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BRAIN RESEARCH

Research Report

Expression of steroid hormone receptors in the fetal sheep brain during the critical period for sexual differentiation

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ABSTRACT

The objective of this study was to examine the expression of receptors for androgen, estrogen, and progesterone in the fetal sheep brain during the critical period for sexual differentiation. We isolated mRNA from the hypothalamus–preoptic area (HPOA), amygdala (AMYG), medulla (MD), frontal cortex (FCTX) and olfactory bulbs (OB) of fetal sheep that were delivered on day 64 of gestation. Using a ribonuclease protection assay and species-specific cRNA probes, we measured mRNA expression levels of androgen receptor (AR), estrogen receptor alpha (ERα) and progesterone receptor (PR). ERα and AR mRNA were expressed in all of the tissues tested and highest in the HPOA. PR mRNA was measured in HPOA and AMYG only and was significantly higher in male than in female fetuses. We conclude that the fetal brain is a target site for circulating steroid hormones. These data have implications for the steroid dependent development of sexually dimorphic brain functions in sheep.

1. Introduction

Exposure of sheep fetuses to steroid hormones during sensitive periods in fetal development known as critical periods causes permanent changes in the sexual phenotype of the brain (Ford and D'Occhio, 1989; Wood and Foster, 1998). Once testes or ovaries develop, differences in their steroid secretions induce sexual differentiation of the brain, as well as non-neural tissue. Testosterone (T), or its metabolite estradiol (E2) generated by aromatase enzyme activity, acts on the brain to induce masculine patterns of development that result in sex differences in reproductive behaviors and gonadotrophin feedback control mechanisms. Because of prenatal androgen exposure, male lambs reach puberty at an earlier age than female lambs (Wood and Foster, 1998). In addition, early androgen exposure causes adult rams to show a greater capacity than females for expression of male-typical sexual behavior (Masek et al., 1999), and inhibits rams from exhibiting LH surges in response to an estradiol challenge or LH suppression in response to progesterone treatment (Robinson et al., 1999).

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In several species, the critical period for sexual differentiation is characterized by the presence of steroid hormone receptors and aromatase enzyme activity within the areas of the brain that regulate sexual function (Vito and Fox, 1982; Vito et al., 1985; Resko and Roselli, 1997; DonCarlos and Handa, 1994; McAbee and DonCarlos, 1998). We reported previously that the fetal sheep brain has the capacity to aromatize androgens to estrogens on day 64 of gestation, a time point that falls in the middle of the critical period for sexual differentiation in sheep (Roselli et al., 2003). Aromatase activity was highest in the hypothalamus–preoptic area (HPOA) and amygdala (AMYG). Male and female fetuses had equivalent levels of aromatase, but serum concentrations of T were approximately 10 times higher in males indicating that the availability of substrate for aromatase is an important factor limiting exposure of the developing female sheep brain to estrogen.

The present experiment extends our analysis of the fetal sheep brain to examine whether androgen receptors (AR), estrogen receptors (ER) and progesterone receptors (PR) are expressed during the critical period when the brain becomes sexually differentiated. For this study, we used a highly specific and sensitive ribonuclease protection assay to measure the concentration of steroid receptor mRNA in discrete fetal brain regions. Half of the experimental subjects were exposed to the aromatase inhibitor 1,4,5-androstatriene-3,17-dione (ATD) for 2 weeks to determine if the disruption of estrogen production alters the pattern of steroid hormone receptor expression. Our results demonstrate that there is a temporal correspondence between the timing of gonadal hormone secretion and steroid hormone receptor expression in the brain of fetal sheep supporting the hypothesis that sex hormones are critical mediators for sexual differentiation of the central nervous system in sheep. We found no evidence that local estrogen synthesis modulates hormone receptor expression. Furthermore, we found the PR expression was significantly greater in males than in females corroborating recent reports in rodents (Quadros et al., 2002a) that implicate progesterone in brain masculinization.

2. Results

AR, ERα, and PR mRNAs were all expressed within the central nervous system of fetal lambs at 64 days of gestation. However, there were no statistically significant effects of ATD exposure on receptor expression despite the fact that aromatase activity was inhibited by >75% in HPOA, AMYG, and medulla (MD) (Roselli et al., 2003). The highest expression of ERα mRNA in both males and females was found in the HPOA, which was approximately twofold greater than the expression of ERα mRNA in the AMYG and MD (Fig. 1A). Low levels of ERα mRNA were measured in the frontal cortex (FCTX). In the olfactory bulb (OB), ERα mRNA was undectectable in 11 out of 13 samples. There was no effect of sex or ATD treatment on ERα mRNA expression in any of the tissues examined. AR mRNA expression was greatest in the HPOA and MD and lower in the AMYG, FCTX and OB (Fig. 1B). Similar to ERα, there was no effect of sex or ATD treatment on AR mRNA expression. In contrast, the expression of PR mRNA in the HPOA (Fig. 2) was significantly greater in males than in females ($F_{1,11}=28.6$; $P<0.001$), but was not effected by ATD treatment ($F_{1,11}=1.0$; $P=0.3$). The levels of PR mRNA expression in the AMYG were one tenth of the levels in the HPOA and also exhibited a significant sex difference ($F_{1,9}=6.5$; $P<0.05$), but not an effect of ATD treatment ($F_{1,9}=0.5$; $P=0.5$).

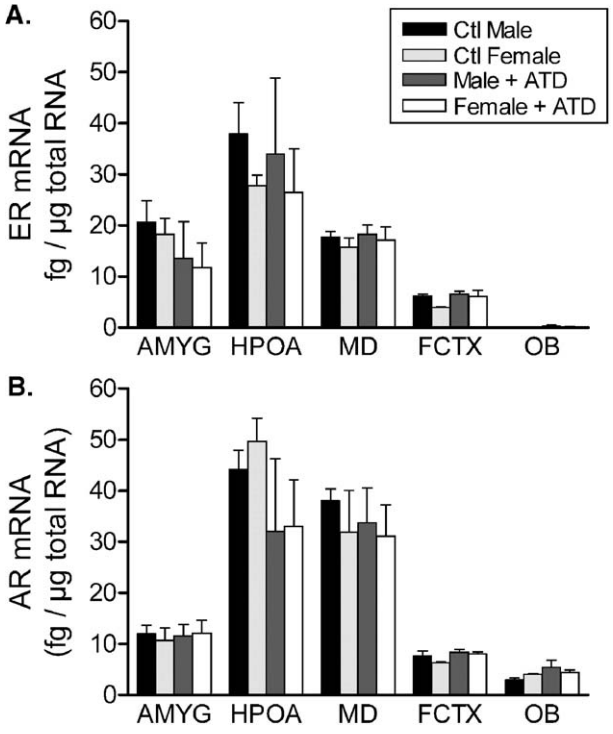

Fig. 1 – (A) Distribution of ERα mRNA in the brain and olfactory bulb of day 64 fetal sheep; (B) distribution of AR mRNA in the brain and olfactory bulb of day 64 fetal sheep. Values represent the mean (bars) ± SEM (vertical lines); *n*=3–4/group. Abbreviations: AMYG, amygdala; FCTX, frontal cortex; HPOA, hypothalamus–preoptic area; MD, medulla; OB, olfactory bulb.

3. Discussion

The results of this study demonstrate that AR, ERα, and PR mRNAs are all expressed in the ovine central nervous system during the critical period of fetal neuroendocrine development that underlies sexual differentiation. The simultaneous expression of all three gonadal steroid receptor genes suggests that circulating hormones produced by the fetus and/or the mother may coordinately regulate developmental processes that lead to long-lasting changes in the structure and function of the brain. The high levels of receptor expression found in the HPOA highlights the central role that this brain region plays for the organization of neural circuitry underlying reproductive behaviors and neuroendocrine control mechanisms (Simerly, 1995). This study also demonstrates that AR and ERα are expressed in AMYG, FCTX, and MD, where they may influence the development of a wide variety of circuits important in reproduction and homeostatic control (Hamson et al., 2004; Scott et al., 1998). Low levels of AR mRNA were present in the olfactory bulb at this early stage of development