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

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THE induction of oxytocin receptor (OTR) synthesis in the periphery and in the brain by estrogen is critical for reproductive success. Oxytocin receptors are involved in the control of parturition, milk ejection, and sexual and maternal behaviors. The discovery of a second estrogen receptor (ERβ) in the brain and the failure of *in vitro* transcription studies using OTR promoter constructs to replicate the *in vivo* transcriptional regulation have raised questions regarding the molecular mechanisms involved in the regulation of the OTR gene by estrogen. Using mice genetically deficient in estrogen receptor α (ERα), we demonstrate that ERα is not necessary for basal OTR synthesis, but is absolutely necessary for the induction of OTR binding in the brain by estrogen. *NeuroReport* 9: 933–936 © 1998 Rapid Science Ltd.

Key words: Estrogen receptor; Gonadal steroids; Oxytocin receptor

Introduction

Oxytocin receptors (OTR) in the brain and periphery are exquisitely sensitive to gonadal steroids. Estradiol treatment in rats results in a several-fold increase in OTR mRNA in the brain,[1,2] pituitary[2] and uterus.[2,3] The induction of OTR in the brain is thought to be crucial for the expression of female sexual and maternal behavior,[4,5] while the induction of OTR in the uterus is thought to be involved in the control of parturition.[6]

The classic view of estrogen action involves activation of the estrogen receptor (ERα), a ligand-dependent transcription factor located in the cell nucleus, which modulates gene transcription through its interaction with specific DNA sequences, termed estrogen response elements (EREs), usually located in the 5′ flanking region of the gene.[7] Although a full palindrome and several half palindrome EREs have been identified on the rat[8] and mouse OTR gene,[9] 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 robust sensitivity to estrogen.[8] Evidence of non-genomic mechanisms of estrogen action,[10,11] and particularly the recent discovery of a second estrogen receptor (ERβ),[12] 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 central 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 13.00 h EDT) in the laboratory in Virginia. Each animal was housed individually at weaning (18 days of age) and had *ad lib* access to food and water. Subjects were generated by crossing heterozygotic mating pairs carrying a single copy of the disrupted ERα gene.[13] The resulting offspring were screened by PCR amplification of tail 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 treatment: All animals were gonadectomized under general anesthesia (20 mg/ml ketamine and 2 mg/ml xylazine; 0.1 ml/20 g body weight) as adults. One month after surgery, animals received a s.c. Silastic implant (1.98 mm i.d.; 3.17 mm o.d.) filled with estradiol (E) dissolved in sesame oil (50 μg in 0.025 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 refrozen 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 effect 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 Superfrost plus slides (Fisher). Receptor autoradiography was performed using [^{125}I]d(CH_2)$_5$[Tyr(Me)$_2$,Tyr-NH_2^9]OVT (NEN; [^{125}I]OTA) as previously described for the mouse[14] with the modification that the sections were lightly fixed in 0.1% 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 ^{125}I-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 absence of this data does not detract from the significance the remainder of the data. Neuroanatomical nomenclature was taken from an albino mouse forebrain atlas.[15] 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 [^{125}I]OTA 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 2,3; $p < 0.001$), 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. [^{125}I]OTA 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 ERα-deficient mice. However, EB 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

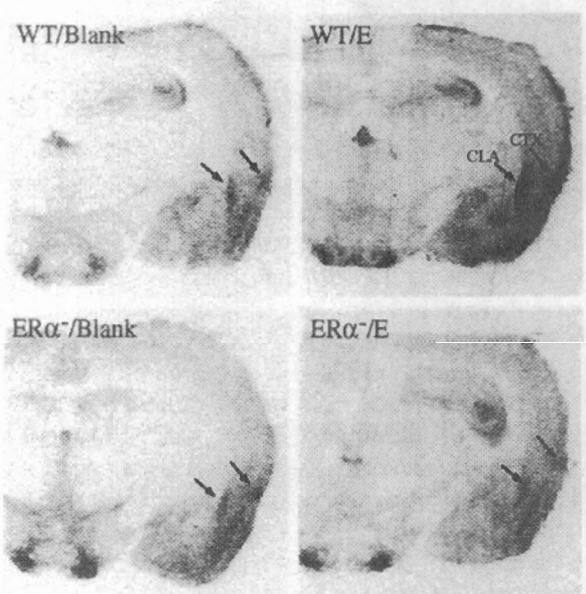

FIG. 1. Photomicrographs illustrating the OTR receptor binding in the claustrum (CLA) and neocortex (CTX) of female wild-type (top panels) and ERα-deficient (ERα⁻) mice (bottom panels). Animals in the left panels received an empty implant while those in the right received an implant containing estrogen. Arrows indicate regions analyzed in the quantitative analysis.

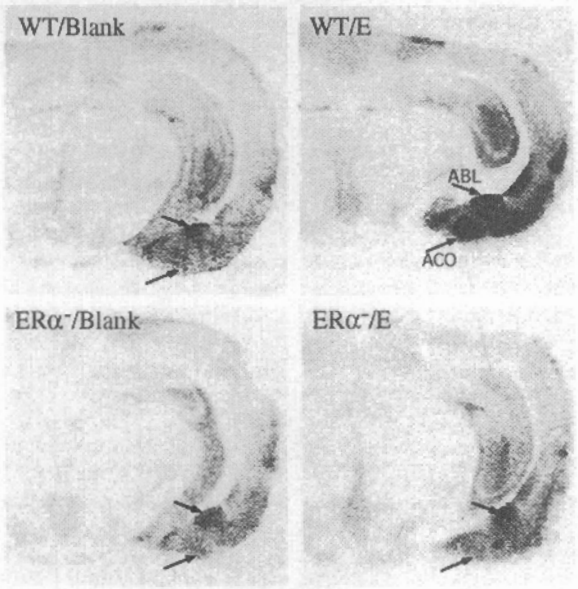

FIG. 2. Photomicrographs illustrating the OTR receptor binding in the basolateral (ABL) and (ACO) cortical nuclei of the amygdala in female wild-type (top panels) and ERα-deficient (ERα⁻) mice (bottom panels). Animals in the left panels received an empty implant while those in the right received an implant containing estrogen. Arrows indicate regions analyzed in the quantitative analysis.

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 receptor (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.[16]

The regulation of central OTR binding and gene expression in the brain by gonadal steroids has been well characterized in the rat.[1,2,17] As noted before, the neuroanatomical pattern of OTR binding in the mouse brain is quite different from that of the rat.[14] 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 effects in the cortex.[14] 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.[18] 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.

Recent molecular studies have raised questions regarding the mechanism by which estrogen modulates OTR binding. A full and several half consensus estrogen response elements (EREs) have been found in the 5′ flanking of the rat[8,19] and mouse[9] OTR genes. The classic mechanism of estrogen action involves estrogen activating the ERα, which in turn enhances transcriptional activity through its direct interactions with the ERβ. The recently discovered ERβ is structurally related to the ERα and apparently modulates gene expression via similar ERE sequences.[12] ERβ appears to make up a significant fraction of the ER mRNA in the brain[20] and is expressed in several brain regions which are known to be estrogen sensitive.[21]

In vitro transcription studies in MCF7 cells using reporter genes driven by the rat OTR 5′ flanking region containing the ERE failed to show robust induction of gene expression in response to estrogen. This raises the possibility that other mechanisms may be acting independently from the ERE *in vivo*. For example, estrogen induces a rapid (i.e. less than

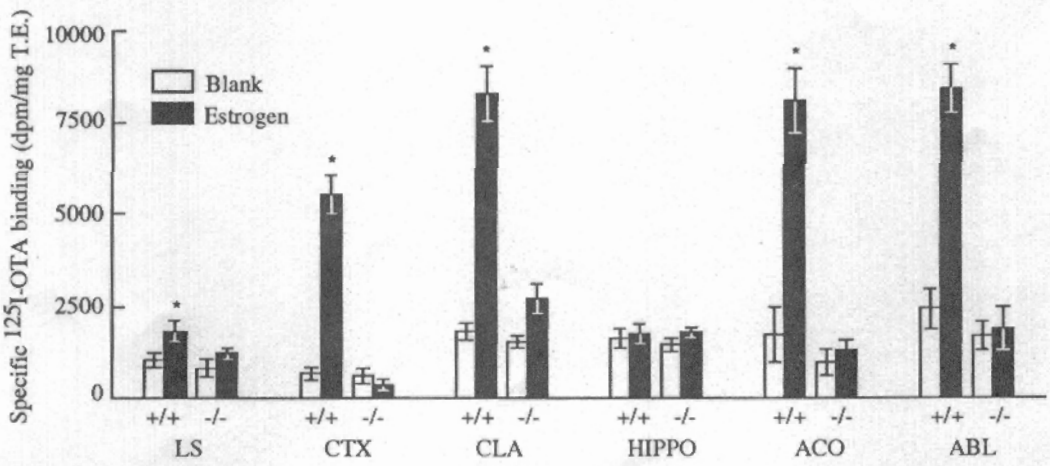

FIG. 3. Quantitative analysis of [^{125}I]OTA binding in wild-type (+/+) and ERα deficient (–/–) mice. Estrogen treatment resulted in increases in binding in the lateral septum (LS), claustrum (CLA), neocortex (CTX), and the basolateral (ABL) and cortical (ACO) nuclei of the amygdala in wildtype but not knockout animals. Receptor binding was unaffected by estrogen in the hippocampus (HIPPO).

15 min) phosphorylation of the cAMP response element binding proteins in the brain,[11] which may influence gene transcription by interacting with the cyclic AMP response elements (CRE).[10] The rat and mouse OTR 5' flanking regions contain CRE sequences.[8,9] It is unclear whether the modulation of gene expression by a cAMP mechanism involves a nuclear ER or perhaps a membrane receptor. The present data can not rule out a potential contribution of alternate mechanisms, however the data clearly demonstrates that ERα is required for the regulation of OTR by estrogen.

Oxytocin and OTR are involved in the control of both male and female sexual behavior as well as other social behaviors.[22] Both male and female ERα-deficient mice exhibit impaired sexual behavior.[23,24] Many of the behavioral effects of oxytocin are potentiated by estrogen. For example, induction of female receptivity in rats requires the upregulation of OTR by E.[4] It is possible that some of the behavioral deficits found in ERα-deficient mice are attributable in part to the lack of induction of OTR by gonadal steroids.

Conclusion

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

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