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Age-related declines in thirst and salt appetite responses in male Fischer 344 × Brown Norway rats

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HIGHLIGHTS

- The effects of age on water and sodium ingestion were tested using male F344 × BN rats.
- Old male F344 × BN rats had decrements in thirst- and salt appetite-related behaviors.
- Behavior declined with age more than did kidney function.
- Sodium homeostasis diminishes less with age in the F344 × BN strain than in other strains.

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ABSTRACT

The F344 × BN strain is the first generational cross between Fischer 344 (F344) and Brown Norway (BN) rats. The F344 × BN strain is widely used in aging studies as it is regarded as a model of "healthy" aging (Sprott, 1991). In the present work, male F344 × BN rats aged 4 mo (young, $n = 6$) and 20 mo (old, $n = 9$) received a series of experimental challenges to body fluid homeostasis to determine their thirst and salt appetite responses. Corresponding urinary responses were measured in some of the studies. Following sodium depletion, old rats ingested less saline solution (0.3 M NaCl) than young rats on a body weight basis, but both ages drank enough saline solution to completely repair the accrued sodium deficits. Following intracellular dehydration, old rats drank less water than young rats, again on a body weight basis, and were less able than young rats to drink amounts of water proportionate to the osmotic challenge. Compared with young rats, old rats drank less of both water and saline solution after combined food and fluid restriction, and also were refractory to the stimulatory effects of low doses of captopril on water drinking and sodium ingestion. Age differences in urinary water and sodium excretion could not account for the age differences in accumulated water and sodium balances. These results extend observations of diminished behavioral responses of aging animals to the F344 × BN rat strain and support the idea that impairments in behavior contribute more to the waning ability of aging animals to respond to body fluid challenges than do declines in kidney function. In addition, the results suggest that behavioral defense of sodium homeostasis is less diminished with age in the F344 × BN strain compared to other strains so far studied.

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1. Introduction

Dehydration is a major health risk for the elderly [5,46], involving decreased thirst sensations [20,21,28] and reduced abilities to conserve water [12,23] and sodium [6]. Thus, when faced with challenges to body fluid homeostasis, the elderly are more susceptible to fluid losses [46] and take longer to restore fluid balance [16,21] than younger people. The success of rat models in examining age-related declines in renal (e.g., [1–3,51]) and cardiovascular (e.g., [8,11,30,35]) function is accompanied by a growing body of literature examining the effects of aging on thirst-related behaviors in rats (e.g., [4,17,24,25,36–38,41,42,47]). We have used the Brown Norway (BN) rat strain, a commonly studied alternative to the Fischer F344 (F344) and Sprague–Dawley (SD) strains for studies on aging, in an extensive series of studies on thirst and salt appetite responses during aging [36–38,41,42,47]. The BN strain lives longer relatively free of disease [13,32] and accumulates less fat than other strains even into old age [22,49]. However, no single strain is without

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age-related pathologies that may confound results. To determine if observed declines in function are generic to the aging process or are strain specific it is beneficial to examine age-related phenomena across multiple strains. We now report the results of a series of experiments using the first generation cross of F344 and BN strains (i.e., F344 × BN). Like the BN strain, the F344 × BN rat has less age-related pathology (e.g., fewer tumors) than the F344 and SD strains [2,32,45], and also lives longer in the absence of disease [13,32]. In addition, the F334 × BN strain is less susceptible to hydronephrosis of the kidney [14,31] and does not develop glomerular sclerosis [19], both of which may have consequences for studies of body fluid homeostasis. This work tests if the F344 × BN strain has significant impairments in thirst- and salt appetite-related behaviors with age.

The considerable age differences involved in aging studies usually mean that old rats are substantially heavier than younger rats which has consequences for administering experimental stimuli and for analyzing subsequent intakes. While intakes are typically adjusted for body weight (BW) in aging studies, there are differences of opinion when normalizing results according to BW is warranted (e.g., [48]). In order to better approximate equivalent challenges for rats of diverse weights in the present work, we administered the treatments on a BW basis and focused the results and discussion on BW-adjusted values.

2. General methods

2.1. Animals

Male hybrid rats of the first generational cross between F344 and BN strains (i.e., F344 × BN) were obtained from Harlan (Indianapolis, IN, USA) through services provided by the National Institute on Aging (NIA Bethesda, MD, USA). They were 4 mo (young; $n = 6$) and 20 mo (old; $n = 9$) at the beginning of testing. The rats were housed singly in hanging stainless steel cages in a room with constant temperature (23 °C) and a 12:12 light:dark cycle (lights on at 7:00 am). They received ad libitum access to Purina rat chow, water and 0.3 M NaCl solution unless indicated otherwise. Intakes of water and saline solution from 100 ml graduated cylinders with attached stainless steel spouts fastened to the front of the cages were recorded daily for the duration of the studies described below. All work was conducted according to procedures approved by the University of Iowa Institutional Animal Care and Use Committee and in compliance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals.

2.2. Drugs

Furosemide (Abbott Laboratories, N. Chicago, IL) was administered subcutaneously at 10 mg/kg BW. Captopril (SQ-14, 225; Bristol-Meyers-Squibb Pharmaceutical Research Institute, Princeton, NJ) was dissolved in tap water at 0.1 mg/ml.

2.3. Experimental protocols

Over a period of 11 weeks, the rats received a series of experimental challenges in the following order, with 6–12 days separating tests.

2.3.1. Experiment 1: extracellular fluid depletion

Rats were weighed in the morning and placed in standard metabolism cages with stainless steel funnels underneath. At 1:00 pm, furosemide was injected subcutaneously (sc; 10 ml/kg BW) to induce natriuresis and diuresis. One hour later, water was provided in 100 ml graduated cylinders attached to the front of the cages. Food was not present. Water intakes were recorded the next morning, 20 h later. Both water and 0.3 M NaCl were then provided from 0.1-ml graduated chemical burettes with sipper spouts and intakes were recorded every 30 min for 4 h. The rats were then returned to the home cage where intakes of both solutions were recorded after another 20 h. Rats were given furosemide injections and then, 20 h later, were tested for water and salt intakes at 8–12 day intervals for a total of 3 times. In test 1, urine was collected into Nalgene® tubes (0.1 ml resolution) for the first hour after furosemide injection. Urine for the remainder of the overnight period was collected into pre-weighed glass beakers and urine volume (UV) and water intakes were recorded in the morning. This UV was calculated as 1 g = 1 ml. For tests 2 and 3, urine for the entire 20-h depletion period was collected into pre-weighed glass beakers. Samples were refrigerated for later analysis of sodium content. Urine was collected neither during the salt appetite portion of testing, nor during the subsequent 20 h when the animals were returned to the home cage. Estimates of plasma sodium concentrations were derived from changes in sodium ingestion and excretion according to a formula that assumes similar basal plasma sodium concentrations between groups [33]. The acute (4-h) period for measuring water and saline intakes the morning after depletion is referred to as the "test" period. Measures obtained before and after this period are referred to as "pretest" and "posttest" values, respectively. Similar nomenclature is used for discussing measures obtained in Exp. 2 and 3.

2.3.2. Experiment 2: intracellular fluid depletion

Two tests, separated by 1 week, studied the effects of intracellular fluid depletion. On test days, rats were weighed and placed in standard metabolism cages as above. The rats were injected sc with hypertonic saline (2 ml/kg BW, 1.0 M NaCl on test 1 and 2.0 M NaCl on test 2), and water was provided immediately from glass burettes. Intakes were recorded every 30 min for 3 h. Urine was collected into Nalgene® tubes. Urine volume was measured at 3 h, and samples were refrigerated for later analysis of sodium content. To minimize potential discomfort from the sc injections, the solutions were made with 0.2% lidocaine [25,42]. The animals showed no signs of discomfort.

2.3.3. Experiment 3: overnight food and fluid restriction

At 10:00 am on test days, rats were weighed and placed in standard metabolism cages as above. Food, water and 0.3 M NaCl were not available. Urine was collected in pre-weighed glass beakers. The next morning, 23 h later, UV was measured, water and 0.3 M NaCl were provided from glass burettes, and intakes were recorded every 30 min for 3 h. Samples of urine were refrigerated for later analysis of sodium content.

2.3.4. Experiment 4: captopril adulteration of drinking water

Rats drink greater amounts of water or saline solutions when angiotensin-converting enzyme (ACE) inhibitors, such as captopril, are added to the drinking fluids or diet at low concentrations [24,25,40,42]. In this experiment, daily intakes of water and 0.3 M NaCl from 100-ml graduated cylinders with sipper tubes were recorded while captopril was added to the drinking water (0.1 mg/ml). In the first part of this experiment, intakes of water and 0.3 M NaCl were recorded for 3 days without captopril, for 4 days with captopril in the drinking water, and for 3 days after removal of captopril from the drinking water. In the second part, saline was removed and only water was available for drinking. Water intakes were recorded for 3 days without captopril, for 4 days with captopril in the drinking water, and for 3 days after removal of captopril from the drinking water.

2.4. Urine analysis

Urine was measured for volume (UV). Urinary sodium concentration (UNa) was determined by ion-specific electrodes (NOVA Biomedical, Waltham, MA) and was used for calculating urinary sodium excretion (UNaV). Relative water balances were calculated by subtracting UV from the total amount of fluid ingested (i.e., water or water + saline). Relative sodium balances were calculated by subtracting UNaV from sodium ingested in the form of 0.3 M NaCl. Fecal losses of water and sodium were not considered.