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Changes in androgen receptor, estrogen receptor alpha, and sexual behavior with aging and testosterone in male rats

Di Wu [a], Andrea C. Gore [a,b,c,*]

[a] Division of Pharmacology and Toxicology, College of Pharmacy, The University of Texas at Austin, Austin, TX 78712, USA
[b] Institute for Neuroscience, The University of Texas at Austin, Austin, TX 78712, USA
[c] Institute for Cellular and Molecular Biology, The University of Texas at Austin, Austin, TX 78712, USA

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ABSTRACT

Reproductive aging in males is characterized by a diminution in sexual behavior beginning in middle age. We investigated the relationships among testosterone, androgen receptor (AR) and estrogen receptor alpha (ERα) cell numbers in the hypothalamus, and their relationship to sexual performance in male rats. Young (3 months) and middle-aged (12 months) rats were given sexual behavior tests, then castrated and implanted with vehicle or testosterone capsules. Rats were tested again for sexual behavior. Numbers of AR and ERα immunoreactive cells were counted in the anteroventral periventricular nucleus and the medial preoptic nucleus, and serum hormones were measured. Middle-aged intact rats had significant impairments of all sexual behavior measures compared to young males. After castration and testosterone implantation, sexual behaviors in middle-aged males were largely comparable to those in the young males. In the hypothalamus, AR cell density was significantly (5-fold) higher, and ERα cell density significantly (6-fold) lower, in testosterone- than vehicle-treated males, with no age differences. Thus, restoration of serum testosterone to comparable levels in young and middle-aged rats resulted in similar preoptic AR and ERα cell density concomitant with a reinstatement of most behaviors. These data suggest that age-related differences in sexual behavior cannot be due to absolute levels of testosterone, and further, the middle-aged brain retains the capacity to respond to exogenous testosterone with changes in hypothalamic AR and ERα expression. Our finding that testosterone replacement in aging males has profound effects on hypothalamic receptors and behavior has potential medical implications for the treatment of age-related hypogonadism in men.

Introduction

In a variety of mammalian species including rats, monkeys and humans, there is an age-related decline in sexual performance that may be attributable to physiological or psychological changes (Chambers and Phoenix, 1983; Nicolosi et al., 2004; Smith et al., 1992). Much of the loss of male sexual function with aging has been correlated with a decline in serum testosterone. This change has been shown in humans (Harman et al., 2001), rhesus monkeys (Downs and Urbanski, 2006), and rats, including Sprague–Dawley (Roselli et al., 1986; Wu et al., 2009; Karpas et al., 1983), Wistar (Bernardi et al., 1998; Taylor et al., 1996), Brown Norway (Chen et al., 1994; Gruenewald et al., 2000), and Fischer 344 strains (Chambers et al., 1991; Luine et al., 2007).

However, not all studies show a clear relationship among testosterone, aging, and sexual behavior, a concept that is particularly important when considering the transitional life period of middle age. In fact, during this life stage, testosterone levels in rats may or may not differ from those in young animals depending on the absolute chronological age, strain, and/or other physiological, behavioral and experimental differences. Thus, whereas some studies have shown age-related declines in testosterone at middle age, others have not demonstrated such a loss (Gruenewald et al., 2000; Frankel and Mock, 1981; Wu and Gore, 2009). Furthermore, testosterone replacement to castrated aging rats does not effectively reinstate sexual performance as it does in young rats (Chambers and Phoenix, 1984, 1986; Chambers et al., 1991; Sato et al., 1998), underscoring the point that absolute testosterone levels do not necessarily predict the capacity to perform sexual behavior, and that there are other changes to the aging brain that may explain this behavioral decline. Furthermore, much of this latter literature is primarily focused on old rats and information about effects of testosterone treatment to middle-aged rats is rather limited, raising the question of what hormonal and neurobiological changes may be occurring at this life stage.

* Corresponding author. Division of Pharmacology and Toxicology, University of Texas at Austin, College of Pharmacy, 1 University Station, A1915, Austin, TX 78712. Fax: +1 512 471 5002.
E-mail address: andrea.gore@mail.utexas.edu (A.C. Gore).

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We hypothesized that the loss of sexual behavior with age may not be due to levels of testosterone *per se* but rather to age-related changes in the actions of testosterone on its androgen receptor (AR) in brain regions involved in the control of masculine sexual behaviors and the hypothalamic–pituitary–gonadal axis (Vagell and McGinnis, 1998; Portillo et al., 2006). Circulating testosterone levels regulate expression of the AR, as in young males, castration can eliminate, and testosterone replacement can restore, AR expression in the brain (Iqbal et al., 1995; Lu et al., 1998, 1999; Wood and Newman, 1993). In comparing young and old male Fischer 344 rats (4 and 26 months, respectively), Chambers et al. (1991) showed that nuclear AR binding is very low in castrated rats, and elevated by testosterone treatment in both ages, albeit to a greater extent in the young group. This suggests that the responsiveness of the aging brain to testosterone is diminished. We showed age-related increases in AR immunoreactive cells within several preoptic–hypothalamic regions between young and middle-aged rats (Wu and Gore, 2009; Wu et al., 2009). However, much more information is needed about the implications of this change at midlife.

It is also important to consider that some actions of testosterone on masculine behaviors and physiology are due to its aromatization to estradiol and subsequent actions on estrogen receptors (ER) (Clancy et al., 2000; Larsson et al., 1973; Morali et al., 1977; Vagell and McGinnis, 1997; Zumpe et al., 1993). Relatively little is known about the interactions among estradiol, aging, and behavior. Even the literature on serum estradiol is quite contradictory depending upon rat strain and absolute age: levels of estradiol have been reported to undergo no change (Goya et al., 1990; Wu et al., 2009), decrease [an effect that was dependent upon prior sexual experience (Wu and Gore, 2009)], or increase (Fujita et al., 1990; Herath et al., 2001; Luine et al., 2007). The hypothalamic ERα may also change with age, although evidence to date shows little change in ERα gene expression or protein immunoreactivity with aging (Bottner et al., 2007; Madeira et al., 2000; Wu and Gore, 2009; Wu et al., 2009). Still, this question merits investigation in the context of sexual behavior and aging.

In the current study, we investigated the effects of aging, testosterone treatment, and their interactions on sexual behavior and expression of AR and ERα in the hypothalamus, focusing on the transition between young and middle-aged rats. Together, these studies were intended to provide novel information about the potential roles of these hormone receptors, their regulation by testosterone, and their relationship to sexual behavior during reproductive aging.

Materials and methods

Animals

Male rats

Male Sprague–Dawley rats were purchased at 2–3 months (young, $N=18$) and 10–11 months (middle-aged, $N=38$) from Harlan Sprague–Dawley Inc (Indianapolis, Indiana; Stock/Strain: Hsd:Sprague–Dawley®™ SD®™). The middle-aged rats were retired breeders. Young rats were given sexual experience by placing them with a receptive female rat (rotated among males) for twenty consecutive nights and checking for sperm in the females' vaginas the next morning. At the end of this period, all young males had mated on average 15 times, estimated to be similar to what middle-aged breeders had experienced in the breeding colony.

Female rats

Female Sprague–Dawley rats (same stock) were purchased at 2–3 months ($N=37$). They were ovariectomized and estradiol-implanted [one silastic capsule (1.98-mm I.D.×3.18-mm O.D. ×5-mm length; Dow Corning Corporation, Midland, MI) packed with 5% crystalline 17β-estradiol (Sigma-Aldrich, St. Louis, MO)]. These female rats received 500 μg progesterone (Sigma-Aldrich, St. Louis, MO) in sesame oil (0.1 ml) injection 3 h before the mating trials. Only females who showed lordosis with male rats were used in mating trials.

Rats were housed in an AAALAC-approved facility (two same-sex animals per cage, cage dimensions 47×20×25 cm) with Rat Sterilizable Diet (Harlan Teklad LM-485 7012, Madison, WI) and water available *ad libitum*. The light cycle was 12 h light, 12 h dark cycle (lights on 2300 h), and temperature was 21±1 °C. All animal procedures were approved by the UT-Austin Institutional Animal Care and Use Committee (Protocol number: 08030101) and studies were performed following the *Guide for the Care and Use of Experimental Laboratory Animals*.

Pre-tests for mating behaviors

All the male rats were given two pre-tests (1 day off between tests) to measure the baselines for each parameter of the mating behaviors, with different receptive females rotated among males. The experimental design is shown in Fig. 1.

Mating tests were performed under dim red light, beginning 3 h after lights out (1400 h–1700 h). Every female was habituated to the chamber for 10 min. Every male was habituated to the chamber for 20 min before the female was introduced. 20-min mating trials took place in a transparent Plexiglas cage [76 cm (L)×32 cm (W)×46 cm (H)]. The following parameters of male sex behavior were recorded in each test (Agmo, 1997): latency to mount; frequencies of mount, intromission and ejaculation; and percentage of rats that intromit or ejaculate in the 20-min test. The test was terminated at 20 min or after a second ejaculation, whichever came first. The choice of this 20-min cut-off was based on the literature using trials of 15–30 min for similar measures (Damassa et al., 1977; Frankel, 1981; Chambers et al., 1991; Vagell and McGinnis, 1998; Wu and Gore, 2009). Mating trials were recorded using a Sony Handycam Hi-Fi Stereo Video 8 XR camcorder (Sony Corporation of America, New York, NY). The videotaped trials were further analyzed by using JWatcher v1.0 computer software (www.jwatcher.ucla.edu, Dan Blumstein's Lab, UCLA and The Animal Behaviour Lab, Macquarie University, Sydney) to quantify the behavior of each experimental animal (Crews et al., 2007).

All the young males reached ejaculation in both of the two pre-tests ($n=18$). For middle-aged male rats, only those who ejaculated successfully in at least one of the two pre-tests were chosen to participate in further experiments ($n=27$). Only these latter rats are described in the remaining sections, in order to focus comparisons on rats with residual capacity to mate and based on the paradigm of Frankel (1981).

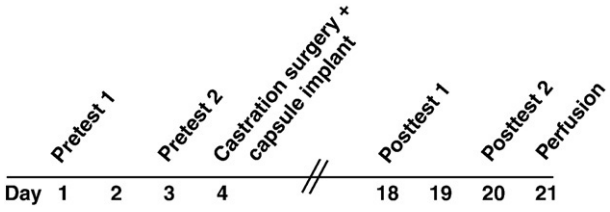

Fig. 1. An experimental timeline is shown. Young and middle-aged rats were given two pretests 2 days apart. One day later, they were anesthetized, and a blood sample was drawn to enable measurement of intact serum hormone levels. Then, rats were bilaterally castrated, and implanted with a Silastic capsule containing either testosterone or vehicle. Two weeks later, the post-tests were performed 2 days apart. One day after testing, rats were euthanized by perfusion; a blood sample collected at that time was used for measuring hormone levels following the castration and hormone treatment.