THE RELATİONSHİP BETWEEN STERİOD RECEPTORS AND AROMATASE İN THE MOUSE BRAİN

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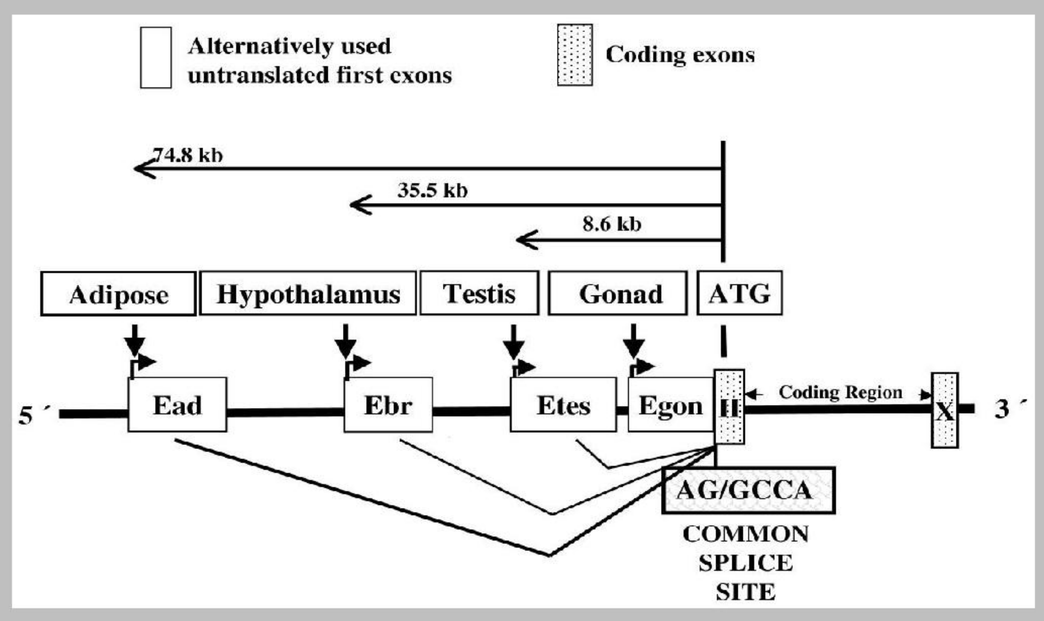
INTRODUCTİON

Sex steroids influence most aspects of cellular organization and mammalian development. They particularly play active roles in shaping the neural functions and reproductive behaviors.[1,2] Sex steroid synthesis begins with the entry of cholesterol into the mitochondria and as a result of six main enzymatic steps, estrogen, progesterone and testosterone occur. These steroids especially estrogen and progesterone have variety of functions on several organs including the brain. Sex steroids are primarily synthesized by male and female reproductive organs and adrenal cortex, but they can also be locally synthesized by various tissues and organs, such as the brain.[2,3]

Aromatase is the key enzyme that catalyzes the most important step of sex steroids synthesis, the conversion of C_{19} steroids to biologically active estrogen, estradiol (E_2).[4]

Aromatase is encoded by a single-copy gene (*Cyp19a1*), the inhibition of which effectively eliminates estrogen production in the entire body, both in human and mouse.[4,5] The mouse *Cyp19a1* gene, which spans a 30 kb coding region and a 75 kb regulatory region (~105 kb in total length), is located on the long arm of chromosome 9. The aromatase protein encoding region spanning 30 kb of the 3'-end contains nine coding exons (II-X).[5,6] The upstream 75 kb of the gene contains a number of promoters that initiate the transcription of alternative first exons, giving rise to aromatase mRNA species with unique 5'-untranslated region.[7] The upstream regulatory region contains two major promoters regulated in a tissue-specific manner: the proximal gonad-specific promoter and the distal brain-specific promoter; which lies ~35 kb upstream of the ATG translational start site in coding exon II.[5,8,9]



In the vertebrate brain, aromatase is primarily expressed in the hypothalamus, hippocampus and amygdala via a highly conserved promoter I.f.[10,11] Aromatase expression in the hypothalamus is primarily localized in the medial preoptic area and the ventromedial nucleus of the hypothalamus, which are the centers that govern reproductive functions of both sexes of different species.[12-16]

The molecular mechanisms regarding regulation of brain-specific aromatase promoter I.f are still not well understood. Recently, E_2 and progesterone were found to alternately upregulate or downregulate hypothalamic aromatase mRNA and enzyme activity via estrogen receptors (*Esr1*) and progesterone receptors (*Pgr*) under both *in vitro* and *in vivo* conditions [23-25] These studies, however, did not establish an *in vivo* connection between aromatase expression and promoter I.f regulation in the brain. In this study, we focused on simultaneous mRNA expression profiles of *c-Jun*, *Esr1* and *Pgr*, which are thought to play key roles in regulating aromatase expression and enzyme activity in the fetal mouse brain at different stages of pregnancy. Additionally, in order to delineate the function of *Esr1* on *Cyp19a1* mRNA expression in *in vivo* settings *Cyp19a1* mRNA expression was assessed in estradiol pellet implanted-castrated mouse hypothalamus and in *Esr1*KO mouse hypothalamus which confers the highest *Cyp19a1* mRNA expression and enzyme activity throughout the brain.

RESULTS

To determine *Cyp19a1*, *c-Jun*, *Esr1*, and *Pgr* mRNA expressions in the fetal mouse brain, pregnant mice were sacrificed at gestational days 9, 11-12, 13-14, 15, 16-17, 19, 21 and RNAs were isolated from their brains for quantitative real-time RT-PCR.

Cyp19a1 mRNA expression levels for various gestational days are shown in Figure 1. The highest *Cyp19a1* mRNA expression was found at days 13-15. Subsequently, *Cyp19a1* mRNA expression levels started to fall back to normal adult levels. In contrast to *Cyp19a1*, *Esr1* (Figure 2) and *Pgr* mRNA expressions (Figure 3) peaked at day 9, and started to decline at day 11-12 and drop far below the adult levels at days 13-15 following a gradual increase up to normal adult levels after days 16-17. However, *c-Jun* mRNA expression remained at similar levels throughout the gestational period with slight fluctuations (data not shown).

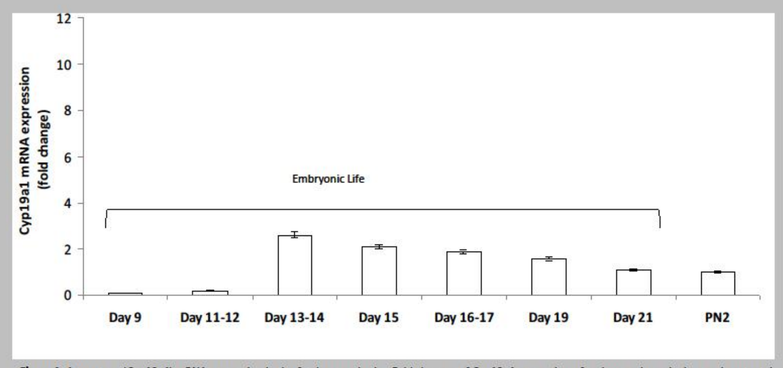


Figure 1. Aromatase (*Cyp19a1*) mRNA expression in the fetal mouse brain. Fold changes of *Cyp19a1* expression of various embryonic days and postnatal day 2 is shown. Results were normalized to the expression levels of *GAPDH* gene as an endogenous control. The results are expressed as mean ±S.E.M.from three independent experiments (P<0.01, paired t test).

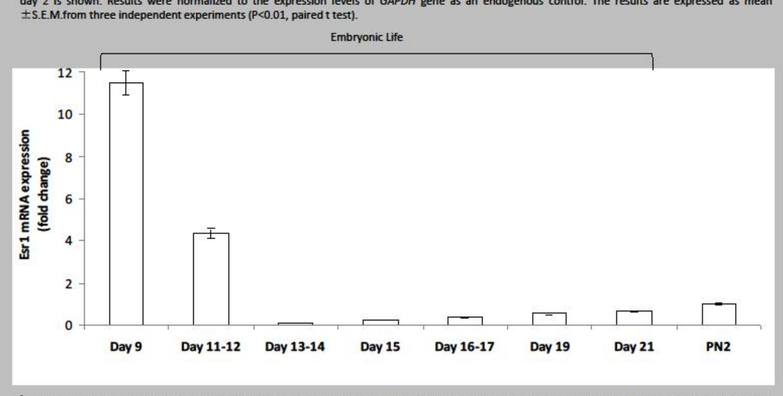


Figure 2. Estrogen receptor (*Esr1*) mRNA expression in the fetal mouse brain. Fold changes of *Esr1* expression of various embryonic days and postnatal day 2 is shown. Results were normalized to the expression levels of *GAPDH* gene as an endogenous control. The results are expressed as mean ±S.E.M.from three independent experiments (P<0.01, paired t test). ca

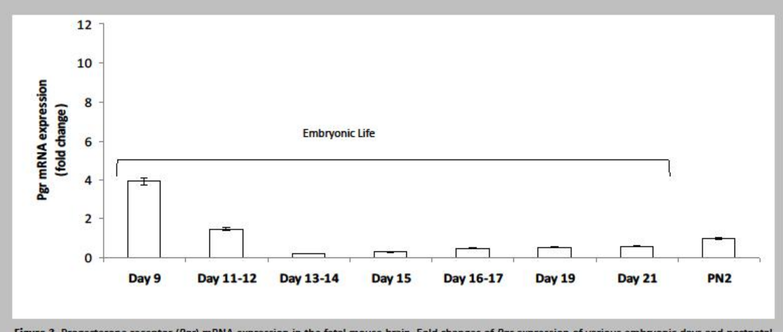


Figure 3. Progesterone receptor (*Pgr*) mRNA expression in the fetal mouse brain. Fold changes of *Pgr* expression of various embryonic days and postnatal day 2 is shown. Results were normalized to the expression levels of *GAPDH* gene as an endogenous control. The results are expressed as mean ±S.E.M.from three independent experiments (P<0.01, paired t test).

In the *Esr1*KO mice hypothalamic *Cyp19a1* mRNA expression increased almost two-fold, *Pgr* and *c-Jun* mRNA expression levels, however, remained at the similar levels compared to controls (Figure 4).

Cyp19a1, but not *Esr1* and *c-Jun*, mRNA expression decreased in the hypothalamus of castrated mice. Estradiol, on the other hand, had no affect on *Cyp19a1* mRNA expression in the hypothalamus of castrated mouse (Figure 5).

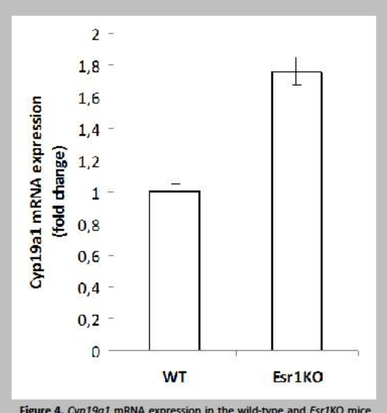


Figure 4. *Cyp19a1* mRNA expression in the wild-type and *Esr1*KO mice. Fold changes of *Cyp19a1* mRNA were normalized to the expression levels of *GAPDH* gene as an endogenous control. The results are expressed as mean ±S.E.M.from three independent experiments (P<0.01, paired t test).

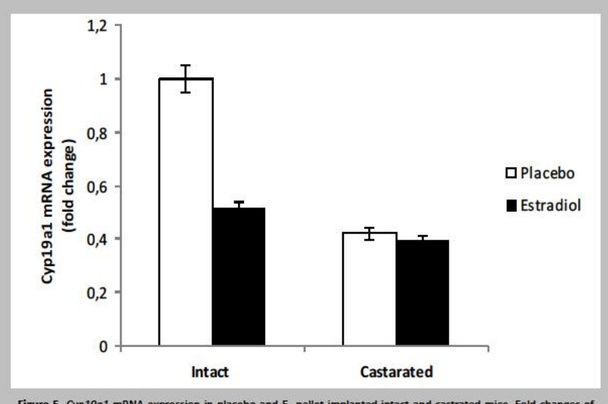


Figure 5. *Cyp19a1* mRNA expression in placebo and E_2 pellet implanted intact and castrated mice. Fold changes of *Cyp19a1* mRNA were normalized to the expression levels of *GAPDH* gene as an endogenous control. The results are expressed as mean ±S.E.M.from three independent experiments (P<0.01, paired t test).

CONCLUSİON

The effects of different steroids on *Cyp19a1* expression have been extensively studied. Estradiol has been reported to alternately regulate *Cyp19a1* mRNA and enzyme activity.[23,24] In accordance with these previous studies, we found that *Cyp19a1* mRNA expression decreased in the hypothalamus of castrated mice without any alterations in *Esr1* mRNA levels. Since DHT (5 alpha dihydrotestosterone), a non-aromatizable form of testosterone, has no effect on *Cyp19a1* mRNA expression in the hypothalamus of primates.[12,28,29] Our results might suggest that testosterone should be available in the circulation to induce its conversion into E_2 via aromatase in the brain. Interestingly, E_2 administration had no affect on *Cyp19a1* mRNA expression in the hypothalamus. Since the dual affects of E_2 on hypothalamic *Cyp19a1* mRNA expression requires high levels of E_2, it is possible that the required E_2 levels were not achieved by E_2 pellet implantation. It should also be kept in mind that in some cases DHT may act with E_2 synergistically to induce *Cyp19a1* mRNA and protein expression.[30,31] Therefore, it is possible to argue that testosterone is more prominent in males for mediating *Cyp19a1* mRNA and protein expression expression. Another aspect of sex steroid dependent regulation is that both brain and hypothalamus are largely composed of distinct cell populations with spatiotemporal steroid receptor expression patterns leading differential regulation via sex steroids at a given developmental stage and/or gender. [32,34] Taken together, our findings, which indicate that in vivo steroid receptors and aromatase expression patterns in the brain are closely linked to each other, might pave the way for gaining detailed understanding of physiologic and pathologic functions of aromatase in the brain.

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