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HYPOTHALAMIC AROMATASE CYTOCHROME P450 AND 5α-REDUCTASE ENZYME ACTIVITIES IN PREGNANT AND FEMALE RATS

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Summary

The metabolism of steroid hormones in the medial basal hypothalamus (MBH) is known to play a critical role in neural development, the modulation of neuroendocrine function and regulating sexual behavior. While the important biological functions of the aromatase enzyme are well established, the importance of brain 5α-reductase has been revealed and elucidated only in the last few years. The distribution and regulation of brain aromatase and 5α-reductase enzyme activities have been investigated for the most part in male rats. Therefore, in the present study, MBH aromatase cytochrome P450 and 5α-reductase activities were characterized in pregnant and female rats during postnatal development under various hormonal conditions. MBH aromatase activity was determined in each tissue sample using the 'tritiated water release' assay, whereas, the 5α-reductase rates were determined by thin layer chromatography and scintillation counting of the isolated 5α-metabolites. Both activities were highest in infantile animals, then declined with increasing postnatal age; whereas, in aged non-cycling or ovariectomized/adrenalectomized (Ovx/Adx) rats high rates of androgen metabolism were seen in MBH tissue. No significant alterations in MBH aromatase were observed when the 5α-reductase pathway was blocked in pregnant animals during late gestation with a known 5α-reductase inhibitor (Proscar). However, plasma estradiol levels were significantly increased in the Proscar-treated animals. These results indicate that: 1) the decreasing MBH aromatase and 5α-reductase profile (in infantile to adult cycling animals) is developmentally regulated, 2) evidently, there is a divergent regulatory mechanism controlling MBH aromatase versus 5α-reductase in aged animals where the aromatase activity increased in aged non-cycling and Ovx/Adx rats while 5α-reductase rates remained at moderate levels and, 3) apparently, the 5α-reductase pathway is not involved in regulating MBH aromatase activity during late pregnancy.

Key Words: aromatase, 5α-reductase, hypothalamus, pregnancy, rat

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In the brain, androgens are metabolized by two major enzymatic pathways (i.e. testosterone can be converted to estrogens by the aromatase cytochrome P450 enzyme or it can be converted by the 5α-reductase enzyme to 5α-dihydrotestosterone) (1,2). Both enzymes are found in specific brain regions, such as the medial basal hypothalamus (MBH) and limbic system (1-8). Although, the highest levels of 5α-reductase in adult rats are found within white matter structures (1,6,8). During perinatal development, local estrogen biosynthesis in the MBH region influences the sexual differentiation of neural structures and modulates neuroendocrine/reproductive functions and regulates sexual behavior (2-5). Brain 5α-reductase has also been reported to alter neuroendocrine mechanism(s) and behavior which apparently involves the 5α-reduction of androgens and progesterone. 5α-Reduced metabolites of androgens and progesterone, such as, tetrahydroprogesterone (THP) mediate these effects through a $GABA_A$ receptor mechanism (1,6,9-12). The distribution and regulation of brain aromatase and 5α-reductase enzyme activities have been investigated for the most part in male rats (1,2,4). In adult male rats, MBH aromatase is significantly decreased in castrates, while androgen replacement therapy restores the activity to or enhances the aromatase enzyme activity above pre-castration control levels (2,3,13-16). This is not the case for 5α-reductase, where gonadectomy does not significantly alter MBH activity levels (1,8,17). Additionally, *in vivo* studies indicate that estradiol along with androgens, such as 5α-dihydrotestosterone, have the ability to stimulate MBH aromatase activity in a synergistic manner in adult rats (2,17). Conversely, in female rats, MBH aromatase decreases with the onset of puberty (during the first estrous cycle) where increased steroid production is observed (12). Moreover, brain aromatase or 5α-reductase enzyme activities are not significantly altered after ovariectomy or during the estrous cycle in adult animals (1,2,4,8,15). However, in reproductive tissues, blocking the 5α-reductase pathway of androgen metabolism significantly increases aromatase activity in vaginal tissue or estrogen levels are significantly increased in 5α-reductase (type 1) knock-out mice during pregnancy (18,19). While the developmental pattern and regulation of brain aromatase and 5α-reductase are well described in male rats, brain aromatase or 5α-reductase in female rats is not well characterized (under different hormonal conditions and during various postnatal developmental intervals). Therefore, the purpose of the present study was to characterize MBH aromatase and 5α-reductase activities in infantile, prepubertal, random cycling, ovariectomized/adrenalectomized (Oxv/Adx), and aged female rats. Furthermore, it is not known whether the 5α-reductase pathway influences brain aromatase activity levels. Finally, MBH aromatase activity was quantified in pregnant rats where the 5α-reductase pathway of androgen metabolism was blocked with a known 5α-reductase inhibitor.

Methods

Sprague-Dawley non-pregnant and time-mated pregnant rats (sperm positive date = day 0 of gestation) from our stock laboratory colony were housed in a controlled environment with free access to tap water and rat laboratory chow (Teklad Rat Diet, Madison, WI, USA). The animals and methods used in this study were approved by the Institutional Animal Care and Use Committee at Brigham Young University. Pregnant animals were divided into three groups: 1) control-untreated, 2) control-injection (corn oil vehicle) or 3) 5α-reductase inhibitor [Proscar (Finasteride) was administered (@ 75 mg/kg/day) from gestational day (GD) 14 through 20. The Proscar was suspended in corn oil and the total volume of oil injected (s.c. at the nape of the neck) over the treatment period did not exceed 1.5 ml]. The brain tissue samples were collected on GD 21. The following groups of rats were also