing toward the end of testing. This latter effect is seen clearly in Fig. 2. On a BW basis, middle-aged rats had significantly lower baseline daily water intake than the other ages (Table 1). As with the absolute intakes, the BW-adjusted daily water intake significantly increased during infusion with ALDO but not during infusion of ALDO/DEX20. In addition, BW-adjusted water intake during ALDO/DEX20 decreased for a few days before rising toward the end of testing.

**Daily total (i.e., water + saline) fluid intake.** On an absolute basis, there was a significant three-way interaction of test period × treatment × age ($F_{9,378} = 5.31; P < 0.001$). Daily total fluid intake increased more during ALDO/DEX20 than during ALDO alone. In addition, old rats had significantly higher average daily total fluid intake during baseline compared with the other age groups (Table 1). However, their daily total fluid intake increased less in response to either treatment compared with the other age groups. When intakes were adjusted for BW, there were significant interactions involving treatment ($F_{1,42} = 6.84; P < 0.05$) and age ($F_{2,42} = 4.11; P < 0.05$). Daily total fluid intake increased significantly more in response to ALDO/DEX20 than to ALDO alone and increased significantly less for old rats compared with the other ages. We analyzed the intakes as change scores, i.e., as average change in daily intake from average baseline levels (Table 2). On either an absolute or BW basis, ALDO increased daily water intake more than did ALDO/DEX20, and ALDO/DEX20 increased daily saline intake more than ALDO did. In addition, by either analysis, the increases in total fluid intake during ALDO are from additional ingestion of both water and saline, while increases in total fluid intake during ALDO/DEX20 are from additional ingestion only of saline. Old rats had significantly smaller changes in total fluid intake than the other ages using either absolute or BW-adjusted values and had significantly smaller changes in saline intake on a BW basis.

**Daily mean arterial blood pressure.** There was a significant three-way interaction of test period × treatment × age for MAP [$F_{2,42} = 21.90; P < 0.001$]. During baseline, MAP was significantly higher with increasing age (Fig. 2). Both treatments significantly increased MAP at all ages. In addition, the combination of ALDO/DEX20 increased MAP significantly more than did ALDO for young and middle-aged, but not for old rats. Lastly, significant increases in MAP were observed earlier, by 1–2 days, during ALDO/DEX20 than during ALDO (interaction, $F_{9,378} = 4.20; P < 0.001$). When analyzed as change scores (i.e., change from average baseline values; Table 2), there were significant main effects of age ($F_{2,42} = 44.83; P < 0.001$) and treatment ($F_{1,42} = 130.72; P < 0.001$) and a significant age × treatment interaction ($F_{2,42} = 21.07; P < 0.001$). During ALDO, MAP increased slightly, but significantly, more for young and middle-aged rats compared with old rats. In addition, the combination of ALDO/DEX20 increased MAP significantly more than did ALDO for young and middle-aged rats, but not for old rats. Lastly, ALDO/DEX20 increased MAP significantly more for young rats compared with middle-aged rats, and for middle-aged rats compared with old rats.

**Daily mean heart rate.** A main effect of age [$F_{2,42} = 12.99; P < 0.001$] indicated that middle-aged rats had the lowest HR, and old rats had highest HR (Fig. 2). In addition, HR was significantly higher for animals in the ALDO/DEX20 treatment condition than for animals in the ALDO treatment condition (main effect, $F_{2,42} = 4.79; P < 0.05$). Significant triple interactions revealed that HR differed between baseline and infusion periods depending on days × treatment ($F_{9,378} = 1.93; P < 0.05$) and on days × age ($F_{18,378} = 2.14; P < 0.01$). Daily mean HR increased for the first 1–4 days of ALDO infusion across the ages. Daily mean HR then significantly
decreased for young and middle-aged, but not for old, rats. However, inspection of the data revealed that the results for old rats were skewed by a single animal with increased HR at this time. In contrast, daily mean HR significantly decreased from baseline levels by the second or third day of treatment for all ages when DEX20 was included in the infusion. The change in average daily HR was significantly greater during ALDO/DEX20 treatment than during ALDO treatment (Table 2).

Fig. 2. Daily intake of 0.3 M NaCl and water, daily mean arterial pressure (MAP), and daily heart rate (HR) for young (4 mo), middle-aged (12 mo), and old (29–30 mo) rats during treatment with aldosterone (ALDO) or ALDO in combination with dexamethasone (ALDO/DEX20). Vertical line denotes start of treatment. All groups had significantly increased saline intake and MAP in response to both treatments. All groups had increased water intake in response to ALDO, but only young and middle-aged rats had increased water intake in response to ALDO/DEX20. All groups had reduced HR in response to ALDO/DEX20, but old rats did not have reduced HR in response to ALDO. Group ns are in parentheses. Values are expressed as means ± SE.

Effects of ALDO and DEX on mean arterial pressure and saline intakes in the first days of treatment. Figure 3 depicts the changes in saline ingestion and MAP during the treatments plotted on the same axes. During infusions of ALDO, levels of daily saline intake and MAP changed together. During infusions of ALDO/DEX20, MAP increased the day before saline ingestion increased. During the combination of ALDO and DEX20, in all individual cases, MAP increased before saline, or water, drinking increased.
**Table 1. Average daily fluid intakes and cardiovascular measures during baseline for young (4 mo), middle-aged (12 mo), and old (29–30 mo) rats during treatment with aldosterone or the combination of aldosterone plus dexamethasone.**

<table>
<thead>
<tr>
<th>Age</th>
<th>Tmt</th>
<th>n</th>
<th>Water Intake, ml</th>
<th>Saline Intake, ml</th>
<th>Tot Flu Intake, ml</th>
<th>Adjusted Water Intake, ml/100 g</th>
<th>Adjusted Saline Intake, ml/100 g</th>
<th>Adjusted Tot Flu Intake, ml/100 g</th>
<th>MAP, mmHg</th>
<th>HR, bpm</th>
</tr>
</thead>
<tbody>
<tr>
<td>4 mo</td>
<td>A</td>
<td>9</td>
<td>15.8 ± 0.7</td>
<td>2.0 ± 0.5</td>
<td>17.8 ± 0.7</td>
<td>5.0 ± 0.4</td>
<td>0.6 ± 0.2</td>
<td>5.7 ± 0.4</td>
<td>85 ± 2</td>
<td>271 ± 3</td>
</tr>
<tr>
<td></td>
<td>A/D20</td>
<td>7</td>
<td>17.2 ± 0.6</td>
<td>2.0 ± 0.4</td>
<td>19.2 ± 0.4</td>
<td>6.0 ± 0.2</td>
<td>0.7 ± 0.2</td>
<td>6.7 ± 0.2</td>
<td>84 ± 2</td>
<td>291 ± 6</td>
</tr>
<tr>
<td>12 mo</td>
<td>A</td>
<td>10</td>
<td>17.0 ± 0.5</td>
<td>1.2 ± 0.5</td>
<td>18.2 ± 0.5</td>
<td>4.3 ± 0.1</td>
<td>0.3 ± 0.1</td>
<td>4.6 ± 0.2</td>
<td>92 ± 1b</td>
<td>257 ± 4b</td>
</tr>
<tr>
<td></td>
<td>A/D20</td>
<td>7</td>
<td>16.8 ± 0.6</td>
<td>1.7 ± 0.7</td>
<td>18.5 ± 1.0</td>
<td>4.3 ± 0.1</td>
<td>0.4 ± 0.2</td>
<td>4.8 ± 0.2</td>
<td>92 ± 1b</td>
<td>269 ± 4b</td>
</tr>
<tr>
<td>29–30 mo</td>
<td>A</td>
<td>6</td>
<td>22.7 ± 1.2†</td>
<td>0.9 ± 0.4</td>
<td>23.6 ± 1.4†</td>
<td>5.5 ± 0.3</td>
<td>0.2 ± 0.1</td>
<td>5.7 ± 0.4</td>
<td>104 ± 3†</td>
<td>284 ± 6†</td>
</tr>
<tr>
<td></td>
<td>A/D20</td>
<td>6</td>
<td>23.2 ± 1.4†</td>
<td>1.1 ± 0.4</td>
<td>24.2 ± 1.8†</td>
<td>5.5 ± 0.2</td>
<td>0.2 ± 0.1</td>
<td>5.7 ± 0.3</td>
<td>101 ± 3†</td>
<td>300 ± 16†</td>
</tr>
</tbody>
</table>

Values are expressed as means ± SE. Each value is the average of the measure over 10 days of baseline. Tmt, drug treatment condition; Tot Flu, total fluid. A, aldosterone; includes data from animals that received the lower (i.e., 2.5 μg/day), ineffective dose of DEX. A/D20, aldosterone plus dexamethasone (i.e., 20 μg/day). *Significant main effect of treatment, P < 0.05. †Significant main effect of age, different from both other ages, P < 0.05. ‡Significant main effect of age, different from young rats, P < 0.05.

**DISCUSSION**

This is the first study to continuously monitor cardiovascular variables in freely behaving animals in a standard model of mineralocorticoid-induced salt appetite using the two-bottle, free-choice procedure. The experiment examined the effects of age on water and sodium ingestion, MAP, and HR during continuous subcutaneous infusion of ALDO or ALDO in combination with DEX, in male BN rats. The main findings were 1) ALDO stimulated comparable levels of sodium ingestion by young (4 mo), middle-aged (12 mo), and old (29–30 mo) rats; 2) the addition of DEX to the infusion treatment (i.e., ALDO/DEX20) increased sodium ingestion more than did ALDO by itself, but less so with increasing age on a BW basis; 3) infusion of ALDO/DEX20 increased MAP and decreased HR, more than did infusions of ALDO; 4) the changes in MAP in response to both treatments depended on age; and 5) increases in sodium ingestion and MAP occurred together during ALDO, but increases in MAP always preceded increases in sodium ingestion during ALDO/DEX20.

Continuous subcutaneous administration of ALDO (60 μg/d) stimulated additional saline drinking by all ages of rats. During baseline, all age groups drank small amounts, generally <3 ml per day, of concentrated (1.8% NaCl) saline solution. Treatment with ALDO stimulated modest, approximately four-fold, increases in salt intake across the age groups on either an absolute or BW basis. We previously demonstrated mineralocorticoid-induced salt appetite in aged rats using daily subcutaneous injections of very high doses (2 mg/day) of DOCA (53). In that study, middle-aged rats drank the most saline solution in response to DOCA, whereas old rats drank less than young rats only on a BW basis. In the present study, the comparable levels of salt appetite achieved across the ages may reflect the use of much lower doses of mineralocorticoid or to differences in mineralocorticoid activity of DOCA and ALDO. Mineralocorticoids stimulate salt appetite by direct effects on mineralocorticoid receptors (MR) in the brain (17, 41, 42, 47). The results of this and the previous study (53) suggest that neural pathways “downstream” from key MR in the brain must retain residual function in aged rats. The present work is the second instance of aged rats reliably responding with salt appetite to an administered mineralocorticoid.

In a series of studies, we observed deficiencies in both osmotic and renin-dependent thirst in old BN rats (51, 52, 55, 56, 60). Notably, the salt appetite response to sodium depletion is greatly reduced—and sometimes absent—in aged rats (3, 40, 54), even after multiple episodes of sodium depletions (56). We have proposed that a key reason why aged rats fail to respond appropriately to thirst and salt appetite challenges is their inability to endogenously produce the relevant hormonal stimuli (51). The diminished capacity of old rats to secrete renin (1, 2, 40, 55) likely prevents them from generating sufficiently high levels of circulating ANG II to stimulate salt appetite to an administered mineralocorticoid.

**Table 2. Average change in daily fluid intakes and cardiovascular measures from average baseline levels for young (4 mo), middle-aged (12 mo), and old (29–30 mo) rats during treatment with aldosterone or the combination of aldosterone plus dexamethasone.**

<table>
<thead>
<tr>
<th>Age</th>
<th>Tmt</th>
<th>n</th>
<th>ΔWater Intake*, ml</th>
<th>ΔSaline Intake*, ml</th>
<th>ΔTot Flu Intake, ml</th>
<th>ΔAdjusted Water Intake*, ml/100 g</th>
<th>ΔAdjusted Saline Intake*, ml/100 g</th>
<th>ΔAdjusted Tot Flu Intake, ml/100 g</th>
<th>ΔMAP*, mmHg</th>
<th>ΔHR*, bpm</th>
</tr>
</thead>
<tbody>
<tr>
<td>4 mo</td>
<td>A</td>
<td>9</td>
<td>6.4 ± 0.8</td>
<td>5.0 ± 0.8</td>
<td>11.4 ± 0.7</td>
<td>1.8 ± 0.2</td>
<td>1.6 ± 0.3</td>
<td>3.4 ± 0.2</td>
<td>7 ± 1</td>
<td>−5 ± 3</td>
</tr>
<tr>
<td></td>
<td>A/D20</td>
<td>7</td>
<td>−1.4 ± 1.0</td>
<td>14.2 ± 3.7</td>
<td>12.8 ± 3.4</td>
<td>0.2 ± 0.4</td>
<td>6.1 ± 2.0</td>
<td>6.3 ± 1.7</td>
<td>23 ± 1†</td>
<td>−10 ± 3</td>
</tr>
<tr>
<td>12 mo</td>
<td>A</td>
<td>10</td>
<td>7.4 ± 0.8</td>
<td>4.6 ± 0.7</td>
<td>12.0 ± 1.1</td>
<td>1.8 ± 0.2</td>
<td>1.1 ± 0.2</td>
<td>2.9 ± 0.2</td>
<td>6 ± 1</td>
<td>−8 ± 3</td>
</tr>
<tr>
<td></td>
<td>A/D20</td>
<td>7</td>
<td>2.0 ± 1.3†</td>
<td>13.1 ± 3.8</td>
<td>15.1 ± 3.1</td>
<td>1.2 ± 0.4†</td>
<td>4.1 ± 1.2</td>
<td>5.3 ± 1.1</td>
<td>17 ± 1†</td>
<td>−12 ± 4</td>
</tr>
<tr>
<td>29–30 mo</td>
<td>A</td>
<td>9</td>
<td>7.8 ± 0.8</td>
<td>3.4 ± 0.7</td>
<td>11.2 ± 1.4†</td>
<td>2.0 ± 0.2</td>
<td>0.8 ± 0.2b</td>
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<td>4 ± 1†</td>
<td>6 ± 9</td>
</tr>
<tr>
<td></td>
<td>A/D20</td>
<td>6</td>
<td>−3.2 ± 1.4</td>
<td>8.7 ± 1.4</td>
<td>5.5 ± 1.5</td>
<td>−0.1 ± 0.3</td>
<td>2.4 ± 0.4b</td>
<td>2.3 ± 0.4b</td>
<td>7 ± 2†</td>
<td>−15 ± 10</td>
</tr>
</tbody>
</table>

Values are expressed as means ± SE. Each value is the difference between the average of the measure over 10 days of treatment from the average measure over 10 days of baseline. Tmt, drug treatment condition; Tot Flu, total fluid. A, aldosterone, includes data from animals that received the lower (i.e., 2.5 μg/day), ineffective dose of DEX. A/D20, aldosterone plus dexamethasone (i.e., 20 μg/day). Treatment with the combination of aldosterone and the higher dose of dexamethasone (i.e., 20 μg/day) produced significantly greater changes in most measures than did treatment with aldosterone. Old rats had significantly smaller changes in total fluid intake than the other ages using either absolute or body weight (BW)-adjusted values, and in saline intake using BW-adjusted values only. *Significant main effect of treatment, P < 0.05. †Significant main effect of age, different from both other ages, P < 0.05. ‡Significant main effect of age, different from young rats, P < 0.05. ††Significant interaction effect: different from the other ages, P < 0.05.
appetite in response to sodium depletion. However, in the mineralocorticoid model of salt appetite, the relevant stimulus (i.e., ALDO or DOCA) is provided to the animal by daily administration. The substantial salt appetite elicited from aged rats in response to mineralocorticoids supports the idea that aged rats do not lack the ability to respond behaviorally but lack the ability to generate the relevant endogenous signal.

The salt appetite response to mineralocorticoids is greatly exaggerated—or “potentiated”—by coadministration of glucocorticoids (33, 45, 50). In the present study, the addition of DEX (20 μg/day) to the ALDO infusate (i.e., ALDO/DEX20) increased daily saline intakes more than did ALDO. For all ages, both peak and average daily saline intakes during infusions of ALDO/DEX20 were more than double those produced by infusions of ALDO (Fig. 2). However, the daily intakes of saline during combined treatment with ALDO/DEX20 also depended on age, with young rats drinking the most and old rats drinking the least. Thus, although the modest levels of salt appetite produced by ALDO were not significantly diminished with age, the potentiated levels of salt appetite produced by coadministering DEX with ALDO were. We previously have shown that one plausible mechanism by which glucocorticoids increase sodium ingestion is through renal actions (50). DEX treatment robustly increases water and sodium excretion within hours (50). By promoting water and sodium excretion, DEX limits volume expansion during mineralocorticoid treatment (50), thereby reducing volume-related inhibitory signals for water drinking and sodium ingestion (28, 58). In addition, glucocorticoids are postulated to potentiate thirst and salt appetite by actions within the brain to increase binding to Type I MRs (33, 65) and by augmenting the central effects of ANG II (45). It is unknown whether the renal and central actions of glucocorticoids to potentiate thirst and salt appetite behavior change with age.

There were striking age-related differences in the ABP responses to treatment. Both treatments significantly increased MAP for all age groups. The inclusion of DEX20 in the infusion regimen caused far greater increases in MAP than did ALDO, but only for young and middle-aged rats. That is, the glucocorticoid had little effect on the ABP response of aged rats. We speculated that ALDO infusions would increase MAP more in aged rats than in younger animals because the relative inability of aged animals to excrete water and sodium (4, 34, 40) would likely cause greater sodium retention and volume expansion. In fact, the change in ABP in response to ALDO was slightly less in aged rats compared with younger rats. This reduced responsiveness to ALDO by aged rats may be due to their higher baseline MAP and a possible ceiling effect for MAP at this dose of ALDO. In addition, the changes in MAP during administration of ALDO are small for all ages, and the ultimate levels of MAP achieved during ALDO by young and middle-aged rats remained lower than baseline levels of MAP of aged rats. We anticipated that ABP would increase further in response to ALDO/DEX20 than to ALDO, which was borne out by the results. This result is consistent with the idea that the mechanisms by which ALDO and DEX increase ABP are somehow additive. However, it is at least possible that the volume-depleting actions of DEX either negated or canceled the volume-retaining actions of ALDO so that the resulting levels of MAP were due exclusively to DEX. We cannot distinguish between these possibilities based solely on the levels of MAP. Regardless of the underlying actions of DEX to affect MAP, there was little effect of DEX on MAP of aged rats. Old rats operate against a background of increased levels of circulating glucocorticoids (43). Thus, old animals may be refractory to DEX and may require higher doses to achieve similar increases in MAP. However, DEX-induced weight loss was identical between groups (Fig. 1), and the DEX-induced increases in salt appetite in response to ALDO were roughly proportional across the ages (Table 2). Therefore, old rats may have greatly attenuated responses only to some effects of glucocorticoids.

The patterns of water and saline drinking across treatment days and the timing of the increases in the various responses were markedly different between the treatments yet strikingly similar across the ages. As can be seen in Fig. 3, saline drinking and MAP increased together after 2–3 days of ALDO treatment for all ages. These concurrent increases in sodium ingestion and MAP are to be expected, as the increase in MAP during ALDO treatment is a product of sodium retention and volume expansion. This pattern of increased MAP together with increased saline consumption is also observed during DOCA/salt hypertension models (6, 36). On the other hand, during ALDO/DEX20 treatment, the latencies for increases in saline drinking and MAP were very different. At each age, there was a significant increase in MAP on day 1 of treatment, and a significant increase in saline ingestion on day 2 of treatment. We previously have shown that DEX dramatically increases urinary excretion of water and sodium within hours.
of administration (50). Therefore, the present time course of effects is consistent with the possibility that one mechanism by which DEX increases sodium ingestion is by increasing ABP, thereby augmenting pressure-induced excretion of sodium (natriuresis), and countering mineralocorticoid-induced water and sodium retention and volume expansion. These results suggest dual actions of ABP on salt appetite. Under conditions of sodium depletion and volume contraction (15, 49), increases of ABP may reduce the salt appetite response by suppressing renin secretion. However, under conditions of sodium excess and volume expansion, increases of ABP may increase the salt appetite response by facilitating the excretion and turnover of sodium and mitigating the rise in volume.

The different patterns of responding to the treatments extend to water drinking, total fluid intake, and HR. Administration of ALDO caused small, but significant, increases in water drinking that preceded significant increases in saline drinking. The increased water intakes likely reflect systemic, renal effects of ALDO to cause sodium retention with ensuing osmotic thirst. After a few days, the central effects of ALDO cause additional sodium ingestion. The ALDO-induced water drinking was delayed by 1 day in aged rats, which may reflect impaired intracellular thirst of older BN rats (3, 51, 56). In contrast, combined treatment with ALDO and DEX20 always increased saline drinking before water drinking. Indeed, water drinking decreased for the first few days of combined treatment, possibly indicating that the animals partly compensated for the additional fluid from ingested saline by drinking less water. The elevated water intakes in the last few days of testing are likely osmotic in nature. The increases in daily total fluid intakes during ALDO derive from increases in both water and saline consumption, while increases in daily total fluid intakes during ALDO/DEX20 are due solely to increases in saline drinking. As noted, sodium retention in response to ALDO stimulates water drinking through osmotic mechanisms, followed days later by increased sodium ingestion. However, when excess glucocorticoid is present (i.e., ALDO/DEX20), the sodium-retaining effects of ALDO are effectively countered (24, 30, 35, 48, 50), leading to greatly increased sodium turnover and a preferential increase in sodium ingestion. The ALDO-induced water drinking was followed days later by increased sodium ingestion. The ALDO-induced water drinking was delayed by 1 day in aged rats, which may reflect impaired intracellular thirst of older BN rats (3, 51, 56). In contrast, combined treatment with ALDO and DEX20 always increased saline drinking before water drinking. Indeed, water drinking decreased for the first few days of combined treatment, possibly indicating that the animals partly compensated for the additional fluid from ingested saline by drinking less water. The elevated water intakes in the last few days of testing are likely osmotic in nature. The increases in daily total fluid intakes during ALDO derive from increases in both water and saline consumption, while increases in daily total fluid intakes during ALDO/DEX20 are due solely to increases in saline drinking. As noted, sodium retention in response to ALDO stimulates water drinking through osmotic mechanisms, followed days later by increased sodium ingestion. However, when excess glucocorticoid is present (i.e., ALDO/DEX20), the sodium-retaining effects of ALDO are effectively countered (24, 30, 35, 48, 50), leading to greatly increased sodium turnover and a preferential increase in sodium ingestion. There were transient increases in HR at the start of each treatment. The increases in HR lasted about 4 days during ALDO and only 1 day during ALDO/DEX20. Heart rates also slowed earlier during treatment with ALDO/DEX20. The reduction in HR appears to correspond with significant increases in MAP, which occur at different times depending on treatment. Overall, the similarities in the patterning of responding across the ages suggest quantitative, and not qualitative, changes in responding with increasing age.

The addition of DEX20 to ALDO treatment was accompanied by significant weight loss consistent with glucocorticoid-induced urinary excretion, suppression of food intake, and catabolism. It is well known that glucocorticoids, such as DEX, when administered at relatively high doses (e.g., 1–2 mg/day sc) suppress food intake beginning the second day of treatment (16, 22, 32, 50). However, weight loss precedes reduction of food intake (16, 22, 32, 50), and this can be explained by glucocorticoid-induced diuresis and negative water balance (50). Chronic reductions in weight are likely due to a combination of reduced food intake, negative water balance, and catabolism of muscle tissue (32). Even at the considerably lower daily doses of DEX employed here (i.e., 20 μg/day), compared with previous work, there was clear evidence of weight loss by 2–3 days (~2% of BW per day).

Perspectives and Significance

Insufficient sodium intake results in chronic underhydration, diminished blood volume and marked vulnerability to environmental challenges such as heat. In elderly humans, declining thirst, sodium ingestion, and renal function become especially important under stressful conditions. For example, heat waves most severely affect persons at the extremes of age. During heat waves in St. Louis (1966), Memphis (1980), and Pittsburgh (1988), virtually all of the increases in mortality from heat illness and circulatory collapse were in persons over the age of 60. With an aging population, an increased understanding of the changes in mechanisms related to thirst and salt appetite as a function of age is critical for the development of therapies to maintain adequate hydration of older people. As with elderly people, aged rats have declining control of body fluid and cardiovascular homeostasis and serve usefully as a model for studying the effects of aging. The present work suggests that mineralocorticoid-treated rats “voluntarily” ingesting aversive concentrations of saline that is available in choice with water have a coincident rise in MAP that is similar in timing to that observed in rats in standard studies of mineralocorticoid-induced hypertension (e.g., 6, 36, 60, 61, 62). Furthermore, both the pattern of increased ABP and increased sodium ingestion appear to be similar in aged and young rats. However, both the sodium ingestion and ABP responses to the addition of glucocorticoid in the treatment were notably diminished in aged compared with young animals, suggesting age-related decline(s) in response to glucocorticoids. Whether these response deficits are due primarily to loss of systemic (50) or central (9, 33, 45, 65) effects of glucocorticoids remains to be determined.

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DISCLOSURES

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AUTHOR CONTRIBUTIONS


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