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論　文　の　要　約

報告番号	甲　　第　　９７１号	氏　名	金谷　萌子
学位論文題目	Mechanism of Sex Steroid Action on Sexual Differentiation of Brain （脳の性分化における性ステロイドホルモンの作用機構に関する研究）		

論文の要約

Sexual differentiation of the brain is essential to achieve sexual reproduction for species survival. Studies performed for many decades revealed that there are three major contribution factors of sexual differentiation of the brain: sex chromosome gene expressing in the brain, testicular testosterone in developing period, and ovarian and testicular hormones during puberty. Of these factors, sex steroids have major role in sexual differentiation of the brain, because hormonal manipulation in rodents such as rats and mice in the perinatal period can be reversed the sex of the brain from the genetic sex. However, it is not clarified the detailed mechanisms, by which the brain is sexually differentiated under the influence of sex steroids in the perinatal and pubertal periods.

Sexually differentiated brain contains nuclei exhibiting morphological sex differences, which are termed sexually dimorphic nuclei (SDNs). SDNs have been identified in the brain of many species including rodents and primates, and they are considered to be a critical component of the neural systems regulating sex- or gender- biased physiological functions. In rodents, there is a female-biased SDN termed the anteroventral periventricular nucleus (AVPV) and a male-biased SDN termed the principal nucleus of the bed nucleus of the stria terminalis (BNSTp). The AVPV is larger and has more neurons in female mice. In contrast, the BNSTp is larger and has more neurons in male mice. In this dissertation, for better understanding of sexual differentiation of the brain, I aimed to determine the mechanisms of sex steroid actions on the formation of the AVPV and BNSTp in mice.

It is well known that ovarian estradiol acts in the brain via two types of nuclear receptors, estrogen receptor-α and -β (ERα and ERβ). Testicular testosterone acts via androgen receptor (AR). In addition to the direct action of testosterone via AR, testosterone can affect the brain by binding with ERs after it was locally converted into estradiol in the brain. It has been demonstrated that masculinization of the BNSTp in mice requires the gene expression of ERα and aromatase, but not ERβ. First, I examined the morphology of the AVPV in transgenic mice lacking aromatase, ERα, or ERβ gene to determine what genes are required for the sexual differentiation of the AVPV. I also examined the AVPV and BNSTp of AR knockout mice to determine the role of AR on the formation of the AVPV and BNSTp. As a result, defeminization of the AVPV in male mice was disrupted by deletion of the aromatase and ERα genes, but not by deletion of the ERβ and AR genes. The volume and neuron number of the AVPV in aromatase knockout and ERα knockout

male mice were significantly smaller than those in wild-type male mice. Masculinization of the BNSTp was disrupted by deletion of the AR gene, although deletion of the AR gene did not have any significant effect on defenimization of the AVPV. These findings suggest that defeminization of the AVPV involves the actions of aromatized testosterone binding with ERα. For masculinization of the BNSTp, direct actions of testosterone via AR are also required in addition to actions of aromatized testosterone binding with ERα.

Second, to investigate the mechanisms of sex steroid actions on the sexual differentiation of the AVPV and BNSTp during the perinatal period, the AVPV and BNSTp were isolated from the brain of wild-type mice on embryonic day 18 (ED18, ED1 = the day of vaginal plug confirmation) and on postnatal day 4 (PD4, PD0 = day of birth), and the mRNA levels of aromatase, ERα, ERβ, and AR in the AVPV and BNSTp were measured. Additionally, the mRNA levels of ARKO mice on ED18 and PD4 were measured to determine whether testosterone actions via AR modulate the mRNA levels. In the AVPV, the aromatase mRNA level was higher on ED18, while the ERα mRNA level was higher on PD4 without any effect of AR gene deletion and sex difference. AR mRNA in the BNSTp and AVPV of wild-type mice was not expressed on ED18 and emerged on PD4. Aromatase and ERα mRNA levels in the male BNSTp were significantly increased on PD4 by AR gene deletion, suggesting that testosterone signaling via AR during the postnatal period attenuates aromatized testosterone signaling via ERα.

Lastly, the mRNA levels of aromatase, ERα, ERβ, and AR in the AVPV and BNSTp of male and female mice from PD20 to PD60 were measured to investigate the mechanisms of sex steroid actions on the sexual differentiation of the AVPV and BNSTp during puberty. Form PD20 to PD60, the mRNA level of ERα in the AVPV was higher in female mice, while the mRNA level of ERβ in the AVPV was higher in male mice, although no sex difference in AR expression was found. The AVPV from PD20 to PD60 did not express aromatase in both sexes. In the BNSTp from PD20 to PD60, the mRNA levels of ERα and ERβ were significantly higher in female mice, while the mRNA level of AR was significantly higher in male mice. The BNSTp expressed aromatase from PD20 to PD60 without sex difference.

In conclusion, this study suggested that defeminization of the AVPV in male mice involves aromatized testosterone signaling via ERα during the perinatal period. Aromatized testosterone signaling via ERα during the perinatal period and testosterone signaling via AR during the puberty are presumably required for masculinization of the BNSTp. Thus, there are regional differences in sex steroid action on the formation of SDNs.