Modulation of Learning and Memory in Dahl Rats by Dietary Salt Restriction

Nelson Ruiz-Opazo, Lyle V. Lopez, John Tonkiss

Abstract—The Dahl rat represents a robust animal model of salt-sensitive hypertension, with Dahl S rats being salt sensitive and Dahl R rats (the Dahl S counterparts) being salt resistant for the development of hypertension. Here we evaluate the effect of reduced dietary salt intake on learning and memory in the Dahl rat model. Salt restriction produced a significant impairment in social transmission of food preference and social recognition memory in Dahl S rats without affecting spatial learning. In contrast, social transmission of food preference and social recognition memory remained unaffected in Dahl R rats, whereas navigation performance was significantly improved. This effect on learning and memory was not generalized because sodium restriction did not influence object recognition memory in either Dahl S or Dahl R rats. The significant decrement in select cognitive functions in Dahl S rats produced by salt restriction are in sharp contrast to the well known positive effect of dietary salt restriction in alleviating high blood pressure and associated target organ complications, suggesting that caution must be exercised when weighing the benefits of salt restriction in improving cardiovascular health in salt-sensitive hypertension against the potential undesirable effects of reduced cognitive function. (Hypertension. 2004;43:797-802.)

Key Words: rats, Dahl ■ sodium

Dietary salt intake is a critical environmental factor influencing genetic susceptibility to a number of complex disorders, including essential hypertension1,2 and heart disease.3-6 As many as 50% of hypertensive patients have been found to be salt-sensitive (ie, exhibit a significant increase in mean blood pressure after high-salt intake).7

The Dahl rat represents a robust animal model of human salt-sensitive hypertension, with Dahl S rats being salt-sensitive and Dahl R rats (the Dahl S counterpart) being salt-resistant for the development of hypertension.8 We have recently developed a transgenic hyperlipidemic Dahl salt-sensitive hypertensive rat model that exhibits coronary artery disease and decreases survival on a regular rat chow (0.4% NaCl), recapitulating most of the pathological characteristics of coronary heart disease observed in humans.9-11 Sodium restriction in this transgenic hyperlipidemic Dahl salt-sensitive hypertensive rat (0.008% NaCl in rat chow) produces a dramatic improvement in disease progression, resulting in increased survival and significant attenuation of the coronary artery disease phenotype.10 Based on the potential clinical significance of these observations, the present studies were undertaken to ascertain whether restriction of dietary salt intake has any positive or adverse effects on cognitive function using the Dahl rat model.

Animals were maintained from weaning on either a regular-salt chow or a low-salt rat chow and then subject to behavioral characterization. Four distinct cognitive tasks were used to evaluate learning and memory performance: 2 hippocampus-dependent learning and memory tasks, the Morris water maze (MWM), and the social transmission of food preference (STFP) tasks;12,13 and 2 hippocampus-independent learning and memory tasks, the social recognition (SR) and the object recognition (OR) tasks.14,15 The results demonstrate that Dahl S and Dahl R strains responded differently to sodium restriction. This treatment produced a significant impairment in STFP and SR performance in Dahl S rats without affecting spatial learning in the MWM. In contrast, STFP and SR performance remained unaffected in Dahl R rats, whereas MWM performance was significantly improved. The effect on learning and memory was not generalized because sodium restriction did not influence OR memory in either Dahl S or Dahl R rats. These results delineate a complex effect of dietary salt intake on learning and memory function in Dahl rats. Moreover, salt restriction produced a significant decrement in select cognitive functions in Dahl S rats. These findings are in sharp contrast to the well-known positive effect of dietary salt restriction in alleviating high blood pressure and coronary artery disease progression,1,10,11 and suggest that maintenance on a low-salt diet may have detrimental effects for the quality of life of salt-sensitive hypertensive patients caused by potential cognitive impairment.
Spatial learning in Dahl S and Dahl R male rats maintained on a 0.4% NaCl diet. A–C, Hidden version of the Morris water maze (MWM) for Dahl R (▫) and Dahl S (◼) male rats. A, Acquisition, mean distance (±SEM) traveled to locate the escape platform. B, Mean percent distance (±SEM) traveled in each quadrant during the probe trial. C, Number of crossings of training site (±SEM) during the probe trial. D, Mean distance (±SEM) traveled to locate the escape platform during a visible version of the MWM. Quadrants are target (training) quadrant (T), adjacent right (A₀), adjacent left (A₀), and opposite quadrant (O).

Methods
An extended Method section can be found in an online supplement available at http://www.hypertensionaha.org.

Results
Water Maze Testing
To examine potential modulation of spatial learning and memory by dietary salt intake, we performed the MWM task on Dahl S and Dahl R rats maintained on a regular-salt or low-salt rat chow. In this test, subjects are placed in a large tank of water and they must learn to find a “hidden” platform by the flexible use of distal cues to the escape platform. As shown in Figure 1, Dahl S and Dahl R rats maintained on regular-salt diet demonstrate comparable ability to locate a hidden platform during acquisition performance of the task (Figure 1A; Dahl S on regular-salt diet versus Dahl R on regular-salt diet, F₁,₂₂ = 0.005, NS; Dahl R on regular-salt diet versus Dahl R on low-salt diet, F₁,₂₂ = 4.308, P < 0.05) and increased platform crossings during the probe trial (Figure 1B; Dahl S on regular-salt diet versus Dahl S on low-salt diet, F₁,₂₂ = 0.259, NS; Dahl R on regular-salt diet versus Dahl R on low-salt diet, F₁,₂₂ = 0.116, NS; Dahl R on regular-salt diet versus Dahl R on low-salt diet, F₁,₂₂ = 12.236, P < 0.01).

To investigate potential effects of sodium restriction on navigational performance, both Dahl S and Dahl R rats maintained on a low-sodium diet were tested on the MWM task. Dahl S and Dahl R rats showed comparable acquisition performance of the task (Figure 2A; Dahl S on low-salt diet versus Dahl R on low-salt diet, F₁,₂₂ = 1.243, NS). However, in the probe trial, although both groups showed target selectivity with “spatial bias” toward the target quadrant (Dahl S, P < 0.0001; Dahl R, P < 0.0001, Tukey pairwise multiple comparison test comparing percent search distance in target quadrant versus each of the other quadrants after one-way ANOVA), the Dahl R group exhibited superior performance when compared with the Dahl S group (Figure 2B; Dahl R versus Dahl S, F₁,₂₂ = 4.349, P < 0.05), indicated by the higher percentage of distance traveled by the Dahl R group in the quadrant where the escape platform had been located during training. Similarly, the Dahl R group showed increased number of platform crossings when compared with Dahl S rats (Figure 2C; Dahl R versus Dahl S, F₁,₂₂ = 7.979, P < 0.01), consistent with their superior search accuracy for the hidden platform. Both groups showed equivalent efficacy in locating a visible platform (Figure 2D; Dahl S, F₁,₂₂ = 0.093, NS), implying absence of motor-sensory differences between the experimental groups.

Direct comparison of MWM performance by genotypes based on dietary sodium intake clearly shows that the modulator effect of sodium restriction on spatial learning and memory depends on genetic background. In Dahl R rats, the decrease of dietary sodium intake improves navigational performance, as evidenced by an increased bias for the correct quadrant (Figure 3A; Dahl S on regular-salt diet versus Dahl S on low-salt diet, F₁,₂₂ = 0.093, NS; Dahl R on regular-salt diet versus Dahl R on low-salt diet, F₁,₂₂ = 4.308, P < 0.05) and increased platform crossings during the probe trial (Figure 3B; Dahl S on regular-salt diet versus Dahl S on low-salt diet, F₁,₂₂ = 0.259, NS; Dahl R on regular-salt diet versus Dahl R on low-salt diet, F₁,₂₂ = 12.236, P < 0.01).

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Performance at the 5-minute (Figure 4A; Dahl S: a regular rat chow demonstrated strong social recognition Rlow, Dahl R rats maintained in 0.008% NaCl diet; S reg, Dahl S/H11005 0.002). P in 0.008% NaCl diet (*F 1,22 utes later (B, D). (* P 0.003, compared with first P 0.0001, **P11005 0.0001; Dahl R: F1,22 0.006) duration of investigation of the juvenile by the inability to recognize a conspecific after 30-minute exposure). Rreg indicates Dahl R rats maintained in 0.4% NaCl diet; Rlow, Dahl R rats maintained in 0.008% NaCl diet; Sreg, Dahl S rats maintained in 0.4% NaCl diet; Slow, Dahl S rats maintained in 0.008% NaCl diet (*F1,22=4.308, P<0.05, **F1,22=12.236, P<0.002).

Social Recognition Testing

This task involves the recognition (memory) of conspecifics expressed mainly through olfactory investigation. The duration of the investigation will depend on the familiarity with the conspecific, with unfamiliar ones being explored longer and more intensively than familiar ones. Thus, difference in social transmission of investigation of conspecifics presented at different retention times is used as a measure of social recognition. Both Dahl S and Dahl R rats maintained on a regular rat chow demonstrated strong social recognition performance at the 5-minute (Figure 4A; Dahl S: F1,22=48.756, P<0.0001; Dahl R: F1,22=62.734, P<0.0001) and at the 30-minute (Figure 4B; Dahl S: F1,22=22.493, P<0.0001; Dahl R: F1,22=28.156, P<0.0001) retention times. In contrast, maintaining the subjects on a low-salt diet resulted in a profound deficit in SR in Dahl S rats, evidenced by the inability to recognize a conspecific after 30-minute retention time (Figure 4D; Dahl S: F1,22=3.325, NS, with a trend toward increased, rather than decreased, investigation of the conspecific during the second presentation), whereas the Dahl R rats performance was unaffected by sodium restriction (Figure 4D; Dahl R: F1,22=30.869, P<0.0001).

Social Transmission of Food Preference Testing

Social transmission of food preference testing is a hippocampus-dependent learning and memory task that is natural and nonspatial. Subjects have a preference for a particularly scented food after an “observer” rat interacts with a “demonstrator” rat that has recently consumed a distinctively scented food. Thus, food consumption of trained and nontrained scented food presented in a pair–choice paradigm at different retention times can be used to measure STFP long-term memory. To assess potential inherited predilection for specifically scented foods that could confound subsequent STFP testing, we measured the inherited preference for each odor within a pair considered for STFP testing, using an independent group of Dahl S and Dahl R male rats maintained either on a 0.4% or on a 0.008% NaCl diet. On a 0.4% NaCl diet, Dahl S rats have inherent preference for cinnamon over cocoa (Figure 5A; F1,22=29.481, P<0.0001) and Dahl R rats demonstrated inherent preference for thyme over marjoram (Figure 5B; F1,22=11.417, P<0.01) and turmeric over nutmeg (Figure 5B, F1,22=38.621, P<0.0001). On a 0.008% NaCl diet, Dahl S rats showed preference for cinnamon over cocoa (Figure 5C, F1,18=106.3, P<0.0001), thyme over marjoram (Figure 5C, F1,18=22.1, P<0.0001), cumin over anise (Figure 5C, F1,18=16.0, P<0.0001), onion over sage (Figure 5C, F1,18=6.7, P<0.02), and turmeric over nutmeg (Figure 5C, F1,18=40.9, P<0.0001). Dahl R rats demonstrated inherent food preference for cinnamon over cocoa (Figure 5D, F1,18=42.1, P<0.0001), onion over sage (Figure 5D, F1,18=12.3, P<0.0003), and turmeric over nutmeg (Figure 5D, F1,18=50.5, P<0.0001). Thus, these pairings were not used in the STFP experiments described, except for anise–cumin, which showed significant bias in Dahl S rats maintained on a low-salt diet. However, this pairing did not affect the results because Dahl S rats maintained on a low-salt diet were found to be unable to perform this task at any of the retention times tested (Figure 5I, 5J, and 5I).

On a regular rat chow, Dahl R rats showed an efficient performance at 5-minute (Figure 5E, F1,22=42.835, P<0.0001), 3-hour (Figure 5F, F1,22=207.757, P<0.0001), and 24-hour (Figure 5G, F1,22=8.513, P<0.01) retention times. In contrast, Dahl S rats exhibited difficulties in performing this task. They were able to show preference for the trained odor at 5-minute (Figure 5E, F1,22=11.577, P<0.01) and 3-hour (Figure 5F, F1,22=8.061, P<0.05) retention times; however, they did not recognize the trained odor after 24 hours of retention time (Figure 5G, F1,22=3.296, NS). Maintaining the subjects on a low-salt diet caused a dramatic decrease in performance in the Dahl S group, such that they were completely unable to perform this task (Figure 5H; 5 minutes, F1,22=1.613, NS; Figure 5I, 3 hours, F1,22=0.700, NS; Figure 5J, 24 hours, F1,22=0.978, NS). In contrast, the Dahl R group demonstrated effective performance in this behavioral task at all retention times tested (Figure 5H, 5 minutes, F1,22=68.171, P<0.0001; Figure 5I, 3 hours, F1,22=15.113, P<0.001; Figure 5J, 24 hours, F1,22=11.535, P<0.01).
Object Recognition Testing

This is a broadly used cognitive test in rodents that relies on the inherent ability of rodents to investigate new objects. Thus, the time spent exploring unfamiliar versus familiar objects can be used as an index of recognition. Imposition of different retention times allows assessment of long-term memory for object recognition.15 In this particular cognitive test, Dahl S and Dahl R groups were able to demonstrate proficient object recognition memory. On a 3-day retention test, Dahl S and Dahl R rats showed strong performance when kept on a regular rat chow and when maintained on a low-salt diet (see the data supplement online at http://www.hypertensionaha.org).

The results obtained in the different behavioral tests are unlikely to be confounded by other physiological effects produced by the different salt diets, because systolic blood pressure (SBP) (tail-cuff BP measurements10) at age 12 weeks were found to be in the normal range (Dahl S[0.008% salt diet] SBP = 117.4 ± 3.9 mm Hg, n = 5; Dahl R[0.008% salt diet] SBP = 117.2 ± 3.5 mm Hg, n = 6; Dahl S[0.4% salt diet] SBP = 136.9 ± 4.9 mm Hg, n = 8; Dahl R[0.4% salt diet] SBP = 123.4 ± 3.0 mm Hg, n = 5) and body weights at age 18 weeks were similar (Dahl S[0.008% salt diet] BW = 370.0 ± 4.8 g, n = 4; Dahl R[0.008% salt diet] BW = 375.3 ± 8.6 g, n = 4; Dahl S[0.4% salt diet] BW = 377.0 ± 2.3 g, n = 4; Dahl R[0.4% salt diet] BW = 357.3 ± 2.1 g, n = 4) on the different salt diets (F1,12 = 2.994, NS).

Discussion

We report modulation of learning and memory in Dahl rats by dietary salt intake. Salt restriction produced a significant decrement in select cognitive functions in Dahl S rats. Specifically, there was a significant decrease in social recognition memory and a profound impairment in the STFP task in which they were completely unable to recognize the trained odor, even after very short (5 minutes) retention intervals. These findings are in sharp contrast to the well-known positive effect of dietary salt restriction in alleviating high blood pressure and coronary artery disease progression1,10,21 and suggest that caution must be exercised when weighing the benefits of salt restriction in improving cardiovascular health in salt-sensitive hypertension against the undesirable effects of reduced cognitive function. In contrast, Dahl R rats maintained on a low-salt diet exhibited an improvement in spatial learning and memory (MWM) with no measurable effect in other forms of cognition (SR, STFP, and OR).

The sodium restriction effect on learning and memory was not generalized because OR was unaffected by other physiological effects produced by the different salt diets, because systolic blood pressure (SBP) (tail-cuff BP measurements10) at age 12 weeks were found to be in the normal range (Dahl S[0.008% salt diet] SBP = 117.4 ± 3.9 mm Hg, n = 5; Dahl R[0.008% salt diet] SBP = 117.2 ± 3.5 mm Hg, n = 6; Dahl S[0.4% salt diet] SBP = 136.9 ± 4.9 mm Hg, n = 8; Dahl R[0.4% salt diet] SBP = 123.4 ± 3.0 mm Hg, n = 5) and body weights at age 18 weeks were similar (Dahl S[0.008% salt diet] BW = 370.0 ± 4.8 g, n = 4; Dahl R[0.008% salt diet] BW = 375.3 ± 8.6 g, n = 4; Dahl S[0.4% salt diet] BW = 377.0 ± 2.3 g, n = 4; Dahl R[0.4% salt diet] BW = 357.3 ± 2.1 g, n = 4) on the different salt diets (F1,12 = 2.994, NS).
sodium diet but that the MWM performance of Dahl S rats was the same whether they were maintained on a regular chow diet or on a low-sodium diet. A previous study has documented that a much higher dietary salt intake (8% NaCl) than was used here impairs spatial learning in Dahl S rats compared with Dahl R rats.22 However, the presence of hypertension in those animals appeared to contribute to this effect, because higher blood pressures were correlated with inferior spatial learning performance.

Our results demonstrate salt-sensitivity of cognitive function in Dahl rats that mimics the well-described salt-sensitivity of blood pressure and associated target organ complications in Dahl rats.8 To what extent salt-sensitivity reflects common underlying mechanisms remains speculative. Whereas hypertension and atherosclerosis are alleviated by salt restriction in Dahl S rats,10 some forms of learning and memory are impaired by dietary salt restriction. This could imply either that there is different sodium-sensitive gene networks underlying the effects on central (brain) and peripheral organ systems or that there is a common and/or overlapping salt-sensitive gene network that exhibits differential pleiotropic effects on central and peripheral organ functions. One hint as to what type of mechanism might be operative in salt-sensitivity could be inferred from our recent studies on the potential role of the Ang II/AVP receptor in salt-sensitive hypertension susceptibility.23 This receptor, coupled with adenylate cyclase,24 could play a significant role in renal and neuronal function based on its prominent expression in kidney25 and its broad expression in neurons throughout the central nervous system.26 An Ang II/AVP receptor gene N119S/C163R variant has been associated with salt-sensitive hypertension in Dahl S rats.23 Moreover, this variant exhibits sodium-induced dysfunction that could have distinct pleiotropic effects in kidney and brain, such as contributing to hypertension pathogenesis via increased renal tubular sodium and fluid reabsorption and modulating cognitive performance through cAMP-driven effects on learning and memory function, respectively. The involvement of the cAMP–CREB signaling pathway in learning and memory has been shown to be operative in different organisms,27–29 supporting the notion that the Ang II/AVP receptor could represent a key component of the gene network modulating cardiovascular and cognitive function by dietary salt intake.

There is substantial evidence supporting a role for Ang II in cognition and behavior,30,31 although the specific effect remains unclear with some studies reporting facilitation32–34 and others inhibition of cognitive function.35,36 Our finding that salt restriction, which is well-known to enhance the circulatory rennin-angiotensin-system (Ang II levels37), produced deficits in SR and STFP in Dahl S rats and enhanced spatial navigation in Dahl R rats suggest that if Ang II is mediating these effects, then its action would depend on the neural substrate underlying the specific cognitive effects and genetic background of the experimental subjects.

**Perspectives**

The association of salt-sensitivity and target organ damage in essential hypertension is well-documented1–3 and constitutes a major risk factor for renal and cardiovascular morbidty.1,38–40 Moreover, salt-sensitivity is associated with an increase mortality in normotensive and hypertensive humans.40 This has given impetus to the use of sodium restriction as an added interventional tool in the treatment of conditions known to be associated with salt-sensitivity, such as hypertension and heart and kidney diseases.1–38 Our finding of a significant decrease in cognitive performance in Dahl S rats induced by dietary salt restriction puts a cautionary note into the use of this strategy to ameliorate morbidity and mortality associated with salt-sensitivity caused by potential detrimental effects on cognitive function. Although there is much to be unraveled in these salt-sensitive cognitive function investigations, it is clear that the Dahl rat could provide a key experimental model to delineate gene networks underlying the modulator effect of dietary salt intake on learning and memory function and its relationship to other sodium responsive systems.

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**References**

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