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5alpha-reductase 2 inhibition impairs brain defeminization of male rats: Reproductive aspects

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Abstract

The present study was carried out to determine whether 5alpha-reductase 2 (5alpha-R2) metabolic pathway plays a key role in brain sexual differentiation. The inhibition of 5alpha-R2 by finasteride (20 mg/kg/day) from gestational day 19 to postnatal day 5 has long-term effects on sexual behavior and reproductive physiology detected only in adult life. Sexual maturation assessed by timing of preputial separation was unchanged. Finasteride-treated males were able to mate with untreated females which became pregnant but exhibited increased rate of pre-implantation loss. The subfertility observed was probably due to abnormally shaped sperm, since the sperm number was not altered. While plasma testosterone was enhanced, LH levels were not changed. The copulatory potential was not affected and all finasteride-treated rats presented male sexual behavior. Despite this, 53% of them showed homosexual behavior when pretreated with estradiol, suggesting an incomplete brain defeminization. These results indicate that 5alpha-R2 acts in brain sexual differentiation of male rats. Moreover, we suggest that 5alpha-R2 not only produces essential metabolites that act together with estradiol in brain sexual differentiation but also protects the brain from the damaging effects of estradiol excess.

Keywords: Brain sexual differentiation; 5alpha-reductase; Sexual behavior; Reproduction; Androgens; Finasteride; Rat

1. Introduction

Sexual differentiation, the process by which permanent sex differences in the brain arise, is regulated by testosterone (T) secreted from fetal and neonatal testes (Quadros et al., 2002). In male rats, T surges markedly during days 18 – 19 of gestation (Weisz and Ward, 1980) and again during the first few hours following parturition (Corbier et al., 1978). Early exposure to androgen from developing testes results in masculinization and defeminization of the brain. The former entails permanent actions that support male-typical copulatory behaviors and patterns of gonadotropin secretion. The latter results in the permanent suppression of female-typical behaviors and the LH surge mechanism (Roselli et al., 2004).

An intriguing characteristic of steroids' mechanism of action is the fact that these compounds do not always act in their native form, but should be locally metabolized into their "active" form. The two major metabolic pathways involved in T activation are aromatase, which converts T into estradiol, and the 5alpha-reductase (5alpha-R) that transforms T into the more potent androgen dihydrotestosterone (DHT) (Negri-Cesi et al., 1996; Lephart et al., 2001a). A central step in the development and sexual differentiation of the brain is the intraneuronal conversion of androgens to estrogens (MacLusky and Naftolin, 1981). Thus, neural aromatase is considered to be crucial for the neonatal imprinting and sexual differentiation of the brain (Gerardin and Pereira, 2002). However, these processes in the rat seem to be mediated not only by estrogens alone, but they also seem to require the participation of androgens per se (Döhler, 1991). More recently, DHT has been shown to be essential for the development and organization of selected neuronal populations and, therefore, is possibly involved in the processes of sexual differentiation of some brain regions (Arnold and Gorski, 1984; Goldstein and Sengelaub, 1994). In fact, much less is known about the 5alpha-R steroid pathway in brain sexual differentiation. The 5alpha-R type 2 isoform (5alpha-R2) appears to be selectively concentrated in classical androgen dependent structures. Furthermore, it shows a clear-cut pattern of expression in the rat brain in late fetal/early postnatal life that overlaps the

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secretory profile of testosterone. 5alpha-R2 transcripts are undetectable on gestational day (GD) 14, increases after GD 18, peaks on postnatal day 2, then decreases gradually, becoming low in adulthood. Moreover, its expression seems to be triggered by androgens. Therefore, 5alpha-R2 may be involved in the control of brain sexual differentiation occurring during a very critical period, when androgen-organizing effects are thought to take place in the CNS (Poletti et al., 1998).

On the basis of these considerations, the aim of this study was to investigate the role of 5alpha-R2 metabolic pathway in brain sexual differentiation focusing on reproductive physiology and sexual behavior. For this, we evaluated the long-term effects of perinatal 5alpha-R2 inhibition by finasteride on the reproductive physiology and sexual behavior in male rats.

2. Methods

2.1. Animals

Male and female Wistar rats were maintained on a 12 h light/dark schedule (lights on at 6:00 a.m.) with free access to food and water within a temperature and humidity controlled colony room. Virgin female rats (200 ± 10 g) were mated overnight. The onset of pregnancy was confirmed by the presence of spermatozoa in vaginal smears on the following morning and was considered day 1 of gestation. These pregnant females were randomly assigned to two groups, according to treatment, as described below. The experimental procedures were not done at the same time, since the animals used for each evaluation was obtained from different mothers, so not necessarily born at the same day. This study was conducted in accordance with Ethical Principles in Animal Research adopted by Ethical Committee for Animal Research from Bioscience Institute/UNESP-Botucatu (Protocol n^{o}. 073/03). The experimental protocol is diagramed in Fig. 1.

2.2. Treatments

Sperm-positive animals, 6 dams per group, were injected sc once a day with sterile peanut oil containing 12% benzyl alcohol or 5alpha-R2 inhibitor finasteride (>99% purity, Aurobindo Pharma Ltd, India) at 20 mg/kg/day dissolved in 12% benzyl alcohol/sterile peanut oil (vol/vol) from gestational days (GD) 19 to 22 as well on the first 5 days of lactation [postnatal day (PND)1-PND5]. The timing of the injections was designed to include both the period of brain sexual differentiation which occurs in rats during the last third of fetal life and continues through the first week after birth (Jacobson et al., 1985) as well as the perinatal 5alpha-R2 expression (Poletti et al., 1998). Finasteride is a competitive 5alpha-R inhibitor that does not bind to the androgen receptor. Besides, it is a more potent inhibitor of type 2 5alpha-R than type 1 (Rittmaster and Finasteride, 1994). The dose level chosen for this study was based on previous data that set a threshold of response to finasteride at 0.1 mg/kg/day (Clark et al., 1990). Moreover, 20 mg/kg/day is an equivalent clinical dose (Lephart, 1995). Also, due to the ability of the 5alpha-R inhibitor to block parturition in rat; at GD22 the pups of both groups were removed from the uterus and fostered to recipient dams which were injected in the first 5 days of lactation. The day of parturition was considered PND1 and each litter was left with 8 pups, keeping all the obtained males (females were kept just to complete the litter). On PND25, male rats from the control and finasteride-treated groups were identified and housed in collective polypropylene cages ($32 \times 40 \times 18$ cm^{3}) each with a bedding of wood shavings, 5 animals/cage.

2.3. Sexual maturation (preputial separation)

Starting on PND42, the male rats of both experimental groups were examined daily for complete preputial separation. It was noted when the prepuce, which is fused to the glans penis until the onset of puberty, could be fully retracted (Korenbrot et al., 1977).

2.4. LH and T plasma levels in adult life

Blood from the abdominal aorta was collected, centrifuged (2500 rpm for 20 min at 2 °C), and the plasma stored at −20

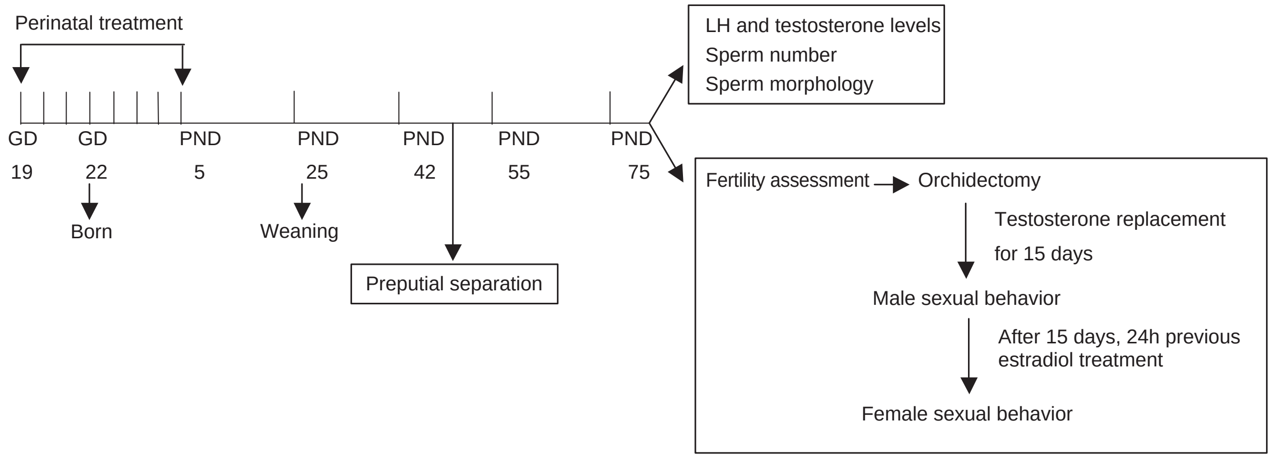

Fig. 1. Diagram of the experimental design: GD, gestational day; PND, postnatal day.

°C until assayed. Plasma hormone levels were measured by RIA using both Coat-A-Count Total T and Coat-A-Count LH IRMA kits (Diagnostic Products Co., Los Angeles, CA) according to manufacturer's instructions. The assay detection limits were 0.15 mIU/ml for LH and 0.04 ng/ml for T. All plasma samples were analyzed in a single assay.

2.5. Fertility assessment in adult life

Male rats of both experimental groups were housed in a large cage with 2 regularly cycling females. Vaginal smears were examined daily for the presence of spermatozoa, indicating copulation. On GD21, all mated females were killed by decapitation. After removing and analyzing the contents of the uterine horns, the proportions of females with pre- and post-implantation losses were quantified, and the mean rates calculated. Thus, pre- and post-implantation losses are couple-mediated endpoints for evaluating male reproductive toxicity. The implantation was scored at different times during the research, but always on GD21. Alterations, if present, could be observed in both groups. The pre-implantation loss was calculated as the difference between the number of corpora lutea minus implantation sites × 100/number of corpora lutea, and the post-implantation loss as the number of implantation sites minus live fetuses × 100/number of implantation sites.

2.6. Sperm number in adult life

Homogenization-resistant testicular spermatids in the testes and sperm in the caput/corpus epididymis and cauda epididymis were enumerated as previously described (Robb et al., 1978). Daily sperm production (i.e. DSP) was determined by dividing the total number of homogenization-resistant spermatids per testis by 6.1, the number of days of a seminiferous cycle in which these spermatids are present. Transit times through the caput/corpus epididymis and cauda epididymis were calculated by dividing the number of sperm within each of these regions by the DSP.

2.7. Sperm morphology in adult life

After sperm was collected from vas deferens and cauda epididymis, sperm smears were done. Two hundred spermatozoa per animal were classified as normal or abnormal, as previously described (Linder et al., 1992).

2.8. Sexual behavior

The evaluation of sexual behavior in male rats was performed under red-light illumination during the dark phase of their cycle. Before this, the same male rats used in the fertility assessment were anaesthetized with sodium pentobarbital (40 mg/kg, ip) and bilaterally castrated. Then, all these males received T propionate (1 mg/day, sc) 3 times a week, for 2 weeks. The T replacement schedule was set up in a way that the first injection was done on the day after orchidectomy, and the last one was always done on the day immediately before the male sexual behavior test. This procedure was done in order to obtain the same hormonal condition in male rats of both experimental groups before the male sexual behavior evaluation. The female sexual behavior was assessed in the same experimental animals 15 days after the male sexual behavior test.

2.8.1. Male sexual behavior

Experimental male rats were allowed to mount female rats whose estrus phase was induced by estradiol benzoate injection (20 μg/kg, ip) in the previous 24h (Arteche et al., 1997). Each male was placed into a Plexiglas cage and after 10 min of adaptation the estrus female was introduced. During 30 min, the following parameters were recorded: mount, intromission, and ejaculatory latencies; number of mounts and intromissions before the first ejaculation; postejaculatory mount and intromission latencies after the first ejaculation; total number of mounts, intromissions, and ejaculations.

2.8.2. Female sexual behavior

For the test, experimental males were treated with estradiol benzoate (20 μg/kg, ip) 24 h before. A sexually experienced intact male rat was first placed into a Plexiglas cage over 10 min for adaptation and then cohabited with each experimental male. The animals were observed during 10 min for female sexual behavior (lordosis).

2.9. Statistical analysis

At the outset, results were analyzed by descriptive statistics for determination of normal distributions of data. Then Student's t-test, Mann–Whitney U-test, and Fisher's exact test

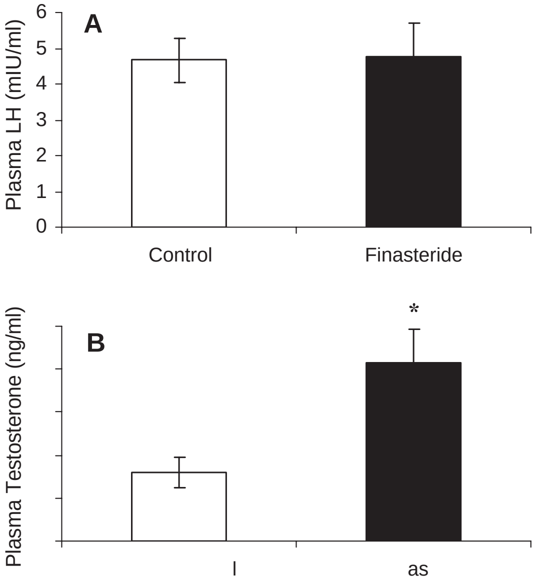

Fig. 2. Panel A: LH plasma levels from control and finasteride-treated males. Panel B: testosterone plasma levels from control and finasteride-treated males. Values expressed as means ± S.E.M. of 5 animals/group. *$p < 0.05$ by Student's t-test.

Table 1
Number of pregnant females, number of pregnant females that showed pre- and post-implantation losses, and rates of pre- and post-implantation losses[†] in untreated females mated with control and finasteride-treated males

Male rats	Untreated female rats (2 females/male)				
	Pregnant females	With implantation losses		Rate of implantation losses	
		Pre-	Post-	Pre-	Post-
Control	20/20	7/20	7/20	8.33 (7.42–11.21)	9.09 (8.17–11.27)
Finasteride	19/20	7/19	7/19	15.38* (10.42–16.03)	9.09 (8.71–12.88)

[†] Values expressed as median ($IQ_{25\%}-IQ_{75\%}$) of 10 males/group.
* $p<0.05$ by Mann–Whitney test.

were employed, with the results considered significant at $p<0.05$.

3. Results

3.1. Anogenital distance

The perinatal treatment with finasteride did not alter the anogenital distance of male pups at birth (mean of litter, means ± SEM): control group = 3.18 ± 0.07/finasteride group = 3.10 ± 0.07, $p>0.05$.

3.2. Sexual maturation

Perinatal 5alpha-R2 inhibition did not affect sexual maturation, assessed by the timing of preputial separation (control group = 48.50 (47.25–50.00) days, $n=10$; finasteride group = 49.50 (48.25–50.75) days, $n=10$).

3.3. LH and T plasma levels in adult life

As shown in Fig. 2, the perinatal 5alpha-R2 inhibition did not change the LH levels (Fig. 2A) but increased testosterone plasma levels [$t(8)=2.92$; $p=0.02$] (Fig. 2B).

Table 2
Sperm number[†] in control and finasteride-treated males

Parameters	Experimental groups	
	Control	Finasteride
No. of spermatids × 10^6/testis	229.04 ± 14.62	217.17 ± 5.95
Daily sperm production	37.54 ± 2.40	35.60 ± 0.98
No. of spermatozoa × 10^6/caput + corpus of epididymis	116.00 ± 6.83	105.30 ± 6.04
No. of spermatozoa × 10^6/cauda of epididymis	129.40 ± 10.83	136.65 ± 6.6
Sperm transit time through caput + corpus of epididymis (days)	3.14 ± 0.18	2.96 ± 0.16
Sperm transit time through cauda of epididymis (days)	3.46 ± 0.18	3.85 ± 0.18

[†] Values expressed as means ± S.E.M. of 10 animals/group.
No significant difference was found ($p>0.05$ by Student's t-test).

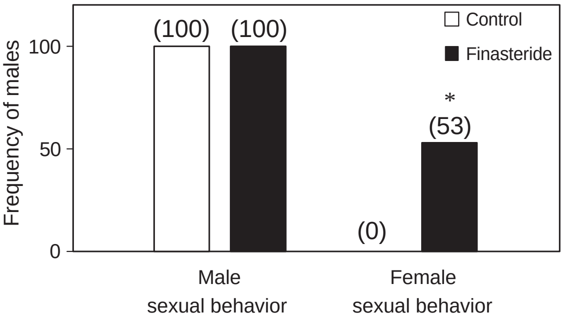

Fig. 3. Frequency of control and finasteride-treated males that showed male sexual behavior after receiving testosterone propionate and that showed female sexual behavior after receiving estradiol benzoate ($n=15$/group). *$p<0.05$ by Fisher's exact test.

3.4. Fertility assessment in adult life

Finasteride-treated males were able to mate with untreated females, as shown in Table 1. The females mated with these males showed rate of post-implantation loss similar to those of control group and higher rate of pre-implantation loss [$U=23.5$; $p=0.04$]. Moreover, the percentage of female rats that showed pre- and post-implantation losses did not differ between the groups. Actually, the females that showed pre-implantation losses were not necessarily the same with those ones that presented post-implantation losses. Occasionally, the same number of females presented pre- and/or post-implantation losses when mated with males of both groups. Indeed, 3 of 20 females mated with control males and 3 of 19 females mated with finasteride-treated males showed pre- and post-implantation losses.

3.5. Sperm number in adult life

As demonstrated in Table 2, perinatal 5alpha-R2 inhibition did not alter the sperm number in male pups.

3.6. Sperm morphology in adult life

Perinatal 5alpha-R2 inhibition increased the percentage of abnormally shaped sperm collected from the cauda epididy-

Table 3
Effects of perinatal treatment with finasteride on sexual behavior[†] of male rats

Parameters	Experimental groups	
	Control	Finasteride
Latency to first mount (s)	67.10 ± 17.57	55.70 ± 13.95
Latency to first intromission (s)	67.50 ± 17.41	56.00 ± 13.85
Latency to first ejaculation	662.80 ± 130.26	684.10 ± 94.86
No. of mounts until ejaculation	34.90 ± 7.11	28.40 ± 4.42
No. of intromissions until ejaculation	24.00 ± 5.64	26.80 ± 3.89
Postejaculatory mount latency (s)	1092.2 ± 123.86	992.30 ± 110.14
Postejaculatory intromission latency (s)	1092.2 ± 123.86	993.00 ± 110.41
Total number of mounts	58.50 ± 6.94	62.60 ± 6.99
Total number of intromissions	42.40 ± 6.52	58.40 ± 5.81
Total number of ejaculations	2.20 ± 0.25	2.70 ± 0.26

[†] Values expressed as means ± S.E.M. of 15 animals/group.
No significant difference was found ($p>0.05$ by Student's t-test).

mis and vas deferens (control group = 91.00 (90.00–95.00), $n = 9$; finasteride group = 84.50 (78.25–88.38), $n = 10$); [$U = 13$; $p = 0.01$].

3.7. Sexual behavior

Fig. 3 shows the frequency of male and female sexual behavior of both experimental groups. All control rats presented male sexual behavior with absence of female sexual behavior. Although all the finasteride-treated animals exhibited male sexual behavior, when castrated and pretreated with estradiol benzoate, 53% of them showed lordosis and accepted mount of another male ($p = 0.01$). As shown in Table 3, none of the male sexual behavior parameters evaluated was altered by perinatal 5alpha-R2 inhibition.

4. Discussion

The present results reveal the utmost importance of 5alpha-R metabolic pathway in brain sexual differentiation. Although the current hypothesis regarding the sexual differentiation of the rodent brain is based upon evidence that certain neural structures are exquisitely sensitive to the organizational effects of the local conversion of T to estrogen the idea that androgen acts entirely via conversion to estrogen seems inconsistent (MacLusky and Naftolin, 1981). Thus, androgenic and estrogenic components seem to be required for complete masculinization and defeminization of sexual brain functions, since hormone antagonists with one or the other component results in incomplete organization of the male brain (Döhler, 1991). In addition, it is known that the 5alpha-R1 expression is significantly higher around birth than prenatally, and that 5alpha-R2 expression appears to be higher in males than in females, particularly just after birth (Colciago et al., 2005). Furthermore, 5alpha-R2 expression increases after gestational day (GD) 18, peaks on postnatal day 2, then decreases gradually, becoming low in adulthood.

In the present study, the finasteride treatment (from gestational days 19 to 22) did not alter the anogenital distance of male pups at birth. However, it was observed as a reduction in anogenital distance at birth in male rats treated prenatally with finasteride. A dramatic decrease in this endpoint occurred in the group treated with finasteride on days 16 to 17 of gestation (Clark et al., 1993). Thus, the results of the present study in which finasteride treatment started on GD19 is expected, since the period of gestational days 16 to 17 is the most critical period for finasteride-induced decrease anogenital distance Clark et al. (1993).

Perinatal finasteride treatment did not disrupt sexual development, as assessed by timing of preputial separation. The latter endpoint is an androgen dependent event necessary for complete copulatory behavior that can be used as an index of male pubertal development as well as an indicator of changes in the hypothalamic-pituitary-testicular axis (Korenbrot et al., 1977). Thereafter, the long-term effects of perinatal 5alpha-R2 inhibition by finasteride on reproductive physiology and sexual behavior of adult rats were detected only in adult life.

Regarding reproductive performance, in the present study, adult male rats treated with finasteride perinatally were able to mate with untreated females which became pregnant but exhibited increased rate of pre-implantation loss. As we had found spermatozoa in the vaginal smear, these results suggest that the damage to the fertility observed can be related to alterations in the morphology and/or motility of spermatozoa or to variation in the fluid from sexual glands. Despite different developmental timing of treatment, the administration of 80 mg/kg/day of finasteride to sexually mature male rats had no effects on mating indices, implants per pregnant female or sperm ability to fertilize. However, it caused an approximate 30% to 40% decrease in fertility due to failure to form copulatory plugs, which are required in rats to transport sperm in the uterus (Cukierski et al., 1991).

In the present study, the sperm production was not affected by finasteride treatment. In fact, DHT does not play a critical role in spermatogenesis. Indeed, finasteride treatment from birth through onset of puberty had no effect on testicular histology or daily sperm production despite the fact that testicular DHT content was lower and testosterone content was higher than those in controls (George et al., 1989). However, 5alpha-R inhibition by finasteride impairs testosterone-dependent restoration of spermiogenesis in adult rats (O'Donnell et al., 1996). Furthermore, the number of spermatozoa in the caput + corpus of epididymis, the sperm reserves in the cauda of epididymis, and the spermatic transit time through both epididymal regions were unchanged. Although androgen action in the epididymis is mediated by DHT, the consequences of inhibiting 5alpha-R activity in sperm maturation, steroid concentrations or epididymal cell functions have been studied only in a limited manner. Besides, the type 2 transcript, though abundant, is not associated with high enzymatic activity in this tissue (Ezer and Robaire, 2002). On the other hand, in the present study, adult males treated with finasteride during perinatal life showed an increase in abnormal spermatozoa form. Sperm morphology profiles are relatively stable and characteristic in a normal individual over time. Considering that abnormally shaped sperm may not reach the oviduct or participate in fertilization, the greater the number of abnormal sperm or the smaller the number of normal sperm in the ejaculate, the greater the probability of reduced fertility (Clegg et al., 2001). Thus, the subfertility represented by the enhanced rate of pre-implantation loss observed in the present study is not related to alterations in quantity spermatozoa, but it seems due to a defect in sperm morphology, since spermatozoa collected from vas deferens and cauda of epididymis presented an abnormal form. To our knowledge, the sperm quality in adult rats treated perinatally with finasteride has never been evaluated. Although, it was shown that inhibition of both isoforms of 5alpha-R in adult rats has consequences on epididymal sperm maturation like decrease in both the percentage of motile and progressively motile sperm and, elevated proportion of abnormal sperm that retained cytoplasmic droplet. Moreover, matings with these males resulted in fewer successful pregnancies and a higher rate of pre-implantation loss due to compromised sperm motility and morphology (Henderson and Robaire, 2005). Thus, sperm morphology defect may be due to inhibition of 5alpha-R. In the present study, the subfertility observed judged as increase in pre-implantation loss of control females mated with males treated perinatally with finasteride can be related to alterations in sperm morphology. Furthermore, it may be due to changes in sperm motility, fluid of sex glands, including changes in fructose, vitamins and enzymes content.

Perinatal 5alpha-R2 inhibition, in the present study, increased T plasma levels in adult life, without changing the LH ones. The plasma T enhancement agrees with what has been reported for endocrine status of steroid 5alpha-R2 deficiency (Wilson et al., 1993; Imperato-McGinley and Zhu, 2002), immature male rats treated with finasteride from birth through the onset of puberty (George et al., 1989), finasteride-treated adult rats (George, 1997), and 5alpha-R knockout mice, although in the latter the increase observed did not reach statistical significance (Mahendroo et al., 2001). On the other hand, the plasma testosterone levels of male rats treated prenatally with flutamide was not altered when these levels were determined at 400 days of age (Casto et al., 2003). Thus, increased T levels in tissue sites of androgen metabolism (e.g. brain tissue) following finasteride treatment may have altered the male hormonal milieu during perinatal life and influenced CNS development (MacLusky and Naftolin, 1981). Moreover, the perinatal 5alpha-R inhibition might have caused an increased sensitivity of Leydig cells to LH in adulthood. Thus, the hypothesis that perinatal finasteride treatment disrupted the hypothalamic-pituitary-testicular axis in adulthood cannot be excluded since DHT has been shown to be important for normal feedback control of T production (George et al., 1989; Lephart and Husmann, 1993; Poletti et al., 2001).

With regard to sexual behavior, all finasteride-treated rats showed normal male sexual behavior although 53% of them presented homosexual behavior after castration and pretreatment with estradiol benzoate. The former result suggests a perfect masculinization and the latter one, an incomplete defeminization of sexual behavior. These observations reinforce the independence of steroid actions on the different differentiating processes (Arnold and Gorski, 1984). Besides, the processes through which the developing CNS actively acquires the potential to execute male copulatory behavior overlap but may be different and independent from defeminization (Ward et al., 2003). Thus, perinatally DHT probably is not involved in masculinization of male sexual behavior even though it is also an androgen receptor-activated event. In fact, it was demonstrated that gonadally intact male rats prenatally exposed to flutamide showed a reduction in nonintromitive mounting probably resulted from effects the androgen antagonist had exerted on sexual differentiation of CNS (Casto et al., 2003). On the other hand, an incomplete defeminization of sexual behavior as observed in the presented study was demonstrated with both pre- or perinatally anti-androgen exposure and seems to be related to increased sensitivity to estrogen in adulthood (Neumann and Elger, 1966; Ward, 1972). Thus, the CNS of males treated perinatally with finasteride might retain functional estrogen receptors that were activated by estradiol benzoate in adulthood and/or the estrogen receptor activation in males that showed female sexual behavior might be different from others that did not present it. We suggest that inhibition of 5alpha-R pathway may have increased the availability of T to the aromatase pathway and/or the expression of aromatase enzyme. This consideration can be supported by the observation that either administration of finasteride (Ladle et al., 1997) or mutation of 5alpha-R gene (Mahendroo et al., 1997) increases plasma estradiol levels. Moreover, whether the DHT "in vivo" has the same inhibitory effect on aromatase expression, as proposed "in vitro" by Negri-Cesi et al. (2001), it might be abolished by finasteride treatment. So, the synergistic action of estradiol excess and the lack of important 5alpha reduced metabolites might disrupt the brain defeminization of finasteride-treated males. It is known that the reproductive function is impaired by exposure to estrogen excess in perinatal life (Pereira et al., 1997). Male adult rats exposed to estradiol exhibit reduced levels of androgen due to an apparent reduction of testicular T biosynthesis by the inhibition of 17–20 desmolase activity (Kalla et al., 1980). Therefore, it is possible that high estradiol levels reduced the postnatal T peak, damaging brain sexual differentiation. Finally, considering that androgen-induced defeminization of feminine behavioral and neuroendocrine responses to estrogen may involve selective reductions in the estrogen sensitivity of critical components of the neural circuitry regulating these responses, mediated in part through a reduction in estrogen receptor biosynthesis, this event might be dependent on synergistic effects of androgen- and estrogen-mediated responses. Moreover, the hypothesis that brain androgen receptor expression and/or activity were not changed in the present study cannot be excluded, since a testosterone replacement schedule was done for male sexual behavior evaluation. On the other hand, the estrogen receptor action in males that showed female sexual behavior might be different from others that did not present it. Early androgen effects might initiate the process of estrogen receptor down-regulation, subsequent exposure to estrogen or aromatizable androgen being required to organize this effect into a permanent change (MacLusky et al., 1997).

As a matter of fact, 5alpha-R enzymes convert a number of delta-4, 3-keto steroids (i.e. androgens, progestagens and glucocorticoids) to their 5alpha-reduced metabolites throughout the brain. However, it should be noted that of all the steroids, progesterone has the highest affinity for the 5alpha-R enzyme and will be preferentially converted before androgens or other steroids (Lephart et al., 2001b). However, the transient, androgen-regulated expression of 5alpha-R2 overlaps the secretory profile of testosterone during critical period of development, which may be important for sexual differentiation of the brain (Poletti et al., 1998). Thus, 5alpha-R2 metabolic pathway should be involved in brain sexual differentiation of male rats, and DHT might be a key metabolite involved. Indeed, the inhibition of 5alpha-R pathway plus addiction of some 5alpha-reduced metabolites (like DHT, THP, and others) would be nice in a study aimed to identify the metabolites involved in brain sexual differentiation.

In summary, the above data indicate that 5alpha-R2 plays a key role in brain sexual differentiation of male rats. Moreover, we suggest that 5alpha-R2 not only produces essential metabolites that act together with estradiol in brain sexual differentiation but also protects the brain from the damaging effects of estradiol excess. Since the molecular and cellular mechanisms responsible for mediating the developmental effects of androgen and its metabolite roles in sexual differentiation of the mammalian CNS remain incompletely understood, further studies with molecular approach should be interesting.

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