Role of sex chromosome complement in the regulation of aromatase expression in developing mice brain

Carla D. Cisternas[1,2], María Ángeles Arévalo[3], Luis Miguel Garcia-Segura[3], María Julia Cambiasso[1,2]

[1]Instituto de Investigación Médica Mercedes y Martín Ferreyra INIMEC-CONICET-UNC. Córdoba, Argentina.
[2]Departamento de Biología Bucal, Facultad de Odontología, Universidad Nacional de Córdoba, Córdoba, Argentina.
[3]Instituto Cajal, CSIC. Madrid, España. cdcisternas2@gmail.com

INTRODUCTION

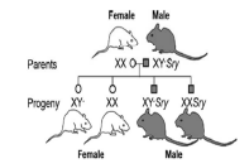
During the critical period of sexual differentiation there are sex differences in brain aromatase expression that are time and regionally specific. Some of these sex differences cannot be explained by organizational actions of gonadal hormones because they occur before exposition to testosterone *in utero*. Previous results from our group using the four core genotype mouse model (FCG) demonstrate that XY neurons from amygdala express higher levels of aromatase and Cyp19a1 than XX neurons of E15 mice independent of gonadal sex. The present study explores the regulation of aromatase in amygdala neurons from E15 mice brain and the role of estrogen (ERα and ERβ) and androgen receptors (AR) in this regulation.

METHODS

The embryos used for this study were obtained from CD1 mice raised in the Cajal Institute (Madrid, Spain) and MF1 four core genotypes (FCG) mice born and reared in the Ferreyra Institute (Córdoba, Argentina).

Amygdala neurons were obtained from E15 mouse embryos. Cells were cultured separately according to the sex and genotype of fetal donors.

Four Core Genotype Model



Gene expression:

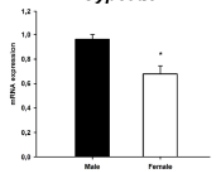
At 3 DIV neurons were treated for 2 h according to the experiment: 17β-estradiol (E2, 10^{-10} M), dihydrotestosterone (DHT, 10^{-10} M), 3β-diol (10^{-10} M), ERα agonist (PPT, 10^{-7} M), a ligand of G protein receptor 30 / G protein-coupled ER (G1, 10^{-7} M), ERβ agonist (DPN, 10^{-9} M), a selective ERβ antagonist (PHTPP, 10^{-7} M) or an anti-androgen (Flutamide, 10^{-7} M).

RNA were extracted and cDNA obtained by reverse transcription. Real-time PCRs were performed using the Sybr Green PCR Master Mix. Relative expression of aromatase mRNA (*Cyp19a1*) and Erβ (*Esr2*) was calculated using the ΔΔCT method.

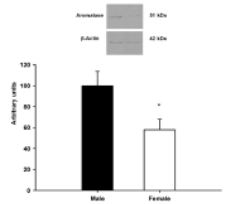
Protein expression:

At 3 DIV neurons were treated for 2 h either with E2 (10^{-10} M) or DHT (10^{-10} M). Homogenates of amygdala neurons raised in medium with/out E2 or DHT were prepared. Protein samples (20 ug/lane) were separated by 10% SDS-PAGE and transferred onto PVDF membranes. Samples were incubated over night with a rabbit polyclonal antibody to aromatase (diluted 1:1000) generated against a 15 amino acid–peptide corresponding to residues 488–502 of mouse aromatase. Specificity of this antibody has been previously described and cross reacts with rat, human and monkey cytochrome P450 aromatase (Garcia-Segura 1999, Ghorbanpoor et al, 2015).

1 *Cyp19a1*

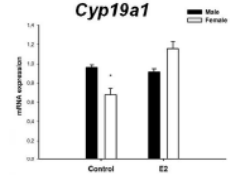


Aromatase

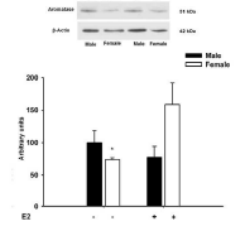


Male neuronal cultures expressed higher levels of aromatase (mRNA and protein) than female cultures (*p<0,05). Data are mean ± SEM. n = 4 - 6 independent cultures.

2 *Cyp19a1*

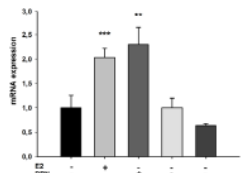


Aromatase

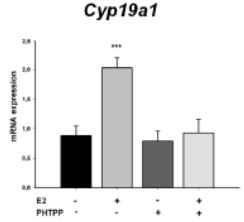


E2 increased *Cyp19a1* and aromatase expression only in female neuronal cultures (*p<0,05). Data are mean ± SEM. n = 4 - 6 independent cultures.

3 *Cyp19a1*

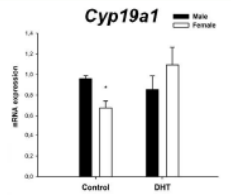


Cyp19a1

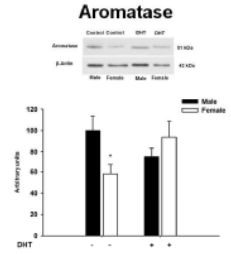


ERβ agonist DPN imitated the effect of E2 on *Cyp19a1* expression in amygdala neuronal cultures of female embryos and PHTPP (ERβ antagonist) prevented these effect. **p<0,01, ***p<0,001. Data are mean ± SEM. n = 4 - 6 independent cultures.

4 *Cyp19a1*

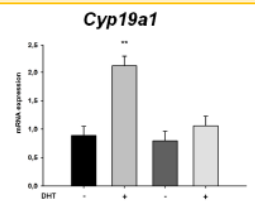


Aromatase

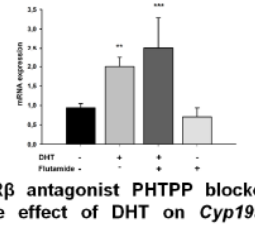


DHT increased *Cyp19a1* and aromatase expression only in female neuronal cultures (*p<0,05). Data are mean ± SEM. n = 4 - 6 independent cultures.

5 *Cyp19a1*

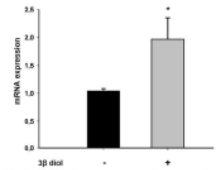


Cyp19a1



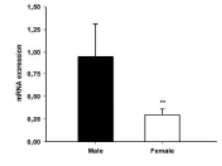
ERβ antagonist PHTPP blocked the effect of DHT on *Cyp19a1* expression while Flutamide (antiandrogen) was not able to prevent these effect in female amygdala neurons at E15. **p<0,01, ***p<0,001. Data are mean ± SEM. n = 4 - 6 independent cultures.

6 *Cyp19a1*



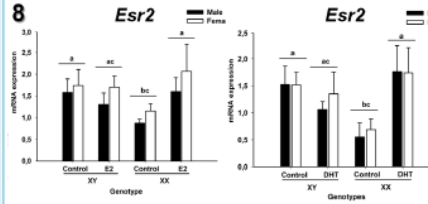
3β-diol increased *Cyp19a1* expression in female amygdala neurons at E15. *p<0,05. Data are mean ± SEM. n = 4 - 6 independent cultures.

7 *Esr2*



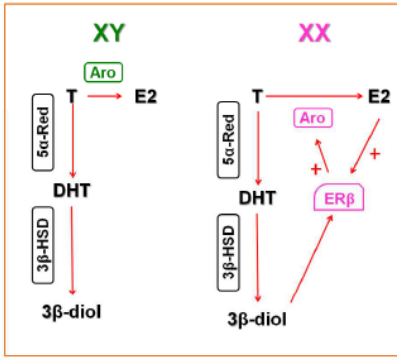
Male neurons expressed higher levels of ERβ mRNA (*Esr2*) in amygdala neurons of E15 embryos. **p<0,01. Data are mean ± SEM. n = 4 - 6 independent cultures.

8 *Esr2* *Esr2*



E2 and DHT increased *Esr2* expression in amygdala neurons of the Four Core Genoty Mouse Model at E15. Different letters indicate signifi differences with p < 0.05. Data are mean ± SEM. n= independent cultures for each genotype and treatment.

CONCLUSIONS



Sex chromosome complement determines expression and regulation of aromatase amygdala neurons of developing mouse brain XX neurons the sex differences in the express of aromatase and ERβ are compensated by effect of E2 and 3β-diol in amygdala neurons .

Given that aromatase is a key enzyme necess for organizational actions of gona testosterone these findings imply that gen and gonadal factors interact in the generation sex differences in some structures of developing rodent brain.