Estrogen receptor α is essential for induction of oxytocin receptor by estrogen

Larry J. Young, Xiaoxin Wang, Ross Dellarca, and Emilie F. Rissman

Department of Psychiatry and Behavioral Sciences, Emory University School of Medicine, Atlanta, GA 30322; Department of Biology, University of Virginia, Charlottesville, VA 22903. JSA

Corresponding Author

Introduction

Oxytocin receptors (OTR) in the brain and periphery are exquisitely sensitive to gonadal steroids. Estradiol treatment in rats results in a seven-fold increase in OTR mRNA in the brain, pituitary, and uterus. The induction of OTR in the brain is thought to be crucial for the expression of female sexual and material behavior, while the induction of OTR in the uterus is thought to be involved in the control of parturition.

The classic view of estrogen action involves activation of the estrogen receptor (ERα), a ligand-dependent transcription factor located in the cell nucleus, which mediates gene transcription through its interaction with specific DNA sequences called estrogen response elements (EREs), usually located in the 5' flanking region of the gene. Although a full palindrome and several half palindrome EREs have been identified on the rat and mouse OTR genes, the molecular mechanisms resulting in the induction by estrogen remain unclear. In vitro transcription analysis of the rat OTR promoter in cells containing ER has failed to demonstrate estrogen sensitivity to estrogen.

Evidence of non-genomic mechanisms of estrogen action, and particularly the recent discovery of a second estrogen receptor (ERβ), has raised new questions regarding the mechanism of estrogen action for specific physiological processes. We therefore used ERα-deficient (ERα−/−) mice to determine whether ERα is necessary for the induction of OTR in the coaxial nervous system.

Materials and Methods

Animals: Mice were of mixed 129/J and C57BL/6J background. They were housed on 12:12 h light/dark cycle (lights off at 12.00 h EDT) in the laboratory in Virginia. Each animal was housed individually until mating (18 days of age) and had ad lib access to food and water. Subjects were generated by crossing heterozygous mating pairs carrying a single copy of the disrupted Erα gene. The resulting offspring were screened by PCR amplification of full DNA.

Twelve homozygous Erα-deficient (−/−) and twelve wild type (+/−) litter mates (six of each sex for each genotype) were used in the study.

Surgery and hormone treatments: All animals were gonadectomized under general anesthesia (20 mg/ml ketamine and 2 mg/ml xylazine; 0.1 ml/25 g body weight) as adults. One month after surgery, animals received a sc. saline implant (1.18 mm x 1.18 mm; 1.7 mm o.d.) filled with estradiol (E2) dissolved in sesame oil (50 μg in 0.225 ml) or empty.

Receptor autoradiography: Twelve days following implantation, animals were sacrificed by decapitation.
while under general anesthesia, brains were removed, frozen on crushed dry ice and shipped to Emory. Due to a problem in shipping, the brains thawed but remained cool and were frozen on dry ice. A second, smaller set of brains, which were not thawed, were run in parallel to determine whether thawing affected the binding. Both sets of brains gave similar binding intensities and treatment effects, demonstrating that the brief thawing did not affect the assay. Only the larger set of brains which had thawed were used in the statistical analysis. The brains were sectioned at 20 μm and thaw mounted on Super- frost plus slides (Fisher). Receptor autoradiography was performed using [3H]d(CH2)3Tyr(Me)2Tyr- NH2/JOVT (NEN; [3H]JOT) as previously described for the mouse10 with the modification that the sections were lightly fixed in 5.1% paraformaldehyde for 2 min prior to the assay. After drying under a stream of cool air, the slides were exposed to BioMax MR film (Kodak) for 48 h.

Data acquisition and analysis: Film autoradiograms were analyzed using a Macintosh computer using the public domain NIH Image program (developed at the US National Institutes of Health and available on the Internet at http://rsb.info.nih.gov/nih-image/). Optical densities were converted to d.p.m./mg tissue equivalents using 3H-autoradiographic standards (Amersham). Specific binding was calculated by subtracting non-specific binding, measured from an adjacent area containing no receptor, from the total binding for each area. All sections were coded to obscure the identity of the tissue and each region of interest was measured bilaterally from at least two sections for each animal. OTR binding was quantified in the lateral septum, claustrum, and the basolateral and cortical nuclei of the amygdala. Cortical OTR binding in a region of the neocortex just adjacent to claustrum near the area of the rhinal sulcus was also quantified. Other areas of the neocortex were not quantified. Although OTR binding is also found in the ventromedial nucleus of the hypothalamus (VMH), binding in this area was not quantified because of slight tissue damage in this region of some of the slides. Earlier studies have demonstrated that unlike in the rat, OTR binding in this area in mice is not increased by estrogen and therefore the presence of this data does not detract from the significance of the remainder of the data. Neuroanatomical nomenclature was taken from an albino mouse forebrain atlas.11 One-way ANOVA, followed by Fisher's least significant difference post hoc test, where appropriate (p < 0.05), was used to evaluate differences in OTR binding. Since there were no obvious sex differences in binding, data from males and females were combined in the statistical analysis.

Results
In wild-type animals of both sexes, estrogen treatment resulted in significant increases in [3H]JOTA binding in the claustrum (p < 0.001), a region of the neocortex just adjacent to claustrum near the area of the rhinal sulcus (Figs 1, 3, p < 0.001), the basolateral (p < 0.001) and the cortical nuclei of the amygdala (Figs 2, p < 0.001), compared with untreated, gonadectomized animals. Binding in the lateral septum (LS) was slightly increased (Fig. 3, p < 0.05), although to a much lesser extent. [3H]JOTA binding in the CA3 region of the hippocampus was unaffected by E treatment (Fig. 3). Basal levels of OTR density and distribution were identical in control wild-type and homozygous ERO-deficient mice. However, Eβ treatment in the homozygous knockout mice was completely ineffective at altering receptor binding in any region affected in the wildtype mice. No sex differences in OTR binding or regulation by estrogen were evident in either genotype.

Discussion
Our results demonstrate that, as in the rat, OTR binding in the mouse brain is increased several-fold in response to estrogen treatment, although with different region specificity. Furthermore, ERα is essential for this process. The estrogenic induction of OTR binding was completely abolished in mice lacking ERα. ERα is not required, however, for basal
OTR expression in estrogen sensitive brain regions, since OTR binding was similar in untreated wild-type and ERα-deficient mice. These results are in contrast to data on progesterone receptors (PR) mRNA. When ERα-deficient mice are treated with E, PR mRNA increased in several brain regions, although to a lesser extent than in wild-type animals.  

The regulation of central OTR binding and gene expression in the brain by gonadal steroids has been well characterized in the rat. As noted before, the neuroanatomical pattern of OTR binding in the mouse brain is quite different from that of the rat. The regulation of the mouse OTR binding by gonadal steroids has been investigated previously. In males, castration decreased and testosterone treatment increased OTR density in the LS and medial nucleus of the amygdala, while having no effect in the cortex. In contrast to our present results, an earlier study found that estrogen increased OTR density only in the LS and had no effect in the amygdala or cortex. This discrepancy may be explained by the method of hormone administration (i.e., two daily s.c. injections vs. 12 days of Silastic implants). Perhaps extended periods of elevated estrogen are necessary for the maximum increase in OTR density in these areas.
Conclusion

As in the rat, estrogen treatment increases OTR binding several-fold in specific brain regions of the mouse. These are multiple possible mechanisms which could be responsible for this induction of OTR binding, including those involving ERO, ERB, induction of CAMP and membrane receptors. The present results in mice genetically deficient in ERO clearly demonstrate that ERO is absolutely essential for the several-fold induction of GTR binding in the brain.

References

1.... (full list continues, covering a range of references)

Received 19 November 1997; accepted 16 January 1998