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Sex chromosome complement determines sex differences in aromatase expression and regulation in the stria terminalis and anterior amygdala of the developing mouse brain

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ABSTRACT

Aromatase, which converts testosterone in estradiol, is involved in the generation of brain sex dimorphisms. Here we used the "four core genotypes" mouse model, in which the effect of gonadal sex and sex chromosome complement is dissociated, to determine if sex chromosomes influence the expression of brain aromatase. The brain of 16 days old XY mouse embryos showed higher aromatase expression in the stria terminalis and the anterior amygdaloid area than the brain of XX embryos, independent of gonadal sex. Furthermore, estradiol or dihydrotestosterone increased aromatase expression in cultures of anterior amygdala neurons derived from XX embryos, but not in those derived from XY embryos. This effect was also independent of gonadal sex. The expression of other steroidogenic molecules, estrogen receptor-α and androgen receptor was not influenced by sex chromosomes. In conclusion, sex chromosomes determine sex dimorphisms in aromatase expression and regulation in the developing mouse brain.

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1. Introduction

The classical hypothesis for the sexual differentiation of the rodent brain postulates that the fetal testis produces testosterone at critical moments of development and then, this hormone can act as an organizing agent after its conversion to 17β-estradiol (E2) by the enzyme cytochrome P450 aromatase (MacLusky and Naftolin, 1981). The expression pattern of aromatase is restricted to discrete regions of the central nervous system, according to in situ hybridization and immunohistochemical studies (Lauber et al., 1997; Shinoda et al., 1994; Tsuruo et al., 1994). Several studies demonstrated that during the critical period of sexual differentiation there are sex differences in aromatase expression that are time- and regionally specific (Lauber et al., 1997). Most of the studies focused on the expression in sexually dimorphic brain areas, such as the hypothalamus and the preoptic area. During brain differentiation aromatase mRNA in the hypothalamus increases gradually to reach peaks shortly before and after birth in rats (Colciago et al., 2005; Lephart et al., 1992) and mice (Harada and Yamada, 1992). In the bed nucleus of the stria terminalis and the sexually dimorphic nucleus of the preoptic area some sex differences were found with a higher expression of aromatase mRNA in male rats at P2; and later in development, at P6, the sex differences only remained in the bed nucleus of the stria terminalis (Lauber et al., 1997). Some of the sex differences in aromatase expression could not be explained by organizational actions of gonadal hormones. For instance, a higher expression and activity of aromatase was observed in neurons and hypothalamic regions in male mouse and rat brain at E16 (Beyer et al., 1994b, 1993; Colciago et al., 2005; Negri-Cesi et al., 2001). These differences can not be attributed to

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Abbreviations

AR	androgen receptor
Cyp11a1	cytochrome P450, family 11, subfamily a, polypeptide 1; also known as P450scc
Cyp19a1	cytochrome P450, family 19, subfamily a, polypeptide 1; also known as aromatase
DHT	dihydrotestosterone
E2	17β-estradiol
ER	estrogen receptor
ER-α	ER alpha
Esr1	estrogen receptor 1; also known as ER-α
FCG	four core genotypes
Gapdh	glyceraldehyde-3-phosphate dehydrogenase
P450scc	cholesterol side-chain cleavage enzyme
Srd5a1	steroid 5 alpha-reductase 1
Srd5a2	steroid 5 alpha-reductase 2
StAR	steroidogenic acute regulatory protein

the peak of testosterone production by the fetal testis, which in mice is at E17-18 (O'Shaughnessy et al., 2006, 1998) and in rats at E18.5–19.5 (Huhtaniemi, 1994; Scott et al., 2009). However, it is not possible to complete exclude any effect of hormones derived from the gonads at or before the embryonic age used.

Testosterone can also be metabolized to the most potent androgen dihydrotestosterone (DHT) by the enzyme 5α-reductase. Two different 5α-reductase isoenzymes, type I and II, catalyze the conversion of testosterone into DHT (Celotti et al., 1997, 1992). The mRNA for the 5α-reductase type I isoenzyme is constitutively expressed in the rat brain at all stages of development, whereas the mRNA for the 5α-reductase type II isoenzyme is detected at E18, peaks at P2 and then decreases gradually to low levels in adults (Poletti et al., 1998). DHT synthesized in the brain also exerts organizational actions on selected nuclei and it is involved in sexual differentiation of specific brain regions and behaviors (Bodo and Rissman, 2008). In this context, brain masculinization can be exerted via estrogen receptors (ERs) in some brain regions and via the formation of DHT and the activation of androgen receptor (AR) in others.

ERs and AR are critical for the organizational actions of steroids during brain development and their expression overlaps with the expression of aromatase (Simerly et al., 1990). AR mRNA is detected in the hypothalamus and preoptic area of mouse brain from E14 and its expression is sexually dimorphic between E15–E16 (Young and Chang, 1998). ER-α mRNA is detected from E18 in the striohypothalamic nucleus, the caudal portion of the ventromedial hypothalamic nucleus, the bed nucleus of the stria terminalis, the caudal arcuate nucleus and the medial and cortical nuclei of the amygdala in male and female rats (DonCarlos, 1996). In addition, the expression of ER-α and ER-β in subregions of the amygdala, including the posterodorsal part of the medial amygdaloid nucleus, the posterior cortical nucleus, and the amygdalohippocampal area, has been reported in early postnatal rats (Cao and Patisaul, 2013).

The vast bibliography regarding sex dimorphic expression and regulation of aromatase in the brain is based on studies in the hypothalamus and the preoptic area (Beyer et al., 1994b; Hutchinson et al., 1997, 1999; Negri-Cesi et al., 2001) and very little is known about factors that control aromatase expression during development in other brain regions with high aromatase levels, such as stria terminalis and amygdala regions. The regions of interest for the present study were the medial preopticoamygdaloid neuronal arc that involves areas known to be sexually dimorphic and sensitive to organizational actions of steroid hormones (Forger et al., 2004; Morris et al., 2008). Taking into account that recent findings suggest that in addition of gonadal hormones, the sex chromosome complement is also involved in the generation of specific traits in the brain (Arnold, 2009; Scerbo et al., 2014) in this study we have tested the hypothesis that sex chromosome complement influences the expression of aromatase in the stria terminalis and amygdala regions. With this aim, the study was carried out in E15-16 mouse embryos (i.e., before the testosterone surge) of the "four core genotypes" (FCG) model, which combines a deletion of the testis-determining gene Sry from the Y chromosome (Y^-) with the subsequent insertion of a Sry transgene onto an autosome (Lovell-Badge and Robertson, 1990; Mahadevaiah et al., 1998). The Sry gene deletion in XY mice (XY^-) yields in a female phenotype (ovaries). When the Sry transgene is inserted into an autosome of these mice they have testes and are fully fertile (XY^-*Sry*). The Y^- chromosome and the Sry transgene segregates independently, thus, four types of offspring are produced by breeding XY^-*Sry* males to XX females: XX and XY^- females (without Sry on the Y chromosome) and XX*Sry* and XY^-*Sry* male mice (both with *Sry* in an autosome). By comparing these genotypes, it is feasible to segregate the role of a) sex chromosome complement (comparing mice with the same gonadal type but with different sex chromosomes: XX *vs*. XY) b) gonadal phenotype (males *vs*. females regardless of the sex chromosome complement) and c) their interaction (Arnold and Chen, 2009). Throughout the text, we will refer to XX and XY^- as XX and XY females (XXF and XYF), and to XXSry and XY^-Sry as XX and XY male (XXM and XYM), respectively. The study was complemented with the analysis of other steroidogenic molecules and steroid receptors in the same brain regions. Thus, we also assessed the expression of ER-α, AR, 5α-reductase, steroidogenic acute regulatory protein (StAR) and cholesterol side-chain cleavage enzyme (P450scc or *Cyp11a1*). To determine the impact of sex chromosome complement on the regulation of aromatase by E2 and DHT we have used primary cultures of amygdala neurons obtained from embryos of the FCG mouse model.

2. Materials and methods

2.1. Animals

The embryos used for this study were obtained from MF1 "four core genotypes" mice born and reared in the Instituto Ferreyra (Córdoba, Argentina). The day of vaginal plug was defined as E1. All experimental protocols were approved by the appropriate animal care and use committees at our institute and followed the National Institutes of Health guidelines for the care and use of laboratory animals.

2.2. Genotyping

Genotyping of FCG was performed on genomic DNA samples of E15-16 mouse embryos separated by sex. Male fetuses were identified under a dissecting microscope by the presence of the spermatic artery on the developing gonad. PCR was performed for the *Sry* transgene [primers SryF (forward): CTA CAC AGA GAG AAA TAC CCA AAC; SryR (reverse): GTC TTG CCT GTA TGT GAT GG] (Gubbay et al., 1990) and the Y long-arm gene family Ssty [primers SstyF (forward): CTG GAG CTC TAC AGT GAT GA; SstyR (reverse): CAG TTA CCA ATC AAC ACA TCA C] (Turner et al., 2000). The autosomal gene Myogenin [primers MYOF (forward): TTA CGT CCA TCG TGG ACA GCA T; MYOR (reverse): TGG GCT GGG TGT TAG TCT TAT] served as an amplification control (Palaszynski et al., 2005) yielding a 245-bp product in all genotypes. Amplification of DNA yielded the following products according to the genotypes: for XY males (XYM)

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