Brain Aromatase in Control Versus Castrated Norway Brown, Sprague-Dawley and Wistar Adult Rats (44394)

LORI J. MATHIAS, NATHAN A. JACOBSON, REUBEN W. RHEES, AND EDWIN D. LEPHART[1]
Department of Zoology, Cellular Biology Division, Brigham Young University, Provo, Utah 84602

Abstract. **Brain aromatase cytochrome P450 converts androgens to estrogens that play a critical role in the development of sexually dimorphic neural structures, the modulation of neuroendocrine function(s), and the regulation of sexual behavior. We characterized the influence of surgical castration on brain aromatase in Norway Brown and Wistar adult rats and compared their responses to Sprague-Dawley rats that were surgically or biochemically castrated (with flutamide, a known androgen receptor blocker). Aromatase enzyme activity was measured by the tritiated water release assay in the medial basal hypothalmus/preoptic area (MBH/POA) and amygdala brain regions. The present results demonstrate that independent of the rat strain examined, MBH/POA aromatase is regulated by androgens (in Sprague-Dawley, Norway Brown and Wistar males). However, intact Wistar animals displayed significantly higher MBH/POA aromatase levels compared to Sprague-Dawley control values. Conversely, in the amygdala region, there was an apparent lack of androgen hormone action upon aromatase enzyme activity in some of the rat strains tested. The importance of brain aromatase regulating estrogen biosynthesis and influencing brain development and function is covered.** [P.S.E.B.M. 1999, Vol 221]

Brain aromatase enzyme activity that catalyzes the conversion of androgens to estrogens is involved in the determination and plasticity of sexually dimorphic neural structures, the regulation of neuroendocrine functions, reproductive behaviors and modulating memory, aging and neurodegenerative processes (1–9). Presumably, aromatase activity has been phylogenetically conserved since it is detectable in brain tissue sites of all mammalian species studied thus far (3). In certain brain areas, the regulation of aromatase activity is controlled by androgens (3, 8, 10–12). For example, aromatase activity significantly declines in the medial basal hypothalamus/preoptic area (MBH/POA) in castrated Sprague-Dawley rats (3, 7, 10, 11). If these animals are given androgen replacement therapy, the activity in these brain regions is restored to or elevated above control aromatase levels (3, 7, 10, 11). Sufficient evidence exists that aromatase in the MBH/POA is mediated by an androgen receptor mechanism, since blocking the receptor(s) with flutamide (a known androgen-receptor blocker) significantly decreases aromatase levels comparable to that of castrated animals (3, 11, 13–15). Whereas, in the amygdala, aromatase is not altered by androgen hormonal action, and this region is apparently independent of androgen receptor regulation (11, 14, 15).

Almost all studies examining the influence of androgen hormonal action on brain aromatase have been performed in Sprague-Dawley rats (3). Previous and preliminary results from our laboratory indicated that brain aromatase levels differ significantly among some rat strains and that in some strains, such as Long-Evans rats, androgens do not regulate brain aromatase activity (16). This latter finding provided the initiative to examine other rat strains and investigate how brain aromatase may be regulated among them. To determine whether androgens regulate brain aromatase in a similar manner to that reported in intact/castrated Sprague-Dawley rats (10, 14, 16), we characterized the influence of

This material is based upon work supported, in part, by the National Science Foundation under Grant No. IBN-9507972.
[1] To whom requests for reprints should be addressed at Department of Zoology, Cellular Biology Division, Brigham Young University, Provo, UT 84602. E-mail: Edwin_Lephart@BYU.EDU

Received July 22, 1998. [P.S.E.B.M. 1999, Vol 221]
Accepted January 11, 1999.

0037-9727/99/2212-0126$14.00/0

surgical castration in Norway Brown and Wistar adult rats and compared their responses to surgically or biochemically castrated Sprague-Dawley rats (with flutamide).

Materials and Methods

All male rats were age-matched and obtained from Charles Rivers Laboratory (Wilmington, MA). Sprague-Dawley, Norway Brown, and Wistar animals were surgically castrated at 40–42 days of age ($n = 6$, $n = 11$ and $n = 12$, respectively). Intact Norway Brown and Wistar rats ($n = 10$ males per group) served as control groups for the surgically castrated animals. Sprague-Dawley ($n = 10$) rats were chemically castrated *via* flutamide injections [at the nape of the neck at 100 mg flutamide (a known androgen receptor blocker) dissolved in ethanol/kg body weight every other day starting at 50 days of age] (13). Sprague-Dawley control injected rats ($n = 12$) received a similar volume of ethanol vehicle that never exceeded 0.12 ml every other day. Sprague-Dawley intact noninjected males ($n = 25$) served as controls for the Sprague-Dawley injected or surgically castrated animals. All male rats were sacrificed at 70 days of age. Body weights were measured in grams (+ 0.1 g), and ventral prostate weights were recorded in milligrams (mg). Since the prostate is an androgen-sensitive organ, it provided an index of the efficacy of the surgical or biochemical castration treatment effects. The animals and methods used in this study were approved by the Institutional Animal Care and Use Committee at Brigham Young University.

The brain tissue sites (i.e., the MBH/POA and amygdaloid regions) were collected using landmark boundaries according to previous published methods for the determination of aromatase activity levels (10, 13, 17). In brief, the brain tissue fragments were incubated for 1 hr in the presence of a saturating concentration of 1β-[3H]androstenedione as the substrate (at 2.4 μM) in 2 ml of DMEM medium. (We have not found a significant difference in brain aromatase levels when DMEM medium is used compared to employing a brain buffer containing potassium phosphate.) At the end of the incubations, the unused substrate was extracted with five volumes of chloroform by vortex mixing. The aqueous phase of the reaction mixture was purified with charcoal, isolated by centrifugation, and then the aromatase activity was quantitated by scintilation counting of the formed tritiated water. This assay is described in detail elsewhere along with the validation of these methods (10, 13, 17, 18). After the aromatase assays were performed, the total protein content of each tissue fragment was determined using the Lowry protein assay with bovine serum albumin as standard (19).

Each biological parameter measured was tested by analysis of variance, followed by Newman-Keuls multiple comparisons to detect potential significant differences by treatment groups (α was set at $P < 0.05$).

Results

To establish the efficacy of the treatments employed, body and ventral prostate weights were determined at the time brain tissue sites were collected (Table I). There were no significant differences in body weights among Sprague-Dawley noninjected intact controls, Sprague-Dawley ethanol-injected intact controls, or Sprague-Dawley flutamide-injected animals. However, in the case of the surgically castrated groups, as expected, there were significant decreases in body weights compared to intact Sprague-Dawley, Norway Brown, or Wistar male rats (Table I). Finally, although the different strains of male rats were age-matched (at 70 days old) the intact Norway Brown rats' weight was significantly less than Sprague-Dawley or Wistar intact control males.

Furthermore, since the ventral prostate gland is an androgen-sensitive tissue, this parameter provided additional evidence for the effects of flutamide (blocking androgen hormonal action) and castration (eliminating the influence of androgens) in the treatment groups (Table I). Regardless of whether the ventral prostate weight (mg) for each animal was standardized (Table I), the Sprague-Dawley ethanol-injected controls displayed a slight but significant decrease when compared to Sprague-Dawley noninjected control values. This result was due presumably to the stress of the injections. However, in Sprague-Dawley castrated or flutamide-injected animals (treated for 20 days from 50–70 days of age), notable significant decreases in ventral prostate/body weight ratios were observed when tested against Sprague-Dawley ethanol-injected or Sprague-Dawley noninjected values (Table I). Although the Norway Brown rats weighed significantly less than Sprague-Dawley or Wistar animals at 70 days of age, when the ventral prostate weights were standardized, similar ventral prostate/body weight ratios were observed in intact males independent of strain. Similar to the changes in body weight by castration, dramatic significant reductions in ventral prostate/body weight ratios were recorded in Sprague-Dawley, Norway Brown, and Wistar males castrated approximately 4 weeks earlier compared to intact animals (Table I).

To compare the effects of surgical or biochemical castration (*via* flutamide) in the Sprague-Dawley strain to that of surgical castration in Norway Brown and Wistar animals, on the regulation of estrogen biosynthesis in the MBH/POA and amygdaloid regions, aromatase enzyme activity levels were determined in 70-day-old age-matched male rats.

As shown in Figure 1, MBH/POA aromatase activity rates were significantly higher in Wistar intact males (at 60 fmol/ hr/mg protein) compared to Sprague-Dawley (noninjected or injected) controls (ranging from 41–44 fmol/ hr/ mg protein). There were no significant alterations in MBH/POA aromatase between Sprague-Dawley noninjected and ethanol-injected controls. However, in general, castration (biochemical or surgical) treatments resulted in significant reductions in MBH/POA aromatase activities compared to

Table I. Body Weight, Ventral Prostate Weight, and Ventral Prostate Weight/Body Weight Ratios in 70-day-old Age-Matched Sprague-Dawley (S.D.), Norway Brown (N.B.), or Wistar (Wis.) Male Rats

Body weight [grams (+ S.E.M.)]							
S.D. noninj $n = 25$	S.D. con-inj $n = 12$	S.D. cast $n = 6$	S.D. fluta $n = 9$	N.B. intact $n = 10$	N.B. cast $n = 11$	Wis. intact $n = 9$	Wis cast $n = 12$
343.4 (5.6)	355.7 (8.8)	258.8* (14.0)	337.2 (6.6)	238.4 (3.4)	212.6* (6.7)	360.5 (5.7)	321.8* (7.6)

* = Significant decrease in body weight in castrated vs. intact S.D., N.B. or Wis. animals.

Ventral prostate weight (mg)							
S.D. noninj $n = 25$	S.D. con-inj $n = 12$	S.D. cast $n = 6$	S.D. fluta $n = 9$	N.B. intact $n = 10$	N.B. cast $n = 11$	Wis. intact $n = 9$	Wis. cast $n = 12$
390.8 (10.6)	362.1▲ (12.8)	14.3● (0.5)	122.4● (3.6)	258.0 (9.3)	12.8* (0.7)	422.2 (10.9)	30.5* (1.3)

Ventral prostate weight (mg)/100 g body weight (+ S.E.M.)							
S.D. noninj $n = 25$	S.D. con-inj $n = 12$	S.D. cast $n = 6$	S.D. fluta $n = 9$	N.B. intact $n = 10$	N.B. cast $n = 11$	Wis. intact $n = 9$	Wis. cast $n = 12$
114.1 (0.5)	97.4▲ (0.4)	5.3● (0.5)	36.3● (0.1)	104.6 (0.4)	6.8* (0.1)	114.7 (0.4)	8.2* (0.1)

▲ = Significant decrease in ventral prostate weight or ventral prostate weight/body weight ratios in S.D.-injected vs. noninjected S.D. control values.

● = Significant decrease in ventral prostate weight or ventral prostate weight/body weight ratios in castrated S.D. or flutamide-injected S.D. vs. S.D.-injected or noninjected S.D. control values.

* = Significant decrease in ventral prostate weight or ventral prostate weight/body weight ratios in castrated N.B. vs. N.B. intact or castrated Wis. vs. Wis. intact values.

Note. Treatments: Noninjected = noninj; ethanol-injected = con-inj; flutamide-injected = fluta; noncastrated = intact and castrated = cast. Sprague-Dawley (S.D.) rats that did not receive any injections (noninj) served as controls for the S.D. castrated (cast), ethanol-injected (con-inj), or S.D. flutamide-injected (fluta) groups. The latter two groups received ethanol or flutamide injections [flutamide (dissolved in ethanol) at 100 mg/kg body weight] every other day from 50–70 days of age. In the Sprague-Dawley, Norway Brown, and Wistar groups, castration was performed at 40 days of age. Body and ventral prostate weights (along with brain tissues) were collected at 70 days of age for all treatment groups.

control intact values (Fig. 1). In the biochemical castrated Sprague-Dawley animals (fluta), a significant 45% decreased in MBH/POA aromatase occurred, whereas in surgically castrated animals significant reductions (ranging from 25%–35%) were seen in MBH/POA activity levels from Sprague-Dawley (cast), Norway Brown (cast), and Wistar (cast) males, compared to control values.

In the amygdaloid tissue site the patterns of aromatase were different from those of the MBH/POA site across the rat strains and treatment groups (Fig. 2). First, in general, the aromatase activity rates were higher in the amygdaloid site compared to the MBH/POA region independent of strain or treatment. Second, in intact animals, there were no significant differences in amygdaloid aromatase levels among Sprague-Dawley (noninj), Sprague-Dawley (ethanol-injected), Norway Brown, or Wistar males (which ranged from 49 to 73 fmol/ hr/mg protein). Third, there were no significant alterations in amygdaloid aromatase activities from surgically castrated Norway Brown or Wistar animals compared to intact control rates. Finally, flutamide-treated or surgically castrated Sprague-Dawley males displayed considerable (but nonsignificant, $P < 0.06$; and $P < 0.1$, respectively) reductions in amygdaloid aromatase when tested against control values (Fig. 2).

Discussion

Ample evidence exists to demonstrate the regulation of MBH/POA aromatase by androgens (7, 8, 10–15), whereas the amygdaloid region, in general, is apparently not influenced by androgen hormonal action (8, 11, 14, 15). While nearly all reports, to date, have studied the Sprague-Dawley rat in this regard, we examined the influence of surgical castration in Norway Brown or Wistar rats on MBH/POA or amygdala aromatase, and a comparison was made to surgical or biochemical castration treatments (using flutamide) in Sprague-Dawley rats. The efficacy of the treatments was confirmed by indexing body weights and standardized ventral prostate weights. In general, the MBH/POA aromatase levels across the different rat strains were comparable to those previously reported by our laboratory (10).

However, there was a difference in MBH/POA aromatase where intact Wistar animals displayed (moderate

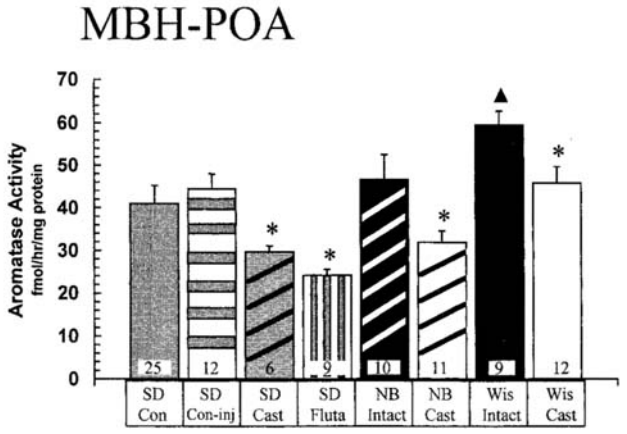

Figure 1. Aromatase activity in medial basal hypothalamic/preoptic area (MBH/POA) tissue (expressed in fmol/ hr/mg protein) in Sprague-Dawley, Norway Brown, and Wistar male rats. The treatments included: Noninjected = Con; ethanol-injected = Con-inj; flutamide-injected = fluta; noncastrated = intact and castrated = cast. Each bar represents the mean + SEM. The number of rats per group is shown at the base of each bar.
* = Significantly lower aromatase activity in (chemically and surgically) castrated animals compared to intact or control-injected animals of the same strain ($P < 0.05$).
▲ = Significantly greater aromatase activity in Wistar control intact animals compared to Sprague-Dawley control noninjected or control-injected rats ($P < 0.05$).

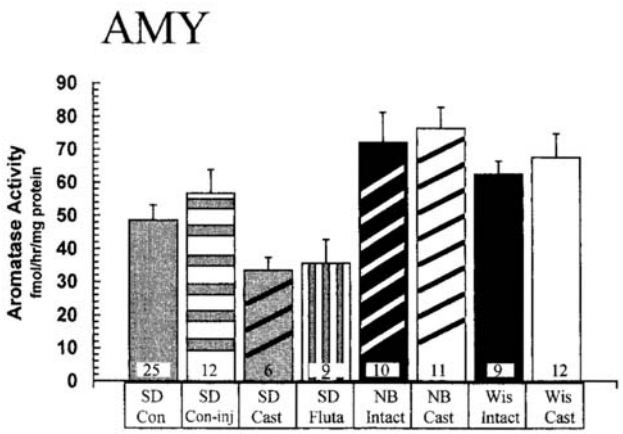

Figure 2. Aromatase activity in the amygdala (AMY) region (expressed in fmol/ hr/mg protein) in Sprague-Dawley, Norway Brown, and Wistar male rats. The treatments included: Noninjected = Con; ethanol-injected = Con-inj; flutamide-injected = fluta; noncastrated = intact and castrated = cast. Each bar represents the mean + SEM. The number of rats per group is shown at the base of each bar. Aromatase activity rates were not significantly altered among intact animals compared to castrated animals within a given strain. However, a notable (but nonsignificant) decline was observed in the activity of flutamide-treated males compared to Sprague-Dawley control injected values and in Sprague-Dawley castrated animals compared to control intact Sprague-Dawley levels ($P < 0.06$ and $P < 0.1$, respectively).

but) significantly higher activity levels compared to intact Sprague-Dawley control values. This was an intriguing finding since increased aromatase activity levels (with adequate substrate availability) would lead one to speculate that increased local estrogen biosynthesis may (in a negative feedback mechanism) reduce the level of gonadotrophin release. This suggests a potential physiological role for the observed strain differences in brain aromatase. Although recent data from our laboratory indicated that the control of estrogen receptor–mediated effects is influenced to a large extent by genetic factors, especially in the Wistar rat strain, rather than *via* steroid hormonal actions (20). Furthermore, the finding that some rat strains display different brain aromatase levels, particularly in this brain region (MBH/POA), implies that the divergent brain aromatase rates may have been established during the important perinatal developmental period (3). This is critical since the aromatase enzyme plays an important role in neuronal growth/proliferation/cellular organization (1, 3, 7), the modulation of neuroendocrine functions (2, 3, 7), and the regulation of sexual behavior (2, 3, 21).

In agreement with other reports, substantiating the regulation of MBH/POA aromatase by androgens (8, 10–15), the enzymatic activity was significantly reduced in flutamide-treated Sprague-Dawley rats. Moreover, in surgically castrated Sprague-Dawley, Norway Brown, and Wistar (age-matched) animals, a similar significant decline in MBH/POA aromatase was observed when compared to controls. Therefore, the effectiveness of the treatments (either surgical or biochemical castration) was indexed by significant reductions in prostate weight (Table I) that correspond to the significant decreases seen in brain aromatase levels. This suggests that aromatase in the MBH/POA region, in Norway Brown and Wistar rats, is regulated by androgens in a similar fashion to that of Sprague-Dawley animals. The only exception, to date, for the MBH/POA brain region that apparently is not influenced by androgens is seen in Long-Evans rats (16). However, even this latter exception must be confirmed by demonstrating a lack of altering aromatase mRNA levels by androgens. In another study, we collected substantial data to suggest that strain differences in rats regulate many important biological parameters, such as brain aromatase and gonadotrophins (20). Finally, it can be concluded from the present results that MBH/POA aromatase, in general, is regulated by androgens regardless of rat strain studied.

In the amygdaloid brain site, for intact animals independent of strain, the activity rates were similar (ranging from 48–73 fmol/ hr/mg protein), where no significant differences were observed. However, in general, amygdala aromatase levels were higher than those seen in the MBH/POA region; this finding is in agreement with other reports (11, 17). There was an apparent lack of androgen regulation for amygaloid brain aromatase activity in Norway Brown and Wistar animals. Whereas, in flutamide-treated or surgically castrated Sprague-Dawley rats, a noted (but nonsignificant) decrease in the activity was observed compared to Sprague-Dawley control values. This observation may be explained by the fact that cortical amygdala aromatase is regulated by androgens whereas aromatase activity in the medial amygdala is not regulated by androgens (11). Since a majority of the amygdaloid tissue fragment isolated in these experiments most likely represents the cortical portion

of this tissue site, this may clarify our results. Finally, for amygdala aromatase there does not appear to be a clear-cut correspondence to the level of sex steroid hormonal status in adult males independent of rat strain.

In summary, androgen steroid hormone metabolism in the limbic and hypothalamic/preoptic regions plays an important role in the development of sexually dimorphic brain structures, the modulation of neuroendocrine function(s), and the regulation of sexual behavior. The present results demonstrate that independent of the strain of rat, MBH/POA aromatase is regulated by androgens (in Sprague-Dawley, Norway Brown, and Wistar males). However, intact Wistar animals displayed significantly higher MBH/POA aromatase levels compared to Sprague-Dawley control values. In the amygdaloid tissue brain region there was an apparent lack of androgen action upon aromatase enzyme activity in any of the rat strains.

1. Celotti F, Negri-Cesi P, Poletti A. Steroid metabolism in the mammalian brain: 5α-Reduction and aromatization. Brain Res Bull **44**:365–375, 1997.
2. Naftolin F. Brain aromatization of androgens. J Reprod Med **39**:257–261, 1994.
3. Lephart ED. A review of brain aromatase cytochrome P450. Brain Res Brain Res Rev **22**:1–26, 1996.
4. McEwen BS, Gould E. Orchinik M, Weiland NG, Woolley CS. Oestrogens and the structural and functional plasticity of neurons: Implications for memory, aging, and neurodegenerative processes. Ciba Found Symp **191**:52–66, 1995.
5. McEwen BS, Alves SE, Bulloch K, Weiland NG. Ovarian steroids and the brain: Implications for cognition and aging. Neurology **48** (Suppl 7):S8–S15, 1997.
6. Naftolin F, Horvath TL, Jakab RL, Leranth C, Harada N, Balthazart J. Aromatase immunoreactivity in axon terminals of the vertebrate brain: An immunocytochemical study on quail, rat, monkey, and human tissues. Neuroendocrinology **63**:149–155, 1996.
7. Resko JA, Roselli CE. Prenatal hormones organize sex differences of the neuroendocrine reproductive system: Observations on guinea pigs and nonhuman primates. Cell Mol Neurobiol **17**:627–648, 1997.
8. Roselli CR, Abdelgadir SE, Resko JA. Regulation of aromatase gene expression in the adult rat brain. Brain Res Bull **44**:351–357, 1997.
9. Vagell ME, McGinnis MY. The role of aromatization in the restoration of male rat reproductive behavior. J Neuroendocrinol **9**:415–421, 1997.
10. Lephart ED, Ojeda SR. Hypothalamic aromatase activity in male and female rats during juvenile peripubertal development. Neuroendocrinol **51**:385–393, 1990.
11. Roselli CR, Horton LE, Resko JA. Distribution and regulation of aromatase activity in the rat hypothalamus and limbic system. Endocrinology **117**:2471–2477, 1985.
12. Roselli CE. The effect of anabolic-androgenic steroids on aromatase activity and androgen receptor binding in the rat preoptic area. Brain Res **792**:271–276, 1998.
13. Lephart ED, Husmann DA. Altered brain and pituitary androgen metabolism by prenatal, perinatal, or pre- and postnatal finasteride, flutamide, or dihydrotestosterone treatment in juvenile male rats. Prog Neuropsychopharmacol Biol Psychiatry **17**:991–1003, 1993.
14. Roselli CE, Resko JA. Androgens regulate brain aromatase activity in adult male rats through a receptor mechanism. Endocrinology **114**:2183–2189, 1984.
15. Roselli CR, Salisbury RL, Resko JA. Genetic evidence for androgen-dependent and independent control of aromatase activity in the rat brain. Endocrinology **121**:2205–2210, 1987.
16. Lephart ED, Butler PC, Mills RH, Jacobson NA, Ladle DR, Bloch GJ. Effects of testosterone and progesterone on brain 5α-reductase and aromatase in Long-Evans (L-E) males and comparison of aromatase in L-E vs. Sprague-Dawley rats. Brain Res **789**:327–330, 1998.
17. Jacobson NA, Ladle DR, Lephart ED. Aromatase cytochrome P450 and 5α-reductase in the amygdala and cortex of perinatal rats. Neuroreport **8**:2529–2533, 1997.
18. Lephart ED, Simpson ER. Techniques for the assay of aromatase cytochrome P450. Methods Enzymol **206**:477–483, 1991.
19. Lowry OH, Rosenfeld RS, Farr AL, Randall RJ. Protein determination with a Folin's phenol reagent. J Biol Chem **193**:265–269, 1953.
20. Lephart ED, Call SB, Rhees RW, Jacobson NA, Teuscher C. Genetic control of the sexual dimorphic nucleus, brain aromatase, reproductive function, and mating behavior in Noble vs. Wistar rats. 4th International Congress of Neuroendocrinology (abstract P261), Kitakyushu, Japan, 1998.
21. Breedlove SM. Sexual differentiation of the brain and behavior. In: Becker JB, Breedlove SM, Crews D, Eds. Behavioral Endocrinology. Cambridge, MA: MIT Press, pp39–70, 1992.