Physiology & Behavior xxx (xxxx) xxx–xxx

Contents lists available at ScienceDirect

Physiology & Behavior

journal homepage: www.elsevier.com/locate/physbeh

Aging affects isoproterenol-induced water drinking, astrocyte density, and central neuronal activation in female Brown Norway rats

Rachel N. Hardy[a], Zinar D. Simsek[a], Brandon Curry[a], Sheri L. Core[a], Terry Beltz[b], Baojian Xue[b], Alan K. Johnson[b], Robert L. Thunhorst[b], Kathleen S. Curtis[a,*]

[a] Oklahoma State University – Center of Health Sciences, Tulsa, OK 74107, United States
[b] University of Iowa, Iowa City, IA, United States

ARTICLE INFO

Keywords:
Subfornical organ
Organum vasculosum of the lamina terminalis
Area postrema
Nucleus of the solitary tract
Supraoptic nucleus

ABSTRACT

Age-dependent impairments in the central control of compensatory responses to body fluid challenges have received scant experimental attention, especially in females. In the present study, we found that water drinking in response to β-adrenergic activation with isoproterenol (30 μg/kg, s.c.) was reduced by more than half in aged (25 mo) vs. young (5 mo) ovariectomized female Brown Norway rats. To determine whether this age-related decrease in water intake was accompanied by changes in central nervous system areas associated with fluid balance, we assessed astrocyte density and neuronal activation in the SFO, OVLT, SON, AP and NTS of these rats using immunohistochemical labeling for GFAP and c-fos, respectively. GFAP labeling intensity was increased in the SFO, AP, and NTS of aged females independent of treatment, and was increased in the OVLT of isoproterenol-treated rats independent of age. Fos immunolabeling in response to isoproterenol was reduced in both the SFO and the OVLT of aged females compared to young females, but was increased in the SON of female rats of both ages. Finally, fos labeling in the AP and caudal NTS of aged rats was elevated after vehicle control treatment and did not increase in response to isoproterenol as it did in young females. Thus, age-related declines in water drinking are accompanied by site-specific, age-related changes in astrocyte density and neuronal activation. We suggest that astrocyte density may alter the detection and/or processing of signals related to isoproterenol treatment, and thereby alter neuronal activation in areas associated with fluid balance.

1. Introduction

The elderly are at major risk for dehydration [1], because of both increased fluid losses [2] and decreased sensation of thirst [3–5]. Indeed, diminished thirst is perhaps the major factor contributing to problems of fluid balance in the elderly [6]. As a result, the elderly are more vulnerable than are the young to the health consequences of dehydration such as confusion, hypotension, and renal dysfunction [1,2]. Accordingly, investigations of water ingestion are critical for understanding declines in body fluid regulation that accompany aging. Notably, the experimental literature contains only a few studies using aging rats to investigate the capacity of behavioral systems, such as thirst, to maintain adequate hydration. Furthermore, most current studies on age-related declines in water intake and body fluid regulation, including our own, have used male rats. In these efforts, we and others have advanced understanding of how the behavioral, renal, and cardiovascular underpinnings of body fluid homeostasis change with age in males [7–13]. However, despite increasing appreciation of the importance of sex differences for body fluid balance in young adult rats (e.g., [14,15]; for review, see [16]), with few exceptions (e.g., [17,18]), aged females have been virtually excluded from studies of body fluid homeostasis.

Isoproterenol (ISOP) is a mixed β-adrenergic agonist that has been extensively employed to investigate thirst mechanisms in rats, including aged rats of both sexes [11,17,18]. ISOP activates the renin-angiotensin system (RAS) both directly by stimulating β_1-adrenergic receptors located on the juxtaglomerular apparatus of the kidney and indirectly from hypotension caused by stimulating vascular β_2-adrenergic receptors [11]. Water drinking in response to ISOP results from high levels of circulating angiotensin II (ANG II) which acts at receptors in the forebrain circumventricular organ, subfornical organ (SFO; [19,20]) to promote thirst. We and others have shown that estradiol (E2) reduces ISOP-induced water intake, neural activation, and ANG II receptors in the SFO of young female rats [21–27], findings that are consistent with an extensive literature focused on central areas involved in body fluid homeostasis [19,20,28–31]. In those studies,

* Corresponding author at: Department of Pharmacology and Physiology, Oklahoma State University – Center of Health Sciences, Tulsa, OK 74107, United States.
E-mail address: kath.curtis@okstate.edu (K.S. Curtis).

https://doi.org/10.1016/j.physbeh.2018.03.005
Received 30 November 2017; Received in revised form 26 February 2018; Accepted 3 March 2018

circumventricular organs in addition to the SFO (i.e., the organum vasculosum of the lamina terminalis (OVLT) and the area postrema (AP)) have been implicated in detecting hormonal signals including ANG II [28–33], while the Nucleus of the Solitary Tract (NTS) receives baroreceptor input related to the hypotension produced by ISOP [30,31]). Moreover, other central areas play a role in compensatory responses to ISOP, with the hypothalamic paraventricular and supraoptic nuclei (PVN, SON) releasing the vasoactive hormones, vasopressin and oxytocin [30,31], and the NTS modifying autonomic function, in part via activation of norepinephrine-containing neurons [35].

Clearly, a wealth of information concerning central controls of water intake has resulted from previous studies. Nonetheless, two oversights are noteworthy. First, nearly all of these studies have been conducted in males, especially male rats (e.g., [19,20]). Much less is known about these central areas in females and, with very few exceptions [17,18], virtually nothing is known about the central controls in *aged* females. Second, this literature has focused primarily on neurons, neurotransmitters, and neural pathways; however, it has become increasingly apparent that non-neuronal cells such as astrocytes play more than a "supporting role". One of the primary functions of astrocytes is their contribution to the blood-brain-barrier via interactions between astrocyte end feet and vascular epithelia [36–38]. In the context of circumventricular organs, which detect circulating hormones by virtue of an incomplete blood-brain-barrier, changes in astrocytes might therefore be expected to alter detection of circulating signals associated with ISOP. In addition, astrocytes may more directly influence neural activity by buffering extracellular potassium concentration, or by the uptake and synthesis of glutamate and GABA [38,39]. In short, astrocytes have the potential to affect ISOP-induced water intake through altering activation in central areas by contributing to neural membrane potential or to levels of neurotransmitters, or by contributing to the blood-brain-barrier.

At present, it is not known whether aging or ovarian hormones such as estrogen may alter astrocytes in circumventricular organs. However, a possible mechanism for age-related changes in astrocytes or astrocyte function that, in turn, might alter neuronal activity is suggested by the neuroprotective effects of the α subtype of estrogen receptors in astrocytes in the central nervous system of mice [40]. Accordingly, the present work examined water drinking, astrocyte density, and neural activation following ISOP treatment in young and aged female rats. In this preliminary assessment of age-related changes in astrocytes associated with ISOP-induced water intake, we focused on SFO, OVLT, and AP (circumventricular organs involved in the detection of ANG II), and the NTS (the primary terminal site of baroreceptor afferents). We also sought to investigate potential functional consequences of any differences in astrocyte density by examining neuronal activation in those areas, in oxytocinergic neurons in the SON, and in norepinephrine-containing neurons in the NTS. To better discriminate between effects due to aging and those due to depletion of ovarian hormones, all experiments were conducted using ovariectomized (OVX) young and aged female rats.

2. Methods

2.1. Animals

Young (5 mo) and aged (25 mo) female Brown Norway rats were obtained from Charles River, Raleigh, NC. They were housed singly in hanging, wire-mesh cages with ad libitum access to standard rodent diet (Teklad) and tap water provided from 100-ml graduated cylinders with attached stainless-steel spouts unless otherwise stated. Room temperature was maintained at 23 °C with a 12:12 light dark cycle. All procedures were conducted in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals, and were approved by the Institutional Animal Care and Use Committees of the University of Iowa and of the Oklahoma State University – Center for Health Sciences.

2.2. Surgical procedures

Under isofluorane anesthesia, bilateral ovariectomy was performed by making a single 2- to 3-cm dorsal midline incision in the skin and underlying muscles. The ovaries were isolated, tied-off with sterile suture and removed, and the incisions were closed. Ovariectomized (OVX) rats were allowed to recover for 8–10 days before testing. Subsets of these OVX rats (aged: $n = 9$, young: $n = 8$) were tested in the following studies; the numbers included in each experimental condition are given in the Results sections pertaining to the individual studies.

2.3. Drugs

The mixed β_1,β_2-adrenergic receptor agonist isoproterenol (ISOP; Isuprel, Hospira, Lake Forest, IL) was diluted 3:1 with isotonic saline. In all experiments, ISOP was injected s.c. (30 μg/kg body weight).

2.4. Experiment 1: Water intake

On the morning of testing, drinking tubes were removed from cages, rats were weighed and then injected with ISOP. A single graduated burette filled with water was placed on the cage and intakes were recorded every 30 min for 90 min.

2.5. Experiments 2 and 3: Immunohistochemistry

One week after the water intake study, rats were randomly subdivided into groups receiving either ISOP or vehicle (VEH; 0.15 M NaCl; 750 μl/kg, s.c.) and then returned to their home cages with no water or food available. Ninety minutes after injections, rats were deeply anesthetized with sodium pentobarbital (Nembutal) and then perfused with 0.15 M NaCl followed by 4% paraformaldehyde. Brains were removed and placed in 30% sucrose until cut into 40-μm serial coronal sections using a cryostat (Leica). Forebrain and hindbrain blocks each were cut in a 1:3 series and stored in a cryoprotectant solution [41] at −20 °C until processed for immunolabeling.

2.5.1. Experiment 2: Glial fibrillary acidic protein (GFAP) immunolabeling

One series each of free-floating forebrain and hindbrain sections was used to label GFAP, a marker of glial cells such as astrocytes after ISOP. Tissue was rinsed with 0.05 M Tris-NaCl, treated with 0.5% H_2O_2 for 30 min, and rinsed again with 0.05 M Tris-NaCl on a rocker at room temperature (RT). Tissue was soaked for 60 min at RT in 10% normal goat serum (NGS) mixed in 0.5% Triton-X in 0.05 M Tris-NaCl, then incubated for ~48 h on a rocker at 4 °C in the primary antibody (Millipore, mouse anti-GFAP; clone GA5, diluted 1:6000 in 2% NGS in 0.05 M Tris-NaCl with 0.5% Triton-X). Sections were rinsed, incubated in Cy3-conjugated goat anti-mouse IgG (Jackson Immunoresearch; diluted 1:200 in 2% NGS) for 4–6 h, and then rinsed multiple times. Sections were ordered, mounted on gelatinized slides, and dried overnight. Tissue was dehydrated in a series of EtOHs, defatted in xylenes, and then coverslipped using Cytoseal 60 (Fisher Scientific).

2.5.1.1. Quantification. The SFO, organum vasculosum of the lamina terminalis (OVLT), area postrema (AP), and the nucleus of the solitary tract (NTS) at the middle of the rostral-caudal extent (mNTS) were identified under brightfield microscopy using a Nikon Eclipse 80i microscope with rhodamine and FITC filter sets based on neuroanatomical landmarks and coordinates [42]. These areas were captured using NIS-Elements AR software 3.2 (Nikon) with exposure times held constant. SFO, OVLT, AP each were traced; a square of uniform size was placed on the mNTS ventral to the lateral extent of the AP and dorsal to the dorsal motor nucleus of the vagus. GFAP