Available online at www.sciencedirect.com

SciVerse ScienceDirect

www.elsevier.com/locate/brainres

BRAIN RESEARCH

Research Report

Changes in estrogen receptor-alpha mRNA in the mouse cortex during development

*Amanda K. Prewitt, Melinda E. Wilson**

Department of Physiology, University of Kentucky College of Medicine, 800 Rose Street, Lexington, KY 40536, USA

ARTICLE INFO

Article history:
Accepted 28 November 2006
Available online 17 January 2007

Keywords:
Estrogen receptor
Cortex
mRNA
Mouse
Development

ABSTRACT

Estrogen plays a critical role in brain development and is responsible for generating sex differences in cognition and emotion. Studies in rodent models have shown high levels of estrogen binding in non-reproductive areas of the brain during development, including the cortex and hippocampus, yet binding is diminished in the same areas of the adult brain. These binding studies demonstrated that estrogen receptors decline in the cortex during development but did not identify which of the two estrogen receptors was present. In the current study, we examined the expression of estrogen receptor alpha (ERα) and estrogen receptor beta (ERβ) in the mouse cortex during the first month of life. Messenger RNA was isolated from cortical tissue taken from C57BL/6 mice on postnatal day (PND) 1, 4, 10, 18 and 25 and expression levels were determined by real-time PCR. ERα mRNA expression in the mouse cortex at PND 25 was significantly reduced as compared to PND 1 ($p<0.01$). ERβ mRNA expression at PND 25 was significantly increased as compared to PND 1 ($p<0.05$). Although the increase in ERβ mRNA was statistically significant, the ERβ levels were extremely low in the isocortex compared to ERα mRNA levels, suggesting that ERα may play a more critical role in the developmental decrease of estradiol binding than ERβ. Additionally, we measured ERα mRNA expression in organotypic explant cultures of cortex taken from PND 3 mice. Explants were maintained *in vitro* for 3 weeks. mRNA was isolated at several time points and ERα and ERβ mRNA was measured by real-time RT–PCR. ERα and ERβ mRNA levels reflected a similar pattern *in vitro* and *in vivo*, suggesting that signals outside the cortex are not needed for this developmental change. This study lays the groundwork for an understanding of the mechanisms of the developmental regulation of ERα mRNA.

1. Introduction

While estrogen is often thought of as being primarily involved in controlling various aspects of reproduction, it also performs a vital role in regulating many aspects of brain function. Estrogen has been implicated in several non-reproductive brain functions, which include involvement in memory and learning (Sherwin et al., 2003), the maintenance of dendritic spine density in the hippocampus (Li et al., 2004; Woolley et al., 1993), modulation of mood (Amin et al., 2005), and protection against brain injury (Azcoitia et al., 1999; Dubal et al., 1998; Goodman et al., 1996; Wilson et al., 2000). Recent studies in postmenopausal humans and aged rats, however, suggest that estrogen may have complex and contradictory actions in the brain in terms of neuroprotection (Espeland et al., 2004; Sohrabji and Bake, 2006).

* *Corresponding author*. Fax: +1 859 323 1070.
E-mail address: melinda.wilson@uky.edu (M.E. Wilson).

0006-8993/$ – see front matter © 2006 Elsevier B.V. All rights reserved.
doi:10.1016/j.brainres.2006.11.069

Sex differences in structure and function of the brain are observed throughout the animal kingdom (MacLusky et al., 1997). A complex cascade of events is initiated by gonadal hormones and results in an extensive array of effects associated with sexual differentiation of the brain. Exposure to estrogen during development is believed to result in the development of a phenotypic male brain. The absence of such a hormone milieu results in a shift in development towards a phenotypic female brain (MacLusky and Naftolin, 1981). In addition to generating sex differences in structural changes in the brain, estrogen is also responsible for the generation of sex differences in cognition and emotion (Arnold and Breedlove, 1985; Berman et al., 1997; Fink et al., 1994; McEwen et al., 1975).

Receptor binding studies have demonstrated that early in postnatal life of male and female rodents, estrogen binds not only in reproductive areas of the brain, such as the hypothalamus, but also shows a unique binding pattern in the cortex (Shughrue et al., 1990; Stumpf and Sar, 1976). In early postnatal development, there are high levels of estradiol binding in the cortex and hippocampus and as the animal approaches puberty, this diminishes. In rats, ERα mRNA expression was shown to correlate with the changes in estrogen binding within specific brain regions, including the hippocampus (Miranda and Toran-Allerand, 1992; O'Keefe et al., 1995; Shughrue et al., 1997; Toran-Allerand et al., 1992). The expression of ERα or ERβ mRNA in the mouse cortex during postnatal development has not been investigated. In the present study we have determined the developmental changes in ERα mRNA and ERβ mRNA in mice and have begun to investigate potential mechanisms of this developmental regulation.

2. Results

2.1. *ERα mRNA levels decrease during development in the male and female mouse cortex*

Male and female C57BL/6 mice were killed on postnatal days (PND) 1, 4, 10, 18 and 25, with day of birth defined as PND 0. Total RNA was isolated from isocortex and reverse transcribed using oligo-dT primers. The resulting cDNA was subjected to quantitative real-time PCR using ERα-specific primers. These primers have previously been shown to produce a single PCR product corresponding to the DNA binding region of the ERα gene (Kuiper et al., 1997; Dubal et al., 1999). Histone 3.1 was included as a housekeeping control gene and all data normalized to its expression as previously described (Wilson and Handa, 1997). ERα mRNA levels significantly declined by PND 10 in male mice (Fig. 1). There was a tenfold decrease in ERα mRNA expression in the male between PND 1 and PND 25 ($F_{[4,19]}=43.82$, $p<0.001$). Females also demonstrated a ten-fold decrease in ERα mRNA expression between PND 1 and PND 25 ($F_{[4,19]}=87.61$, $p<0.001$). There was no statistically significant effect of sex ($p=0.708$).

2.2. *ERβ mRNA levels increase during development in the mouse cortex.*

To quantify ERβ mRNA expression, C57BL/6 male and female mice were killed on postnatal days (PND) 1, 4, 10, 18 and 25, with day of birth defined as PND 0. Real time PCR for ERβ was performed as described for ERα with primers specific to ligand binding domain of the ERβ gene (Dubal et al., 1999; Kuiper et al., 1997). ERβ mRNA levels increased in the cortex of the developing male mouse ($F_{[4,19]}=12.40$, $p=0.039$) (Fig. 2). A similar increase was observed in females ($F_{[4,19]}=7.644$, $p=0.0009$). There was no statistically significant difference in ERβ mRNA expression between male and female mice ($p=0.88$).

2.3. *ERα mRNA levels decrease over developmental time points in organotypic explant cultures of the cortex*

To determine if the changes in estrogen receptor expression occur in the absence of inputs from other regions of the brain, ERα and ERβ mRNA expression were examined in organotypic explant cultures. Organotypic explant slices contain both neurons and glia and maintain local cytoarchitecture yet are independent of synaptic input from other regions of the brain.

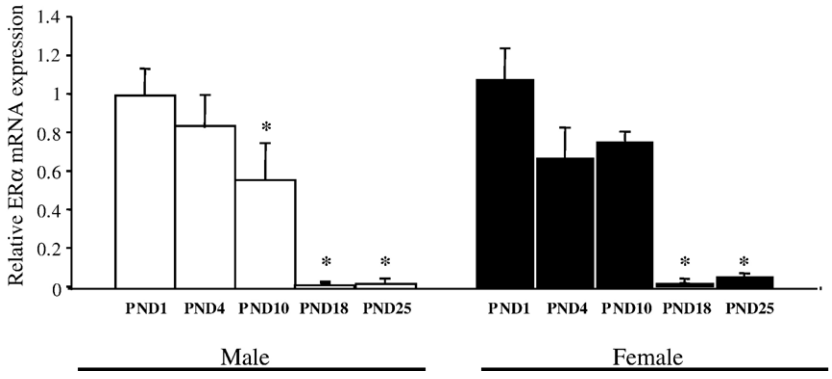

Fig. 1 – ERα mRNA expression in mouse cortex decreases during development in both male and female mice. Quantitative real-time PCR was performed on RNA isolated from PND 1, PND 4, PND 10, PND 18 and PND 25 mouse cortex. Data was normalized to the housekeeping gene Histone 3.1 and expressed relative to PND 1. Bars represent the mean ± SEM, $n=4$. *Significantly different from PND 1.