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Age-related changes in serum and brain levels of androgens in male Brown Norway rats

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Abstract

Age-related depletion of androgens in men results in functional impairments in androgen-responsive tissues, such as the brain, resulting in increased risk for Alzheimer's disease. To investigate the relationship between normal age-related hormone loss and Alzheimer's disease risk, we evaluated the brain and serum levels of androgens and estrogen in aging male rats. We observed that increasing age was associated with a significant reduction in brain levels of the potent androgen dihydrotestosterone and a trend toward decreased testosterone. Brain levels of soluble β-amyloid were observed to increase with age. Collectively, these findings highlight differences in brain and circulating levels of androgens during aging, and identify an inverse correlation with β-amyloid levels that may be relevant to Alzheimer's disease risk.

Keywords

aging; Alzheimer's disease; β-amyloid; dihydrotestosterone; estrogen; testosterone

Introduction

During normal aging, men experience a significant decline in circulating levels of their primary sex steroid hormone, testosterone [1]. Age-related testosterone depletion in men is associated with increased risk of disease and dysfunction in androgen-responsive tissues including brain [2]. As testosterone induces both androgenic and estrogenic actions, the senescent effects of testosterone loss may reflect androgen and or estrogen deficits. Induction of androgen and estrogen actions of testosterone is determined in large part by its

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enzymatic conversion in tissues. Testosterone is reduced to the potent androgen dihydrotestosterone (DHT) by the enzyme 5α-reductase and converted to the estrogen 17β-estradiol (E_2) by aromatase. Thus, understanding how age-related testosterone loss affects the brain requires knowledge of androgen and estrogen levels in brain.

Although tissue levels of sex steroid hormones can parallel circulating levels, factors including the presence of sex hormone-binding globulin in blood and steroidconverting enzymes in tissues often yield differences between serum and tissue levels [3,4]. Therefore, levels of hormones in specific tissues are generally thought to provide a more accurate measure of bioavailable hormones than serum levels [4]. Serum levels of testosterone, but neither DHT nor E_2 are decreased in aging men [1]. How normal male aging affects tissue levels of sex steroid hormones has not been well studied, although there is evidence of age-related decreases in androgen levels in heart, lung, and pubic skin [5]. Earlier study from our laboratory found that brain levels of testosterone [6] and DHT [7] decrease in aging men.

In brain, age-related testosterone depletion in men is associated with impaired cognition [8] and increased risk of Alzheimer's disease [9]. Androgens have numerous protective actions in brain, the loss of which may contribute to the senescent effects of low testosterone. Of particular relevance to Alzheimer's disease, androgens are implicated in reducing levels of amyloid-β (Aβ) [10–13], a protein implicated as a causal factor in Alzheimer's disease pathogenesis [14]. For example, experimental depletion of androgens in male rodents significantly increases Aβ accumulation in brain, an effect prevented by androgen treatment [15,16]. However, the relationships between normal aging, Aβ accumulation, and circulating and brain levels of sex steroid hormones remain undefined. In this study, we investigated these issues in aging male Brown Norway (BN) rats, a model of male reproductive aging [17].

Materials and methods

Animals

Male BN rats (National Institute on Aging; Bethesda, Maryland, USA) were housed in individual cages with *ad libitum* access to food and water under a 12 h on/12 h off light cycle. Rats were anesthetized and killed by decapitation at ages 3, 13, and 23 months ($N = 7$/group). At the time of killing (i) trunk blood was collected and separated into the serum fraction; (ii) seminal vesicles were dissected, blotted, and weighed as a bioassay of androgen activity; and (iii) brains were rapidly dissected, bisected in the sagittal plane, and snap-frozen for Aβ and hormone determinations.

Hormone assays

Steroid hormones were purified from frozen brain tissue and serum using Celite chromatography [18,19], and then quantified by radioimmunoassay as described earlier [7]. Briefly, frozen (−80°C) brain tissue was thawed, weighed, and homogenized in 1 ml of ice-cold PBS. Trace levels of ^{3}H-labeled testosterone, DHT, and E_2 were included as internal standards to correct for procedural losses in both the tissue homogenates and serum samples. Samples were extracted with hexane/ethyl acetate (3 : 2) and separated from interfering

steroids by Celite column partition chromatography with ethylene glycol as stationary phase. DHT and testosterone were eluted with 10 and 35% toluene in isooctane, respectively, whereas elution of E_2 was accomplished with 40% ethyl acetate in isooctane. Levels of purified hormones were determined by validated radioimmunoassays. These assays have been shown to be sensitive, accurate, precise, and specific; interassay and intra-assay coefficient of variation were less than 12% [18,19]. The sensitivity of the radioimmunoassays is 15 pg/ml for testosterone, 8 pg/ml for DHT, and 3 pg/ml for E_2; all collected data exceeded these detection limits. Data from triplicate readings are presented as a fraction of starting tissue weight or per unit volume serum. Data were statistically compared using analysis of variance followed by between-group comparisons using the Fischer's Least Significant Difference test.

Amyloid-β enzyme-linked immunosorbent assay

Brain levels of soluble Aβ1-40 were determined by enzyme-linked immunosorbent assay (ELISA) as described earlier [20]. In brief, hemi-brains were processed for Aβ ELISA after extracting soluble protein by homogenization in buffer (0.2% diethylamine, 50 mM NaCl; 1 ml/100 mg tissue) using a polytron for 1 min on ice. Homogenates were centrifuged at 100 000*g* at 4°C for 1 h and the supernatants collected and neutralized (1/10th volume of 0.5 M Tris–HCl pH 6.8). This soluble protein fraction was used to measure Aβ with a sandwich ELISA using 13.1.1 as the Aβ40 specific capture antibody and 32.4.1 as the rodent-specific detection antibody. Raw data (expressed as pmol Aβ/g protein) were statistically analyzed by analysis of variance followed by between-group comparisons using the Fischer's Least Significant Difference test.

Results

Circulating and brain levels of sex steroid hormones during aging

To investigate the effects of normal male aging on sex steroid levels, we measured circulating and brain levels of testosterone, DHT, and E_2 in male BN rats at ages 3, 13, and 23 months. Serum levels of testosterone decreased by nearly 50% between 3 and 13 months of age and by 63% at the age of 23 months (Table 1). In contrast, we observed no age-related changes in circulating levels of DHT (Table 1). The estrogen E_2 showed a nonsignificant increase in middle age (Table 1). Analysis of sex steroid hormone levels in hemi-brain homogenates showed a different pattern of age-related changes. Brain levels of testosterone showed a statistically nonsignificant trend of reduction with an approximately 40% decrease between ages 3 and 23 months. In comparison, the androgen metabolite DHT showed a relatively stronger age-related decrease. In comparison with brain levels in young adult 3-month-old rats, DHT decreased by 80% at the age of 13 months and more than 90% at the age of 23 months (Table 1). Brain levels of E_2 showed a statistically nonsignificant trend of age-related depletion (Table 1). The observation of significantly decreased DHT in brain, but not serum suggests the possibility of age changes in testosterone metabolism. To investigate this possibility, we evaluated precursor/product ratios for testosterone and its metabolites DHT and E_2. The testosterone/DHT ratio in brain increased from 3.57 at the age of 3 months to 9.29 at the age of 13 months and 9.71 at the age of 23 months ($F = 5.6$, $P < 0.02$). In comparison, the testosterone/E_2 ratio in brain did not significantly change with age ($F = 0.4$,

$P = 0.68$). Similarly, the correlation between brain levels of testosterone and DHT decreased from $r = 0.80$ ($P < 0.04$) at the age of 3 months to $r = 0.04$ ($P = 0.65$) at the age of 13 months and $r = 0.23$ ($P = 0.66$) at the age of 23 months. Brain levels of testosterone and E_2 were not significantly correlated at any age.

Age-related changes in androgen-sensitive measures

To evaluate whether androgen function was impaired by age-related hormone losses, we first measured a biomarker of androgen levels, size of the seminal vesicles. We found a significant, age-related decrease in seminal vesicle weight normalized to body weight ($F = 59.1$, $P = 0.001$). In comparison with the age of 3 months ($1.74 \pm 0.13 \times 10^3$), normalized seminal vesicle weight decreased by approximately 35% at the age of 13 months ($1.12 \pm 0.13 \times 10^3$) and by nearly 50% at the age of 23 months ($0.93 \pm 0.15 \times 10^3$). Next, we measured brain levels of Aβ, a protein negatively regulated by androgens [9]. We observed that brain levels of $A\beta_{1–40}$ significantly increased ($F = 4.8$, $P = 0.03$) by approximately 300% at the age of 13 months and nearly 400% by the age of 23 months in comparison with levels observed in young adult 3-month-old rats (Fig. 1).

Discussion

In this study, we examined age-related changes in circulating and brain levels of androgens and estrogen. We found that increasing age was associated with a nonsignificant decrease in circulating testosterone, no obvious changes in circulating levels of DHT, and a transient increase in E_2 at middle age. In contrast, brain levels of all the three hormones showed evidence of reduction, although only the robust depletion of DHT achieved statistical significance. In parallel, significant impairments were observed in androgen-sensitive seminal vesicle weight and brain levels of Aβ, suggesting functional consequences of age-related androgen loss.

Our results are consistent with reported age changes in circulating levels of sex steroids in male rats. Testosterone levels in young adult male rats are fairly consistent across strains with mean levels normally between 2 and 3 ng/ml [17,21,22]. An approximately 50% or greater age-related reduction in testosterone has been reported in Sprague–Dawley [23], Wistar [21], and Fischer [22] rat strains. Similarly, earlier study in male BN rats showed a significant, approximately 75% loss in circulating levels of testosterone between 6 and 28 months of age [17]. Consistent with this established literature, we observed a trend of decreased testosterone levels with aging (although this effect did not meet statistical significance) and no evidence of age changes in DHT. Although testosterone levels can be affected by illness and chronic disorders, age-related depletion of circulating testosterone in male BN rats is a normal consequence of aging resulting from testicular atrophy as well as dysregulation of the hypothalamic–pituitary–gonadal axis [17,24]. The effects of aging on circulating level of E_2 are less clear, with reports indicating no change [24] or increased levels [22]. Interestingly, in a recent report, circulating E_2 levels in male Sprague–Dawley rats were observed to increase during middle age followed by a decrease at the age of 22 months, whereas circulating testosterone levels progressively declined across ages [25]. Our

findings in male BN rats are most consistent with the latter observations of transient increases in serum E_2 during middle age.

An important new finding in our study is that age changes in sex steroid hormones differ between brain and blood. Earlier studies of sex steroid hormones in animals largely focused on serum rather than brain levels and have not investigated the effects of age on brain levels in either males or females. Our findings suggest that the male brain is particularly vulnerable to age-related changes in sex steroid hormone levels. Specifically, we observe evidence of decreased levels of testosterone, DHT, and E_2 in brain. In recent studies, we examined levels of sex steroid hormones in the frontal cortex of aged men and found that brain levels of testosterone decreased with increasing age, however, there was no change in brain levels of E_2 [6]. Similar to our findings with the male BN rats, we also found an age-related decrease in brain levels of DHT in men [7]. Together, these data suggest that the male brain is particularly vulnerable to age-related androgen depletion although the underlying factors are unknown. Two potential causes are age-related changes in sex hormone binding globulin, which could reduce neural accumulation of testosterone, and altered activity of converting enzymes [19,26]. Our data show changes in the precursor/product ratios between testosterone and DHT as well as changes in the correlation between these two androgens, both of which suggest decreased neural conversion of testosterone to DHT with increasing age. Interestingly, we did not observe a relationship between testosterone and E_2 levels, suggesting that brain levels of E_2 may be the result of another prohormone, such as estrone. Further research is needed to investigate the roles of hormone-converting enzymes and other factors contributing to the age-related decrease in brain levels of androgens.

Earlier study from our lab suggests that androgens may modulate risk for the development of Alzheimer's disease through regulation of Aβ [15,16]. In humans, age-related androgen depletion is inversely correlated with elevated Aβ levels [7,12]. In this study, we also observe that aging is associated with both a decrease in brain levels of androgens and an increase in brain levels of the Alzheimer's-related protein Aβ, however, the design of this study does not permit for the assessment of a potential causal relationship between these factors. Although advancing age is the greatest risk factor for idiopathic Alzheimer's disease, the age-related changes underlying this risk are unknown. The present data are consistent with the theory that age-related loss of androgens in brain may increase Alzheimer's disease risk in men by facilitating the Aβ accumulation.

Conclusion

This study shows that normal aging in male BN rats is characterized by decreased brain levels of sex steroid hormones and increased levels of the Alzheimer's-related protein Aβ. These findings may be important for understanding the role of sex steroid hormones in brain aging and age-related neurological disorders including Alzheimer's disease.

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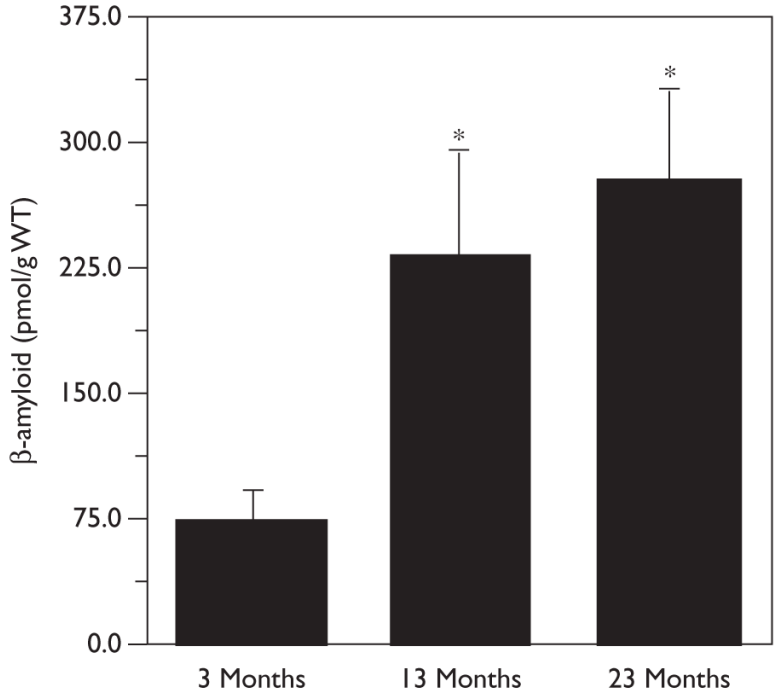

Fig. 1. Brain levels of soluble β-amyloid protein increase during normal male aging. Data show mean levels (±SEM) of $A\beta_{1–40}$ protein as measured by enzyme-linked immunosorbent assay from hemi-brain homogenates of male Brown Norway rats at ages 3, 13, and 23 months (*N* = 7/ group)

* *P*<0.05 relative to the 3 month group. WT, wet tissue.

Table 1

Age-related changes in serum and brain levels (mean ±standard error) of testosterone, DHT, and estrogen (E_2) in male Brown Norway rats

	Age of male rats			
	3 Months	**13 Months**	**23 Months**	***P* value**
Serum testosterone (ng/ml)	2.9 ± 0.9	1.5 ± 0.4	1.1 ± 0.2	0.07
Serum DHT (pg/ml)	57.2 ± 10.1	50.0 ± 9.4	68.0 ± 10.1	0.45
Serum E_2 (pg/ml)	5.0 ± 8.0	21.0 ± 8.7	7.6 ± 8.7	0.41
Brain testosterone (ng/g WT)	2.1 ± 0.5	1.4 ± 0.5	1.3 ± 0.6	0.50
Brain DHT (ng/g WT)	0.65 ± 0.1	0.13 ± 0.1*	0.08 ± 0.1*	0.004
Brain E_2 (pg/g WT)	79.6 ± 15.9	50.7 ± 15.9	27.7 ± 17.1	0.11

DHT, dihydrotestosterone; WT, wet tissue.

*P<0.05 relative to 3 months.