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GONADAL HORMONE INDEPENDENT SEX DIFFERENCES IN STEROIDOGENIC FACTOR 1 KNOCKOUT MICE BRAIN

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Summary

Sex differences in brain morphology have been described in a number of species including humans. Gonadal hormones were shown to provide a major influence on brain sexual differentiation more than 50 years ago. A growing number of studies is providing evidence for roles of genetic factors, in particular sex chromosome complement, on brain sexual differentiation in mammals. In this review, hormone-independent brain sexual differentiation, with the emphasis on mice with a disruption of the SF-1 gene (SF-1 knockout, SF-1 KO) are discussed.

Keywords

brain sexual differentiation; sex chromosomes; SF-1 KO mice; preoptic area and hypothalamus; neuronal NO synthase; calbindin D-28k; neuropeptide Y

Introduction

Permanent effect of sex hormones on sex specific brain development was clearly described in 1959 when Phoenix and coworkers reported the organizational effect of prenatal exposure to exogenous testosterone on brain function in adult guinea pig females (1). Today it is widely accepted that gonadal steroid hormones are the major factors that shape brain development and function in a sex specific manner.

Male and female cells differ in their genetic blue-print consisting of autosomal and sex chromosomes. However, the direct effects of genes located on specific chromosomes on brain sexual differentiation are difficult to study due to the multiple factors contributing to sex specific brain structure and function. In the last decade, development of specific mouse genetic models has energized studies of direct effects of genetic factors on brain sexual development.

Genetic factors in brain sexual development and function

Genes encoded on sex chromosomes are expressed in the brain where they could regulate expression of neural proteins, presumably in a sex specific manner. For example, *Sry* gene

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expression was found in tyrosine hydroxylase expressing neurons in the substantia nigra of adult mice and rats (2). However, the effects of genetic factors during brain sexual development are often masked by the actions of sex steroids.

One way to study genetic effects independently of steroid hormones is to study them in experimental systems before exposure to steroid hormones. In mice, gonadal primordia differentiate into testes or ovaries between embryonic day 10.5 and 12.5. Testes become steroidogenically active on embryonic day 13.0, while ovaries do not secrete steroid hormones until the first week after birth. Consequently, sex differences developed in the brain before gonadal differentiation must be caused by genetic factors. Genomic studies of brains from embryonic mice before or shortly after gonadal differentiation indeed found sex differences in gene expression of X and Y linked genes (3,4) suggesting that sex chromosomes could have a role in brain sexual differentiation during early embryonic development. A study by Carruth and co-workers (5) showed sex differences in the expression of tyrosine hydroxylase in mesencephalic tissue slices dissected from the fetal mouse brain before gonadal development. A different approach to study genetic influences on brain sexual development is using animal models that are exposed to the same gonadal steroid hormones but differ in sex chromosome complements. Such are four core genotype (FCG) mice, developed by translocation of the *Sry* gene onto the autosome together with the deletion of the *Sry* from the Y chromosome (6). Comparisons of XX and XY gonadal males and XX and XY gonadal females showed the effect of sex chromosome complement on density of fibers expressing arginine vasopressin in the lateral septum (7) and on some behavioral traits (8).

SF-1 KO mice; an animal model for studying gonadal hormone independent brain sexual differentiation

Steroidogenic factor 1 (SF-1) is a transcription factor that regulates expression of a plethora of genes involved in development and function of endocrine organs. In mice with disruption of the *SF-1* gene (SF-1 knockout, SF-1 KO) gonadal and adrenal primordia regress early during development (9). The absence of adrenal glands makes the SF-1 KO genotype lethal after birth. With adrenal transplantation, SF-1 KO mice can be kept alive into adulthood (10) and since these mice are not exposed to the endogenous gonadal steroids, they represent a useful animal model for studying genetic and hormonal contributions to brain sexual development independently. Studies of SF-1 KO mice in adulthood have identified some sex differences in brain morphology and behavior traits in which genetic factors, acting independently or in concert with gonadal hormones, are likely contributors (11,12).

The anteroventral periventricular nucleus (AVPV) in mice is sexually dimorphic for many traits such as volume, number of neurons (13) and in the size of chemically defined neuronal populations (e.g. number of cells expressing tyrosine hydroxylase or kisspeptin) (14,15). All of the sex differences in the AVPV can be manipulated by changing gonadal hormonal milieu, showing the effect of gonadal hormones. In our study (11) we found that the number of neurons expressing neuronal nitric oxide synthase (nNOS) was higher in wild type (WT) males in comparison to WT females. A similar sex difference was found in SF-1 KO mice and since SF-1 KO mice are not exposed to gonadal hormones this suggests gonadal hormone independent sexual differentiation. We also studied expression of nNOS in the medial preoptic area (POA) where sex differences in brain morphology have been described previously in various species (rev. in (16)). As in the AVPV, SF-1 KO mice males had higher nNOS immunoreactive area than females, similar to the sex difference in WT mice (11). The stands in stark contrast to the grouping of cells containing immunoreactive calbindin in the same POA, for which the sex difference found was completely hormone-dependent (found in WT, but not KO) (11).

The ventromedial hypothalamus (VMH) is involved in regulation of various behaviors and endocrine processes (17). Structure of the VMH is sexually dimorphic and most sex differences have been found to be gonadal hormone dependent. Studies of VMH sex differences in SF-1 KO mice are complicated by alterations in its cyto-architecture (18). Nevertheless, similar sex differences in the number of calbindin D-28k immunopositive cells were found in WT and SF-1 KO mice suggesting gonadal hormone independent sexual differentiation (11).

Neuropeptide Y (NPY) is an orexigenic metabolic peptide, highly expressed in the arcuate nucleus, from where NPY neurons project to the paraventricular nucleus (rev. in (19)). NPY is also expressed in many other brain areas including the lateral septum where its function is not yet known. There are suggestions that the lateral septum may have important role(s) in social/ affiliative behaviors or in the regulation of food intake, at least in female rats (20). Interestingly, we found gonadal hormone independent sex difference in NPY expression in the lateral septum, similarly to previously described sexual dimorphism in arginine vasopressin expression in this area (7).

Conclusions

It is widely accepted that gonadal steroid hormones are the major factor influencing sex-dependent brain development. However recent studies have indicated that some genes may also have effects on shaping the brain acting independently or together with gonadal hormones in synergistic or antagonistic manners during development. Initial studies with two mouse models, SF-1 KO and FCG mice have already revealed several sex differences that are likely dependent on sex chromosome gene complement, and future studies with these and other models will undoubtedly further reveal an interplay between sex hormones and genetic factors in shaping male or female brain structure and function.

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