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ALTERED BRAIN AND PITUITARY ANDROGEN METABOLISM BY PRENATAL, PERINATAL OR PRE- AND POSTNATAL FINASTERIDE, FLUTAMIDE OR DIHYDROTESTOSTERONE TREATMENT IN JUVENILE MALE RATS

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Abstract

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1. The authors investigated the administration of finasteride, a 5α-reductase inhibitor; flutamide, an androgen receptor blocker and; exogenous dihydrotestosterone (DHT) during intervals covering different portions of the "critical period" of neural development (*i.e.* prenatal, perinatal or pre- and postnatal development) to determine the long-term effects of these agents on altering androgen metabolism in hypothalamic and pituitary tissue of juvenile (30 day-old) male rats.
2. The efficacy of the treatments and hypothalamic-pituitary axis function was monitored by measuring luteinizing hormone levels by radioimmunoassay. 5α-Reductase and aromatase activity was determined in hypothalamic and pituitary tissue.
3. Significant alterations in pituitary 5α-reductase activity was detected in DHT-treated animals, whereas, hypothalamic 5α-reductase activity was significantly decreased by finasteride treatment and significantly increased by DHT treatment. Hypothalamic aromatase activity was significantly decreased in flutamide-treated animals.
4. These results suggest that: a) prenatal exposure to exogenous DHT stimulates hypothalamic (but inhibits pituitary) 5α-reductase activity long-term and b) basal 5α-reductase activity levels can be inhibited by finasteride treatment in hypothalamic but not in pituitary tissue, suggesting that a different regulatory mechanism exists for 5α-reductase in hypothalamic verses pituitary tissue.

Key Words: aromatase, development, hypothalamus, pituitary, rat, 5α-reductase, reproductive behavior, sexual differentiation

Abbreviations: aromatase cytochrome P450 ($P450_{AROM}$), dihydrotestosterone (DHT), gestational day (GD), medial basal hypothalamus (MBH), preoptic area (POA), postnatal day (PND), testosterone (T)

Introduction

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 testosterone to estrogen(s) (MacLusky and Naftolin 1981, Gorski 1989). Estrogens *in vitro* can induce neural growth and aborization in perinatal hypothalamic explants (Toran-Allerand, 1976). Testosterone also stimulates neurite growth in hypothalamic explants *in vitro* via aromatization to estrogens, since DHT, a non-aromatizable androgen, does not increase neurite extension beyond that seen in control cultures (Toran-Allerand, 1976). These hypothalamic structures play important roles in brain differentiation (Toran-Allerand 1976, MacLusky and

Naftolin 1981, Gorski 1989), control of gonadotropin secretion and sexual behavior (Parson et al., 1984, McGinnis and Dreifuss, 1989), suggesting that local formation of estrogens play an integral role in the structural organization of these areas. Furthermore, there is a "critical period " of perinatal development [gestational day (GD) 18 to postnatal day (PND) 5 (MacLusky and Naftolin 1981, Gorski 1989)] in rodents during which neurons are responsive to estrogens.

However, there are two major enzymatic pathways of androgen metabolism in the brain (Martini 1982, Lephart and Ojeda 1990, Lephart et al., 1990). In addition to the conversion of testosterone to estrogens via the aromatase cytochrome P450 ($P450_{AROM}$) enzyme, it can also be metabolized to 5α-androstane-17β-ol-3-one [dihydrotestosterone (DHT)] via the 5α-reductase enzyme. 5α-Reduced metabolities of testosterone have been detected postnatally in various brain regions (Martini 1982, Lephart and Ojeda 1990) and pituitary tissue (Martini 1982, Denef 1983). The 5α-reductase enzymatic activity in the pituitary [unlike the brain (Lephart and Ojeda 1990)] appears to be regulated by gonadal hormones (Martini 1982, Denef 1983), suggesting that androgen action may be mediated via the androgen receptor (Hiemke et al., 1992).

While 5α-reductase appears to be an element in the sexual differentiation mechanism of CNS development in man (MacLusky and Naftolin 1981), it would be premature to conclude that 5α-reduced androgens are not involved in the sexual differentiation of the rodent brain since, the activity has been detected in both neurons (Martini and Melcangi 1991) and glial cells (Melcangi et al., 1988). This is especially intriguing since previous studies using neonates suggest that 5α-reductase activity in oligodendrocytes may be associated with myelin sheath formation for the insulation of axonal projections (Melcangi et al., 1988, Martini and Melcangi 1991). However, to date, the physiological function of 5α-reductase during any developmental period remains to be determined. Furthermore, only one previous study, from one of our laboratories, has examined the pattern of brain 5α-reductase mRNA levels and enzymatic activity during prenatal development (Lephart et al., 1990). In general, in that study, the developmental profile displayed low to moderate levels before and after the peak in 5α-reductase during late gestation (day 18 of gestation). Surprisingly, the 5α-reductase pattern paralleled that of brain $P450_{AROM}$ mRNA and enzyme levels (Lephart et al., 1992), but, the 5α-reductase mRNA content and activity values were several fold higher that those observed for $P450_{AROM}$. This suggests that alterations in the enzyme activities that metabolize testosterone during the "critical period" of CNS development could drastically alter the hormonal milieu of the developing fetus that can influence the sexual differentiation of the rodent brain (MacLusky and Naftolin 1981, Gorski 1989) and subsequent sexual behavior (Parsons et al., 1984, McGinnis and Dreifuss 1989).

It is important to understand the regulation of these enzymes during embryogensis since the deficiency of 5α-reductase in man results in one of the most unusual forms of male pseudohermaphrodites (Walsh et al., 1974, Imperato-McGinley et al., 1979). These individuals are frequently reared as females following birth, and are only diagnosed correctly as males when phallic enlargement occurs at puberty (Imperato-McGinley et al., 1979). The conversion of these children from a female to a male gender identity at adolescence appears to occur with relative ease, and offers one of the most intriguing of clincial questions: To what extent does the hormonal milieu of a child effect sexual identity and performance rather than the sex of rearing. Until recently the ability

to evaluate 5α-reductase deficiency and its importance on brain function was precluded by the lack of specific inhibitors. With the advent of finasteride, (MK-906; Merck Research Laboratories, Rahway, N.J.) a potent reversible 5α-reductase inhibitor that has no affinity for the androgen receptor (Rasmusson et al., 1986); it is now possible to examine the importance of 5α-reductase on brain and pituitary development and function. Finasteride is currently used clinically to treat benign prostatic hyperplasia in man. Inhibition of 5α-reductase by finasteride appears to be effective in reducing DHT levels and prostatc size without affecting fertility or libido (Stoner 1990).

The purpose of this study was to examine the effects of administering finasteride, a 5α-reductase inhibitor; flutamide, an androgen receptor blocker and; exogenous dihydrotestosterone (DHT) during intervals covering different portions of the "critical period" of neural development (*i.e.* prenatal, perinatal or pre- and postnatal development) and quantify the long-term effects of these agents on altering androgen metabolism in hypothalamic and pituitary tissue of juvenile (30 day-old) male rats.

Methods

Treatments

To examine the potential "long-term" effects of the treatments on brain and pituitary 5α-reductase three separate protocols were used which covered different time intervals of CNS development. Time-mated pregnant rats were divided into four groups and received the following treatments: 1) oil & ethanol-injected controls, 2) the 5α-reductase inhibitor, finasteride was suspended in oil and injections were administered @ 300 mg/kg body weight/day, 3) Flutamide, an androgen receptor blocker was dissolved in ethanol and injected @ 100 mg/kg body weight/day and, 4) DHT suspended in oil was injected @ 2.0 mg/kg body weight/day (*i.e.* ≈ 0.5 to 0.8 mg/animal/day) for the injection intervals described below. The volume of oil or oil-containing DHT or finasteride injected ranged from 0.05 to 0.4 ml per day dependent upon the weight of the animal. The volume of ethanol vehicle injected never exceeded 0.08 ml per day.

Protocol 1: Complete (pre- and postnatal) treatment exposure: Gestational day 13 to postnatal day 30 (GD 13 to PND 30). Maternal animals were injected with the various substances (as described above) beginning on GD 13 with subsequent injections administered to the rat pups after birth through 29 days of age.

Protocol 2: Perinatal treatment exposure: Gestational day 13 to postnatal day 14 (GD 13 to PND 14). Maternal animals were injected by treatment groups as described in protocol 1 with the exception that the injections were discontinued after the 14th postnatal day of life.

Protocol 3: Prenatal treatment exposure: Gestational day 13 to birth (GD 13 to birth). Maternal animals were injected starting at GD 13 by treatment groups as before until birth. No injections were given to any pups following delivery.

All aninals were killed on postnatal day 30 of life using protocol 1, 2 or 3 and the sex of each animal was confirmed by examination of the internal gonadal structures (only male animals were used in this study). There were at least three pregnant animals per treatment and 8 to 10 pups per

mother. Also, due to the ability of the 5α-reductase inhibitor to block parturition in the rat; at GD 22 all finasteride treated pups were delivered by caesarean section under ether anesthesia. This operation was performed only after timed matched mothers had delivered. The finasteride treated pups were rapidly removed from the uterus, and the mother killed by intracardiac injection of Nembutal (25 mg/100 g body weight). The timed mated pups of the surrogate mother were killed by ether anesthesia, and the finasteride treated pups were placed with the surrogate lactating dam. Finally, due to the inability to correctly sex both the 5α-reductase inhibited and the DHT-treated newborn animals; due to either a feminized or masculinized anogential distances, all pups were injected with the respective treatments until completion of protocol 1 or 2.

Evidence of the effectiveness of the treatments was demonstrated by the observations that abnormal prostatic and phallic growth (hypospadias) were recorded in the finasteride and flutamide animals (data not shown). Furthermore in the DHT-treated animals androgen-responsive tissues (*i.e.* Wolffian duct derived structures) displayed increased organ weight (hypertrophy) to that of controls (data not shown).

Luteinizing Hormone Assay

At the time tissue specimens were obtained, central venous puncture was performed to collect serum for the determination of luteinizing hormone values. Luteinizing hormone levels were measured by double antibody radioimmunoassay (Lephart and Ojeda, 1990), using antisera supplied by the national pituitary program. Serum samples from all treatments were analyzed in a single assay. The intra-assay coefficient of variation was less than 10%.

Dissection of Tissue

Dissection of the medial basal hypothalamic (MBH) and preoptic areas (POA) tissue were performed as previously described (Lephart and Ojdea, 1990, Lephart et al., 1990). The pituitary was freed from the dura matter, removed from the skull, and the posterior pituitary was dissected free. The MBH-POA and pituitary tissue samples were assayed for 5α-reductase whereas $P450_{AROM}$ activity was determined only in MBH-POA tissue samples.

Enzyme Activity Assays

In brain and pituitary tissue samples, $P450_{AROM}$ and 5α-reductase activities were measured simultaneously using [1α,1β-^{3}H]T as the substrate. To quantify $P450_{AROM}$ activity the "tritiated water" assay was utilized since only the tritium atom in the beta position is incorporated into water during the aromatization reaction (*i.e.* isolated and quantified). Whereas, the tritium atom in the alpha position is retained in the steroid structure (*i.e.* after extraction into the organic solvent, 5α-reduced metabolities are resolved and isolated by thin layer chromatography and quantified by scintillation counting) that can be utilized for the determination of 5α-reductase activity. Both assays have been described in detail elsewhere (Lephart and Ojeda, 1990, Lephart et al., 1990). As previously demonstrated in our laboratory, maximal rates of $P450_{AROM}$ and 5α-reductase activities were obtained at substrate concentrations of 1.0 μM and 2.0 μM, repectively (Lephart and Ojeda, 1990, Lephart et al., 1990). Also, the identity of the [^{3}H]estrogen or [^{3}H]5α-DHT formed during the

incubation has been confirmed by recrystallization of the derivatized products to constant specific activity (Lephart and Ojeda, 1990, Lephart et al., 1990).

Statistical Analysis

The results were analyzed by one-way analysis of variance, followed by the Newman-Keuls multiple comparison test, p values < 0.05 were considered significant.

Results

Luteinizing Hormone Levels

To monitor the function of the hypothalamic-pituitary axis and provide an index of the efficacy of the treatments LH levels were determined in each animal (Fig. 1).

Whether DHT was administered during prenatal, perinatal or pre- and postnatal development a significant suppression in LH levels (*i.e.* < 0.2 ng/ml) was recorded in these males compared to controls (≈ 2.0 ng/ml). Conversely, recovery from the inhibitory effects of the 5α-reductase inhibitor (finasteride) or the androgen receptor blocker (flutamide) on LH levels was evident by the normalization of gonadotrophin levels (≈ 2.0 ng/ml) at postnatal day 30 in prenatally or perinatally treated animals (Fig. 1). Finally, if finasteride or flutamide were administered continually during the pre- and postnatal interval (*i.e.* GD 13 to PND 30) a significant increase in LH levels were observed (11.0 and 32.0 ng/ml, respectively), compared to control values (≈ 2.0 ng/ml).

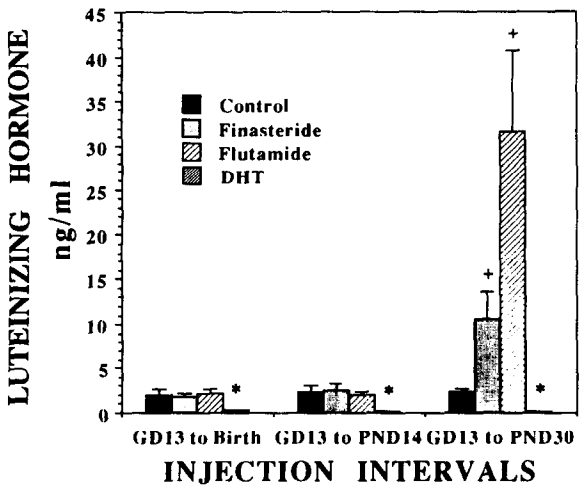

Fig. 1. Effect of finasteride, flutamide or DHT on luteinizing hormone (LH) levels. Each bar represents the mean value ± S.E. of 5 to 10 animals (see Fig. 2 for the exact number of animals per treatment per injection interval). * = significant decrease in LH levels compared to control values for each injection interval. + = significant increase in LH levels compared to control values (@ GD 13 to PND 30).

Pituitary 5α-Reductase Activity

As shown in figure 2 A, treatment with the 5α-reductase inhibitor (finasteride) or the androgen receptor blocker (flutamide) did not significantly alter pituitary 5α-reductase activity for any injection interval tested. However, prenatal, perinatal or pre- and postnatal treatment with DHT significantly decreased the activity by ≈ one-half to one-fifth of control values (≈ 64 pmol/hr/mg protein). A greater suppression in pituitary 5α-reductase activity was observed in animals treated with DHT during the perinatal and pre- and postnatal intervals compared to only prenatal treatment.

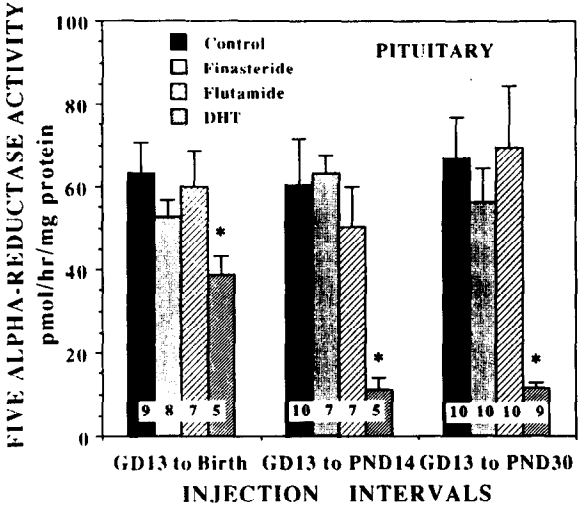

Fig 2 A. Effect of finasteride, flutamide or DHT on pituitary 5α-reductase activity. Each bar represents the mean value ± S.E. and the number of animals per group are shown at the base of each bar. * = significant decrease in 5α-reductase activity levels compared to control values.

Brain 5α-Reductase Activity

Similar to the results obtained in pituitary tissue, flutamide treatment did not significantly alter 5α-reductase activity in brain (MBH-POA) tissue for any injection interval tested (Fig. 2 B). However, in contrast to its lack of effect on 5α-reductase activity in the pituitary, finasteride administered during prenatal or perinatal development resulted in a substantial "rebound" effect of the activity in MBH-POA tissue when assayed at postnatal day 30. While the finasteride levels (≈ 19.0 pmol/hr/mg protein) were notably higher than control values (≈ 11.0 pmol/hr/mg protein) they approached but did not reach statistical significance (Fig. 2 B). Although when finasteride was given throughout pre- and postnatal development (*i.e.* GD 13 to PND 30) the activity was significantly decreased by the 5α-reductase inhibitor (to ≈ 2.5 pmol/hr/mg protein).

On the other hand, rats treated with DHT during prenatal and perinatal development, in these animals, 5α-reductase levels were similar to finasteride-treated males for the same injection intervals [*i.e.* these levels were elevated (≈ 18.8 pmol/hr/mg protein) above controls (≈ 11.0 pmol/hr/mg protein); which approached but did not reach statistical significance]. However, when DHT was administered chronically (*i.e.* GD 13 to PND 30) MBH-POA 5α-reductase levels were significantly greater than control values (DHT = 21.1 ± 2.1 pmol/hr/mg protein vs. control = 9.9 ± 1.1 pmol/hr/mg protein; see Fig. 2 B).

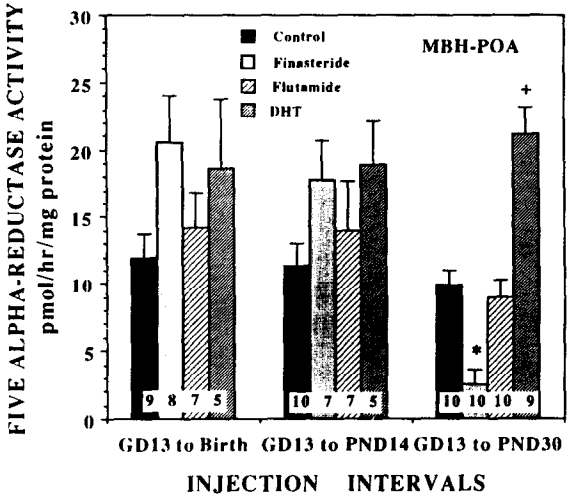

Fig 2 B. Effect of finasteride, flutamide or DHT on brain 5α-reductase activity. Each bar represents the mean value ± S.E. and the number of rats per group are shown at the base of each bar. * = significant decrease, + = significant increase in 5α-reductase activity levels compared to control values (@ GD 13 to PND 30).

Brain $P450_{AROM}$ Activity

In addition to recording 5α-reductase levels, $P450_{AROM}$ activity also was measured in MBH-POA tissue as affected by the treatments (Fig. 3). During the prenatal or perinatal injection intervals $P450_{AROM}$ activity was not significantly altered in MBH-POA tissue by any of the treatments. Notably, males receiving DHT treatment prenatally displayed higher levels of $P450_{AROM}$ activity (≈ 187 ± 24.5 pmol/hr/mg protein) compared to controls (≈ 143 ± 10.3 pmol/hr/mg protein), however, this difference was not significant (Fig. 3). Animals treated with flutamide during the pre- and postnatal interval were the only affected group. Treatment with the androgen receptor blocker significantly reduced brain $P450_{AROM}$ activity (105 ± 13.0 fmol/hr/mg protein) to that of controls (153 ± 14.2 fmol/hr/mg protein).

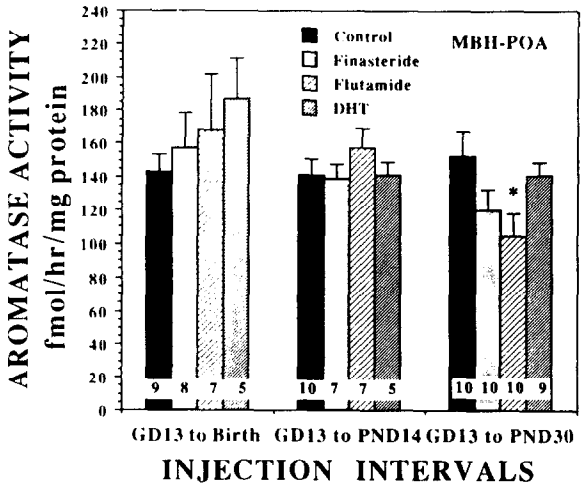

Fig 3. Effects of finasteride, flutamide or DHT on brain aromatase activity. Each bar represents the mean value ± S.E. and the number of rats per group are shown at the base of each bar. * = significant decrease in aromatase activity levels compared to control values (@ GD 13 to PND 30).

Discussion

In the present study, the authors have examined the effects of administering several compounds (*i.e.* finasteride, flutamide or exogenous DHT) during intervals covering different portions of the "critical period" of neural development (*i.e.* prenatal, perinatal or pre- and postnatal development) and recorded the long-term effects of these agents in altering androgen metabolism in the brain and pituitary of juvenile (30 day-old) male rats. The activity rates for brain 5α-reductase and $P450_{AROM}$ observed in this study are similar to previously reported values from our laboratory (Lephart and Ojeda, 1990).

PITUITARY 5α-REDUCTASE ACTIVITY

EFFECTS OF DHT TREATMENT

The inhibitory effect of exogenous DHT treatment on pituitary 5α-reductase activity in the present study is in agreement with previous studies (Martini, 1982, Martini and Melcangi, 1991). Whereas, castration during postnatal development has been shown to increase pituitary 5α-reductase (Martini, 1982, Martini and Melcangi, 1991) and decrease the basal levels of brain $P450_{AROM}$ activity (Lephart and Ojeda, 1990). However, in the present study, prenatal DHT treatment decreased 5α-reductase activity in pituitary tissue long-term resulting in a sustained suppression of LH levels. Since 5α-reductase activity has been characterized in the rat pituitary (Martini, 1982, Denef, 1983) and that other steroids (*i.e.* progesterone and corticosterone) may act as physiological substrates (Martini, 1982, Lephart et al., 1991) [in male rats the adrenal gland secretes significant amounts of progesterone into the systemic circulation (Lephart et al., 1991)], it is

reasonable to consider the involvement of other 5α-reduced metabolites in regulating pituitary function. 5α-Reduced metabolities of progesterone not only appear to modulate the neuroendocrine release of gonadotrophins (Brann et al., 1990) but can act as anesthetic agents (Bixo and Backstrom, 1990) and hence affect behavior. In both cases these 5α-reduced metabolites act in a specific manner to modulate the action of GABA at the level of the $GABA_A$ receptor (Harrison et al., 1989, Brann et al., 1990). In this regard, the long-term suppression of pituitary 5α-reductase activity by prenatal DHT treatment may represent an addition level of control (by decreasing 5α-reduced metabolites) in the prolonged decrease of LH levels.

EFFECTS OF FLUTAMIDE TREATMENT

To our knowledge, flutamide, the androgen receptor blocker has not been tested previously in its ability to alter 5α-reductase activity in the pituitary or brain. To our surprise, for any injection interval tested, pituitary or brain 5α-reductase activity was not significantly affected in flutamide-treated animals. This suggests that while pituitary 5α-reductase can be influenced by gonadal hormones (Martini, 1982, Denef, 1983) the mechanism of regulation may not involve an androgen receptor mediated event. Furthermore, pituitary 5α-reductase apparently is not influenced by gonadotrophins levels (long-term) since LH values were restored to a normal range during the prenatal or perinatal treatment intervals or were significantly increased during pre- and postnatal flutamide-treatment exposure. This is in contrast to a putative connection between luteinizing hormone releasing hormone (LHRH) stimulating pituitary 5α-reductase postnatally (Martini, 1982, Martini and Melcangi, 1991). However, in agreement with previous reports flutamide treatment has been shown to inhibit basal levels of brain $P450_{AROM}$ activity (Roselli and Resko, 1984) as demonstrated in the present study during the pre- and postnatal treatment interval.

EFFECTS OF FINASTERIDE TREATMENT

In finasteride-treated animals, the inability of this 5α-reductase inhibitor to significantly reduce pituitary 5α-reductase activity was observed in all three injection intervals. Conversely, in the brain 5α-reductase activity was significantly decreased by finasteride treatment during the pre- and postnatal treatment (*i.e.* concomitant with significantly increased LH levels) but not during the prenatal or perinatal intervals. The restoration of LH levels in finasteride-treated animals (*i.e.* during prenatal and perinatal periods) suggests that a long-term alteration was not established upon the hypothalamic-pituitary axis. However, several possible explanations exist to elucidate the novel finding that pituitary 5α-reductase may be under a different control mechanism to that found in brain. First, tissue-specific control for 5α-reductase may exist, for example, in the liver 5α-reductase activity may be regulated by thyroid hormones (Kato et al., 1970), whereas, in the prostate, DHT apparently has the ability to regulate 5α-reductase (Andersson et al., 1989, George et al., 1991, Wilson, 1992). Second, the nature of neural verses endocrine tissue composition may play a role in determining the effectiveness of this 5α-reductase inhibitor [*i.e.* due to the steroid structure of finasteride (Rasmusson et al., 1986) and the lipophilic characteristics of neural tissue, the inhibitor may be concentrated in brain tissue while the vascular-endocrine characteristics of pituitary tissue may prevent an accumulation of finasteride]. Indeed, this may be the case since finasteride does not bind the androgen receptor (Rasmusson et al., 1986). Third, since two different 5α-reductase genes have been identified in man (Jenkins et al., 1992), another possible explanation would be if a

separate isoform of 5α-reductase exists in rat pituitary compared to neural tissue. In fact, studies on the existence of two 5α-reductases in the rat prostrate have been reported (Martini et al., 1986). It is likely that two distinct 5α-reductase enzymes are present in the brain and pituitary, however, finasteride may have the ability to effectively inhibit only one of the isoforms (George et al., 1991, Jenkins et al., 1992, Wilson, 1992). Although, the inability of finasteride to alter pituitary 5α-reductase is not understood. Notably this inability of finasteride to block pituitary 5α-reductase in the rat, must temper our enthusiasm for directly comparing this animal model to the 5α-reductase deficiency that occurs in man (Imperato-McGinley et al., 1979, Wilson, 1992). In this latter situation, presumably decreased 5α-reductase activity exists in both the brain and pituitary sites, however this remains to be determined. Finally, with finasteride treatment testosterone levels are presumably increased above basal levels in tissue sites of androgen metabolism (*e.g.* brain tissue) that can create an altered state of the hormonal status of the developing fetus, neonate and juvenile rat that may possibly influence CNS development (MacLusky and Naftolin, 1981, Gorski, 1989) or possibly modulate the 5α-reductase enzyme itself (George et al., 1991). However, in finasteride-treated animals MBH-POA $P450_{AROM}$ enzyme activity was not significantly altered during any of the injection intervals tested.

BRAIN 5α-REDUCTASE

EFFECTS OF DHT TREATMENT

In contrast to the long-term inhibitory effects of DHT on pituitary 5α-reductase and LH levels (Martini, 1982) in DHT-treated animals brain 5α-reductase activity was significantly increased above control values during the pre- and postnatal treatment interval. Of distinct concern is the continued elevation of 5α-reductase activity in MBH-POA tissue at 30 days after fetal exposure. Recently it has been reported that DHT (and to a less extent, testosterone) induces expression of 5α-reductase in rat prostate (George et al., 1991). Several mechanisms may be involved in the enhancement of 5α-reductase activity by DHT [*e.g.* stabilization of the 5α-reductase mRNA, direct stimulation of gene transcription, post-translational modifications, or a decreased rate of catabolism (George et al., 1991)] . However, further investigations are warranted to elucidate this autocatalyic regulation by DHT (which may account for the "rebound" in brain 5α-reductase activity seen in prenatal and perinatal finasteride-treated animals). Although DHT treatment has not been investigated previously during prenatal development, it is intriguing to speculate that when brain 5α-reductase mRNA expression and activity levels are initiated and maximal during prenatal development (Lephart et al., 1990) exogenous DHT administered prenatally may alter the basal level of brain 5α-reductase activity that is sustained during postnatal development. This finding in conjunction with a significant decrease in pituitary 5α-reductase activity, and diminished LH values, is an indication that this treatment apparently reset the basal level of MBH-POA 5α-reductase activity. The importance of brain 5α-reductase activity is readily apparent from the current hypothesis regarding the gonadostat theory for the onset of puberty (Lephart and Ojeda, 1990). The fundamental aspect of this theory is based upon the assumption that high 5α-reductase or $P450_{AROM}$ enzyme activity levels in the hypothalamus rapidly forms DHT or estradiol, respectively, either of which could serve as a more potent, biologically active, factor in the negative feedback mechanism (Piacsek and Hostetter, 1984, Feigelson, 1986). We and others have previously demonstrated that a decrease in the

enzymatic activities of 5α-reductase (and $P450_{AROM}$) occurs during the transition of pubescence (Martini, 1982, Lephart and Ojeda, 1990). The persistent elevation of brain 5α-reductase activity along with suppression of the pituitary 5α-reductase activity and co-existing significantly low LH levels at 30 days of age is consistent with an immature hypothalamic system. The experimental design of this study precludes the evaluation of whether or not we have induced a permanent or transient state of hypogonadotrophism. Furthermore, we did not detect any long-term effects of exogenous DHT treatment on brain $P450_{AROM}$ activity. However, whether this treatment can alter MBH-POA $P450_{AROM}$ prenatally (short-term) is unknown.

Conclusions

The present findings reveal that a different regulatory mechanism exists for 5α-reductase activity in MBH-POA verses pituitary tissue. This report is the first to demonstrate that prenatal exposure to DHT stimulates 5α-reductase activity (long-term) within the hypothalamus and the basal activity levels can be inhibited by finasteride treatment. However, whether these effects are permanent or transient after sexual maturation and whether alterations in androgen metabolism during development can effect sexual behavior remains to be determined .

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