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The effects of aging on biosynthetic processes in the rat hypothalamic osmoregulatory neuroendocrine system

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ABSTRACT

Elderly people exhibit a diminished capacity to cope with osmotic challenges such as dehydration. We have undertaken a detailed molecular analysis of arginine vasopressin (AVP) biosynthetic processes in the supraoptic nucleus (SON) of the hypothalamus and secretory activity in the posterior pituitary of adult (3 months) and aged (18 months) rats, to provide a comprehensive analysis of age-associated changes to the AVP system. By matrix-assisted laser desorption/ionization time-of-flight mass spectrometry analysis, we identified differences in pituitary peptides, including AVP, in adult and aged rats under both basal and dehydrated states. In the SON, increased Avp gene transcription, coincided with reduced Avp promoter methylation in aged rats. Based on transcriptome data, we have previously characterized a number of novel dehydration-induced regulatory factors involved in the response of the SON to osmotic cues. We found that some of these increase in expression with age, while dehydration-induced expression of these genes in the SON was attenuated in aged rats. In summary, we show that aging alters the rat AVP system at the genome, transcriptome, and peptidome levels. These alterations however did not affect circulating levels of AVP in basal or dehydrated states.

1. Introduction

As we age, disorders of body salt and water composition become more commonplace. Cases of hyponatremia/hypernatremia are much more prevalent in the elderly, where they have been linked to increased incidences of falls, fractures, and osteoporosis, thus contributing to increased hospital admissions and morbidity and mortality (Cowen et al., 2013). To promote healthy living well into old age, it is thus necessary to determine why such imbalances occur. Age-associated changes to both peripheral and central mechanisms that control salt and water homeostasis are deemed responsible. There is a progressive age-related decline in renal function, with less urine concentrating capacities in the elderly compared with younger subjects (Ishunina and Swaab, 2002). Such impaired capacity to conserve body water, together with reports of reduced thirst and inadequate fluid intake after periods of fluid deprivation, makes the elderly more susceptible to dehydration (Mack et al., 1994; Phillips et al., 1993). Inappropriate release of the antidiuretic hormone arginine vasopressin (AVP) into the systemic circulation has been highlighted as one of the causes of irregular water homeostasis in aging (Swaab and Bao, 2011).

AVP is synthesized in magnocellular neurons of the supraoptic nucleus (SON) and paraventricular nucleus (PVN) of the hypothalamus. A change in plasma osmolality is detected by osmosensitive neurons in circumventricular organs of the brain that provide direct inputs to shape the firing of AVP magnocellular neurons that are osmosensitive themselves and to coordinate AVP synthesis and secretion from the posterior (neural) lobe of the pituitary gland (Mecawi Ade et al., 2015; Nissen et al., 1994; Zhang and Bourque,

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2003). Once released, following incidences such as rise in plasma osmolality or decrease in blood volume (Kondo et al., 2004), AVP promotes sodium and water reabsorption by the kidney (Ares et al., 2011; Breyer and Ando, 1994). When placed under stress, the capabilities of the AVP system have been shown to decrease with age (Frolkis et al., 1999; Keck et al., 2000; Sladek et al., 1981).

The AVP system has been interrogated on multiple levels, from synthesis to secretion, in aged subjects with differing results. For example, basal circulating AVP levels have been found to decrease, to remain unchanged, and to increase with age in humans, as well as rodents (Frolkis et al., 1999). These discrepancies have been attributed to genetic, age, and strain differences. Although there are many disputations, 1 area of agreement is that, while in many brain areas neuronal activity decreases with age (Burke and Barnes, 2006), paradoxically AVP neurons become more active (Palin et al., 2009; Terwel et al., 1992). This hyperactivity is thought to be a compensatory mechanism for decreased responsiveness to AVP in the kidney due to decreased receptor abundance in aged subjects (reviewed by Ishunina and Swaab, 2002), although this theory has been questioned (Preisser et al., 2004) and is not intuitive with the profound difference in circulating AVP described in aging models. The AVP magnocellular neurons undergo numerous morphological changes as they age including increased size of perikarya, nucleoli, and Golgi apparatus in humans and rodents (Ishunina and Swaab, 2002), analogous to morphological changes in these neurons with dehydration (Hatton and Walters, 1973). Increased AVP neuron size in states of dehydration is recognized as a necessary measure to meet cellular demands for increased transcription and protein synthesis under hypertonic stimulation where circulating levels of AVP are robustly increased (Zhang et al., 2001). It has been suggested that such hyperactivity of AVP neurons may in itself lead to electrolyte disorders in the elderly (Swaab and Bao, 2011), but the relationship between the activity of AVP neurons and circulating levels of AVP is poorly understood.

Transcriptional changes have been identified in AVP neurons with aging. A study by Palin et al. (Palin et al., 2009) showed increased expression of immediate early gene c-Fos, a commonly used marker of neuronal activity, in the rat SON, consistent with hyperactivation of the AVP neurons. In contrast to increased activity under basal conditions, reports have described an attenuation of the evoked AVP secretion in response to osmotic stress with aging in rodents (Sladek and Olschowka, 1994; Swenson et al., 1997). This has led some to suggest that deficits in mechanisms controlling transcription, mRNA stability, or translation in the aging SON magnocellular neurons may be responsible (Lucassen et al., 1997). Moreover, we recently showed that dehydration initiates the formation of new methylation marks on the rat Avp promoter (Greenwood et al., 2016a), suggesting that altered methylation patterns could lie beneath these transcriptional changes in aging AVP neurons.

Few studies have sought to combine information on the physiological aspects of aging with analyses of the molecular changes occurring in the hypothalamus. The reasons why old AVP neurons have elevated basal activity, or why they can fail to adequately respond under stress, is not well understood. In particular, the molecular basis for these changes in relation to circulating levels of AVP has received little attention. We reasoned that aging-associated deficits in the AVP system may be due to a combination of changes at the genome, transcriptome, and peptidome levels, and that these changes might be responsible for disturbances in osmotic stability. In this study, we have interrogated the physiological aspects of aging, performed metabolic measurements, and analyzed peptide levels in plasma and pituitary, correlating the results with molecular events occurring within the hypothalamus of aged rats. Furthermore, we have used our extensive knowledge of the transcriptome of the adult rat SON in euhydrated and dehydrated states to uncover novel changes surrounding altered Avp transcription in aging.

2. Materials and methods

2.1. Animals

All experiments were performed under a Home Office UK licence held under, and in strict accordance with, the provisions of the UK Animals (Scientific Procedures) Act (1986); they had also been approved by the University of Bristol Animal Welfare and Ethical Review Board. We choose to use male Wistar Han rats from the international genetic standardization program (IGS) in our aging study (Charles River, France). The carefully managed breeding program for these animals helps to manage genetic drift, so colonies bred in different locations around the world are not significantly divergent from each other giving a level continuity in aging studies performed in laboratories worldwide. The Charles River Han Wistar rats have been extensively studied at 2 years of age when these rats are reaching the end of their natural life spans. Incidences of neoplastic and non-neoplastic lesions were high in tissues including the kidney and pituitary gland at this age. Furthermore, rats surviving to this age varied from 30% to 80% across 20 control studies (www.criver.com). Therefore, in this aging study, we opted for rats of 18 months of age to minimize pathophysiological effects and thus allow investigation of the aging process in healthy animals. All adult rats used in this study were free of pituitary tumors; however, 6 of 50 aged animals were removed from this study because of tumors on the pituitary gland. On arrival, rats were 2 weeks younger than the desired ages, 3 months (adult) and 18 months (aged), to enable sufficient time for acclimatization before experimentation. Rats were housed at a constant temperature of 22 °C and a relative humidity of 50%–60% (v/v) under a 14:10-hour light/dark cycle (lights on at 0500) with food and water *ad libitum* for 2 weeks. To induce hyperosmotic stress, both adult and aged rats were randomly assigned to 2 groups: control (free access to drinking water) and dehydrated (removal of drinking water for 3 days). All rats were humanely killed by striking of the cranium (stunning) and then immediately decapitated with a small-animal guillotine (Harvard Apparatus, Holliston, MA). Trunk blood was collected in heparin-coated tubes. Brains were rapidly removed from the cranium and immediately frozen by covering with powdered dry ice (within 3 minutes of stunning). The pituitary gland was removed from the base of the skull within 2 minutes after decapitation. The neurointermediate lobe (NIL) was carefully separated from the anterior pituitary using a scalpel blade and then either placed into 1.5-mL tubes containing 500 μL of 0.1 M of HCl or 0.2-mL tubes containing 150 μL of 15 mg/mL of 2, 5-dihydroxybenzoic acid (DHBA) solution. Frozen brains and NIL in HCl solution were stored at −80 °C, whereas NILs in DHBA solution were stored at 4 °C. Animal experiments were performed between 9 AM and 2 PM.

2.2. Metabolic measures in adult and aged rats

For metabolic measurements, animals were individually housed in metabolic cages (Techniplast, Italy) to allow precise daily measures of fluid and food intake and urine output. A plastic gnawing disc was suspended from the lid of the cage to provide environmental enrichment throughout the study. Animals were first acclimatized to metabolic cages for 48 hours. Measures of food hoppers, water bottles, and urine collection tubes were performed for 3 consecutive days, by weight. Extrarenal secretion of fluid was calculated by subtracting water intake from urine output. Plasma