Published in final edited form as:

J Neuroendocrinol. 2012 February ; 24(2): 285–291. doi:10.1111/j.1365-2826.2011.02232.x.

Anatomically-specific actions of estrogen receptor in developing female rat brain: Effects of estradiol and SERMs on progesterin receptor expression

Keith L. Gonzales, Princy Quadros-Mennella, Marc J. Tetel, and Christine K. Wagner
Department of Psychology, Behavioral Neuroscience Program, University at Albany – State University of New York, Albany, NY 12222 (KLG, CKW); Department of Biological Sciences, Delaware State University, Dover, DE 19901 (PSQ-M); Neuroscience Program, Wellesley College, Wellesley, MA 02481(MJT)

Abstract

Steroid hormones largely exert their actions by activating nuclear receptors, which as transcription factors, powerfully influence fundamental processes of neural development. Often, steroid receptor action demonstrates remarkable specificity under different developmental, anatomical or hormonal conditions. Yet, the mechanisms underlying such specificity are poorly understood. The present study examined the anatomically-specific regulation of progesterin receptor (PR) expression by estrogen receptor (ER) activation in the ventromedial nucleus (VMN) of the hypothalamus and the medial preoptic nucleus (MPN) of the neonatal female rat brain, using the selective estrogen receptor modulators (SERMs), tamoxifen and ICI 182, 780 (ICI), in the presence or absence of estradiol benzoate (EB) treatment. The results demonstrate that PR immunoreactivity (PRir) in the neonatal female MPN was significantly increased by EB and this increase was abolished by either tamoxifen or ICI treatment. In contrast, within the VMN of the same animals, EB had no effect on PRir and the SERMs only modestly decreased PRir. Interestingly, ICI acted as a true antagonist regardless of EB treatment, whereas tamoxifen acted as an ER agonist in the absence of EB in the MPN, but not the VMN, representing one of the first *in vivo* demonstrations of tissue-specific and estradiol-independent effects of tamoxifen on ER activation. The present results indicate that PR expression is highly dependent on oestradiol and its receptor in the MPN, but is independent of both oestradiol and ER activation within the neonatal VMN. These findings demonstrate anatomically-specific actions of estradiol and its receptor to induce PR in two brain regions controlling different aspects of female reproductive behaviors in adulthood.

Keywords

estradiol; estrogen receptor; progesterin receptor; SERMs; tamoxifen; development; preoptic; hypothalamus; ventromedial nucleus; VMN; VMH

Introduction

Steroid hormones influence the developing brain through activation of their cognate nuclear receptors which, as transcription factors, regulate gene expression to alter fundamental processes of neural development. Often steroid hormones act with anatomical specificity, exerting differential effects on gene expression in different brain regions despite similar levels of receptor expression (e.g., (1, 2)). Although the specificity of steroid receptor action

is likely critical for normal neural development, the mechanisms by which this specificity is achieved remain poorly understood.

One of the best studied examples of steroid induced gene transcription is the regulation of progesterin receptor (PR) expression by the actions of estradiol at estrogen receptor α (ER α) (2–5). *In vitro* work has demonstrated that estradiol can bind directly to estrogen response element(s) located in the promoter of the PR gene to initiate gene transcription (6–10). The idea that these same processes occur in brain is supported by evidence that the reduction of circulating estradiol levels by ovariectomy reduced PR mRNA (11–13) and protein (1, 14, 15) in both the ventromedial nucleus of the hypothalamus (VMN) and the medial preoptic nucleus (MPN) of adult female rats and estradiol replacement restored PR expression to baseline levels (1).

In stark contrast to the adult female, regulation of PR by estradiol in the neonatal female brain demonstrates both developmental and anatomical specificity. Exogenous estradiol benzoate (EB) treatment significantly increased levels of PR immunoreactivity (PRir) in both the MPN and VMN of ovariectomized female rats from postnatal day 14 (P14) through P67 (1). However, at P7, while EB treatment increased PRir approximately 50-fold in the MPN, PR levels were unaltered by EB in the VMN of the same animals (1, 2, 16), despite high levels of ER α expression in both regions (2). Recent work from our lab suggests that the inhibition of ER α activation by ER β in the VMN, but not the MPN, may be an underlying mechanism by which this anatomical-specificity is achieved (2).

These findings suggest that PR expression is “disengaged” from estradiol regulation in the neonatal female VMN, but is highly dependent on estradiol and ER α in the MPN. In further support of this idea, PR expression is virtually absent in the MPN prior to the onset of ovarian steroidogenesis, and circulating estradiol levels are extremely low (17). However, in the VMN of the same animals, levels of PR expression are relatively high despite low levels of estradiol in circulation (1, 2). Taken together, these findings suggest that PR expression is differentially regulated by estradiol in the neonatal MPN and VMN and that ER α exerts anatomically-specific actions in the developing female brain.

There are several possible mechanisms by which PR expression may occur in the neonatal VMN in the relative absence of ovarian-derived estradiol. One possibility is that PR expression is completely independent of ER α activation in the neonatal female VMN and is constitutively expressed in this region during early postnatal life. Alternatively, PR expression may be dependent on ER α activation, but independent of serum estradiol levels. For example, estradiol has been shown to be synthesized within the brain from pregnenolone within the adult hippocampus (18) and within various regions of the neonatal female brain (19). This locally produced estradiol could act in an anatomically specific fashion to induce PR within the VMN. Furthermore, the possibility that the local conversion of testosterone to oestradiol via aromatase activity is possible as well and indeed, aromatase mRNA is expressed within the developing VMN(20). Along the same lines, ligand independent activation of ERs induces the expression of PR within the developing brain (e.g., (21)) is another possible mechanism. Therefore, to distinguish between these possibilities, we examined levels of PR in the MPN and VMN of neonatal females treated with the ER antagonist ICI 182,780 (ICI) or tamoxifen in the presence or absence of exogenous estradiol. Although tamoxifen is known to have true SERM like actions and ICI 182,780 is more commonly known to be a pure antagonist, ICI 182,780 has been shown to have agonist like effects in specific preparations(22, 23), and therefore the term SERMs will be used for both compounds. It was predicted that the inhibition of ER α activation should reduce PR levels, if indeed, PR expression in the neonatal VMN is dependent on ER α activation. Conversely, PR expression would remain unaltered following SERM treatment if PR expression in the

neonatal VMN is independent of ER α activation. The present results indicate that PR expression is highly dependent on estradiol and its receptor in the MPN, but is independent of both estradiol and ER activation within the neonatal VMN suggesting the existence of anatomically specific mechanisms to regulate the expression of steroid receptors in the developing female brain.

Materials and Methods

Animals

Subjects were offspring of timed pregnant female Sprague Dawley rats (60–80d of age; Taconic Laboratories, Germantown NY). Animals were housed on a 10-h light, 14-h dark cycle at a constant temperature of 25 $\pm$ 2°C with food and water available *ad libitum*. All animal procedures used in this study were approved by the Institutional Animal Care and Use Committee at the University at Albany. All females were permitted to deliver their pups normally. The day of birth was designated postnatal day 1 (P1).

Treatments

Female neonates received daily injections of tamoxifen (Sigma; 100 μ g/animal), ICI (Tocris Bioscience; 1.5mg/kg), or an equal volume of the vehicle alone (0.01cc/g body weight; 10% dimethyl sulfoxide (DMSO) in sesame oil, s.c. (Vehicle) on P1, P2 and P3. In addition, female neonates received a single injection of estradiol benzoate (EB; 20 μ g/kg) or an equal volume of vehicle (sesame oil, s.c. (Oil)) on P2, 48 hours prior to tissue collection. The dose of tamoxifen was effective at reducing ER activation within neonatal female brain as reported previously (24). In preliminary studies, the dose of ICI was shown to be the most effective at reducing levels of PR within the MPN of males and is consistent with effective doses reported previously (25). Doses of EB consistently induce PR in the MPN of neonatal females in previous studies from our lab (1, 2, 26).

Tissue collection

On P4, neonates were anesthetized by hypothermia by placement on ice and sacrificed by rapid decapitation. Brains were removed from the skull and immersion fixed in 5% acrolein in 0.1M phosphate buffer (PB, pH 7.6) for 6 hours, then cryoprotected in 30% sucrose in 0.1M PB. Brains were sectioned at 50 μ m in the coronal plane using a rotary freezing microtome. Sections were stored in cryoprotectant (30% sucrose, 0.1% polyvinylpyrrolidone-40 in 30%ethylene glycol and 0.1M PB) at –20°C until immunocytochemical processing.

Immunocytochemistry

Immunocytochemistry was performed, as previously described (2, 16, 17) on free floating sections using a rabbit polyclonal antiserum (Dako Corp. Inc., Glostrup, Denmark) directed against the DNA binding domain of the human PR. This antisera detects both the A and B isoforms of PR and its specificity has been well-documented (27, 28). All incubations were performed at room temperature unless otherwise stated. Sections were rinsed in tris-buffered saline (TBS; pH 7.6; 3 $\times$ 5min each), incubated in 1% sodium borohydride in TBS (10 min), rinsed in TBS (4 $\times$ 5min), incubated in TBS containing 20% normal goat serum (NGS), 1% H₂O₂ and 1% BSA for 30 minutes. PR antiserum was diluted 1:1000 in TBS containing 0.3% Triton X-100, 2% NGS (TTG) for 72h at 4°C. Sections were rinsed in TTG (3 $\times$ 5min), incubated in biotinylated goat anti-rabbit IgG (Vector Laboratories, Burlingame, CA) at a concentration of 5 μ g/ml in TTG for 90min. Sections were rinsed in TTG (2 $\times$ 5min), in TBS (2 $\times$ 5min) then incubated in avidin-biotin complex reagent (Vectastain Elite Kit, Vector Laboratories) for 60min. Sections were rinsed in TBS (3 $\times$ 5min), then incubated in

TBS containing 0.05% diaminobenzidine, 0.75mM nickel ammonium sulfate, 0.15% β -D-glucose, 0.04% ammonium chloride, and 0.2% glucose oxidase for approximately 10 minutes. Sections were rinsed in TBS (3×5 min), mounted on gelatin-coated slides and coverslipped with Permount (Fisher Scientific, Pittsburgh, PA).

Image and Statistical Analysis

A representative, anatomically-matched section through the MPN and ventrolateral VMN of each animal was selected for image analysis by an experimenter blind to treatment group. Animals for which an anatomical match could not be found were excluded from analysis. Microscopic images of the MPN and VMN were captured with a Nikon Eclipse E600 microscope fitted with a SPOT Insight camera (Diagnostic Instruments, Sterline Heights, MI) connected to a Dell Inspiron 8600 laptop. NIH Image software (NIH, Bethesda, MD) was used to analyze captured images. The relative amount of PRir in the MPN and VMN was determined as described previously (1, 2, 16, 29) by measuring the area (μm^2) covered by “thresholded” pixels [i.e. those pixels with a gray level higher than a defined threshold density (specific immunoreactive staining)]. “Threshold” was determined as a constant function of the background optical density defined as the mean optical density three to five times the standard deviation higher than the mean background density (1, 2, 16). The mean background density was measured in a region devoid of PRir, immediately lateral to the analyzed region containing PRir. Statistical analyses were performed using a two-way ANOVA (SERM $\times$ EB; $p < 0.05$), followed by pre-planned, pairwise comparisons using Student-Newman-Keuls post hoc analysis ($p < 0.05$).

Results

MPN

In the MPN, levels of PRir were highly dependent on exogenous estradiol and ER activation (Figures 1 and 2). Two-way ANOVA revealed a significant main effect of EB treatment ($F_{1,62} = 12.888$ ($p < 0.001$)); a significant main effect of SERM treatment ($F_{2,62} = 23.837$ ($p < 0.001$)), and a significant interaction between EB and SERM treatment ($F_{2,62} = 31.091$ ($p < 0.001$)).

In the absence of SERMs, EB treatment increased levels of PRir within the MPN compared to oil-treated controls ($p < 0.001$). This EB-induced increase of PRir in the MPN was attenuated by both SERMs. Levels of PRir were lower in EB-treated females receiving either tamoxifen ($p < 0.001$) or ICI ($p < 0.001$) compared to females receiving EB alone. In the absence of EB treatment, ICI had no effect on levels of PRir ($p = 0.583$). In contrast, tamoxifen increased levels of PRir in the MPN in the absence of EB compared to oil treated controls ($p < 0.001$), suggesting that tamoxifen had agonistic properties in the absence of estradiol, consistent with its classification as a SERM.

In summary, EB treatment significantly increased levels of PRir compared to oil treated animals and both tamoxifen and ICI acted as potent ER antagonists, blocking the induction of PR by EB. These findings served as proof of principle that these agents were readily accessing the brain and in the presence of EB were acting as potent ER antagonists in our model. These controls are specifically important for ICI 182,780, a compound that has only recently been shown to pass the BBB(25). Our results support the findings of Alfinito et al., (2008) in that the systemic administration of ICI abolished the ability of exogenous estradiol to induce PR-ir within the MPN. These results demonstrate that ICI was capable of passing the immature BBB and act as a potent antagonist to ER α function.

VMN

In the VMN, levels of PR_{ir} were independent of estradiol and largely independent of ER activation (Figure 3). Two-way ANOVA revealed a main effect of SERM treatment ($F_{2,57} = 13.554$ ($p < 0.001$), but no main effect of EB treatment ($F_{1,57} = 0.0952$), and no significant interaction between EB treatment and SERM treatment ($F_{2,52} = 1.127$). EB treatment had no effect on levels of PR_{ir} compared to oil treatment in animals that did not receive SERM treatment. In the absence of EB treatment, both tamoxifen and ICI modestly reduced levels of PR_{ir} ($p < 0.05$). However, in EB treated animals, there was no effect of ICI treatment compared to vehicle controls, but there was a small, but significant effect of tamoxifen treatment decreasing PR_{ir} levels ($p < 0.05$).

Discussion

The present findings suggest that the expression of a transcription factor, the steroid receptor PR, is regulated in an anatomically specific manner by estradiol and ER activation within the developing brain. More specifically, PR expression is highly dependent upon estradiol within the MPN, but not within the VMN of the *developing* female rat. This is in stark contrast to the *adult* female rat in which PR is highly regulated by estradiol within both brain areas (1), suggesting a developmentally-specific regulation of PR expression as well. The present results are consistent with previous work from lab (1), demonstrating that 1) in the absence of circulating estradiol, PR_{ir} is at relatively high levels in the neonatal VMN, and 2) exogenous estradiol administration dramatically increased PR in the MPN, but had no effect on PR in the VMN. Interestingly, results from the present study uncovered that SERMs (tamoxifen and ICI) attenuated the ability of estradiol to increase levels of PR_{ir} in the MPN, but had little effect on the actions of estradiol in the VMN. Taken together, these results demonstrate that PR expression in the MPN is highly dependent on estradiol and ER activation, but that in the VMN of the same animals, PR expression is “disengaged” from estradiol and ER, thereby elucidating a striking anatomical specificity in steroid receptor action in the developing brain.

Dependence of PR_{ir} on estradiol is anatomically-specific: actions of the SERM, ICI 182,780

ICI is widely accepted as a potent ER antagonist with a similar binding affinity for both ER α and ER β (30) and has recently been shown to cross the blood brain barrier (25). To increase the ability of ICI to enter the brain, animals were treated with ICI on P1–P4, prior to the full maturation of the blood brain barrier (31). Estradiol induces PR in the MPN (Figure 1 and (1, 2)), while systemic administration of ICI completely blocked this ability of EB to induce PR_{ir} in the MPN, demonstrating that ICI readily and effectively entered the brain. Despite the ability of ICI to act as a potent ER antagonist in the MPN, ICI did not attenuate levels of PR_{ir} in the VMN in the presence of estradiol. Interestingly, ICI elicited a small decrease in PR_{ir} in control animals (Figure 2), indicating that ICI decreases PR expression in the absence of ER activation. These findings suggest, that in stark contrast to the adult female VMN, PR expression in the neonatal VMN may be independent of estradiol and ER activation.

Agonist/antagonist properties of tamoxifen are anatomically-specific and hormonally-dependent

Tamoxifen is a true SERM in that it can exert both agonist and antagonist effects on ER activation depending on tissue type, presence or absence of estradiol (32) or availability of coactivators (33, 34). One possible mechanism by which this happens is the differential expression of specific coregulatory proteins such as SRC-1 and SMRT. For example, Klinge *et al* (33) demonstrated that the expression of SRC-1 and SMRT, *in vitro*, mediated the agonist like effects of tamoxifen in CHO-k1 cell lines. In further support, tamoxifen bound

ER α recruited SMRT at the PR promoter in chromatin immunoprecipitation assays using MCF-7 cells (35). As SRC-1 and SMRT are expressed in brain (36), and SRC-1 has been shown to mediate levels of PR-ir within the adult hypothalamus (37), these mechanisms may mediate tamoxifen's agonist like behavior in brain. In the present study, tamoxifen acted as an ER antagonist in the MPN in the presence of estradiol treatment, but acted as a relatively robust ER agonist in the MPN in the absence of exogenous estradiol. In comparison, tamoxifen treatment decreased levels of PRir in the VMN suggesting that this drug acted as a weak antagonist both in the presence and absence of estradiol (figure 4). To our knowledge, these findings represent one of the first *in vivo* studies to demonstrate tissue (i.e., brain region) specific effects of tamoxifen independent of estradiol levels which has considerable implications for the use of this drug in the clinical setting.

Conclusions

The actions of estradiol and ER on the developing brain have been well studied, particularly in regards to the masculinization of the male rodent brain (for reviews see (38–41)). In the developing male MPN, relatively high levels of estradiol aromatized from testosterone of testicular origin, result in high levels of PR in MPN, whereas PR is virtually absent in the female MPN prior to ovarian estradiol synthesis (17). The present findings suggest that, unlike the neonatal female MPN, PR expression is high in the VMN and this expression is independent of circulating estradiol and ER activation. An anatomically specific regulation of steroid receptor induced gene expression suggests a mechanism for the differential development of two brain regions that control different reproductive behaviors in adulthood; the VMN is important for female sexual behavior (42–46) and the MPN for maternal behavior (16, 47, 48).

Although the expression of PR protein does not imply that PR is active within the developing VMN, the present findings implicate an important role for PR in the development of the female VMN. Consistent with this idea, PR expression is not increased by estradiol in the VMN, perhaps due to a potential for ER β to inhibit ER α activation (2) and PR expression in the VMN occurs at high levels independent of estradiol and ER activation suggesting that PR expression is constitutively expressed or regulated by an unknown factor within the neonatal female VMN. The disengagement of PR expression from estradiol and ER is transient during early perinatal life, as PR expression becomes increasingly dependent on estradiol after P14 (1). This generates the intriguing idea that progesterone and PR may play an active and important role in the feminization of the VMN, a process that has typically been viewed as independent of steroid hormone action.

Acknowledgments

Supported by NSF IOB0447492 and a March of Dimes National Research Grant to CKW and a NIH NRSA MH08344601A2 to KLG and NIH DK61935 to MJT

The authors wish to thank Kate Shea and Jessica Wess for their assistance on this project.

References

1. Quadros PS, Wagner CK. Regulation of progesterone receptor expression by estradiol is dependent on age, sex and region in the rat brain. *Endocrinology*. 2008;2007–1133.
2. Gonzales KL, Tetel MJ, Wagner CK. Estrogen receptor (er) {beta} modulates er{alpha} responses to estrogens in the developing rat ventromedial nucleus of the hypothalamus. *Endocrinology*. 2008; 149:4615–4621. [PubMed: 18511514]
3. Wagner CK, Pfau JL, Vries GJD, Merchenthaler IJ. Sex differences in progesterone receptor immunoreactivity in neonatal mouse brain depend on estrogen receptor α expression. *J of Neurobiol*. 2001; 47:176–182. [PubMed: 11333399]

4. Blaustein JD, Turcotte JC. Estradiol-induced progesterin receptor immunoreactivity is found only in estrogen receptor-immunoreactive cells in guinea pig brain. *Neuroendocrinology*. 1989; 49:454–461. [PubMed: 2657476]
5. Moffatt CA, Rissman EF, Shupnik MA, Blaustein JD. Induction of progesterin receptors by estradiol in the forebrain of estrogen receptor- α gene-disrupted mice. *J Neurosci*. 1998; 18:9556–9563. [PubMed: 9801392]
6. Frasor J, Barnett DH, Danes JM, Hess R, Parlow AF, Katzenellenbogen BS. Response-specific and ligand dose-dependent modulation of estrogen receptor (er) $\{\alpha\}$ activity by er $\{\beta\}$ in the uterus. *Endocrinology*. 2003; 144:3159–3166. [PubMed: 12810572]
7. Kraichely DM, Sun J, Katzenellenbogen JA, Katzenellenbogen BS. Conformational changes and coactivator recruitment by novel ligands for estrogen receptor- $\{\alpha\}$ and estrogen receptor- $\{\beta\}$: Correlations with biological character and distinct differences among src coactivator family members. *Endocrinology*. 2000; 141:3534–3545. [PubMed: 11014206]
8. Harrington WR, Sheng S, Barnett DH, Petz LN, Katzenellenbogen JA, Katzenellenbogen BS. Activities of estrogen receptor α - and β -selective ligands at diverse estrogen responsive gene sites mediating transactivation or transrepression. *Mol Cell Endocrinol*. 2003; 206:13–22. [PubMed: 12943986]
9. Kraus WL, Montano MM, Katzenellenbogen BS. Identification of multiple, widely spaced estrogen-responsive regions in the rat progesterone receptor gene. *Mol Endocrinol*. 1994; 8:952. [PubMed: 7997237]
10. Kraus WL, Montano MM, Katzenellenbogen BS. Cloning of the rat progesterone receptor gene 5'-region and identification of two functionally distinct promoters. *Mol Endocrinol*. 1993; 7:1603. [PubMed: 8145766]
11. Shughrue PJ, Lane MV, Merchenthaler I. Regulation of progesterone receptor messenger ribonucleic acid in the rat medial preoptic nucleus by estrogenic and antiestrogenic compounds: An in situ hybridization study. *Endocrinology*. 1997; 138:5476. [PubMed: 9389534]
12. Lauber A, Romano G, Pfaff D. Sex difference in estradiol regulation of progesterin receptor mRNA in rat mediobasal hypothalamus as demonstrated by in situ hybridization. *Neuroendocrinology*. 1991; 53:608–613. [PubMed: 1876238]
13. Simerly RB. Distribution and regulation of steroid hormone receptor gene expression in the central nervous system. *Adv Neurol*. 1993; 59:207. [PubMed: 8380515]
14. Blaustein JD, Feder HH. Cytoplasmic progesterin-receptors in guinea pig brain: Characteristics and relationship to the induction of sexual behavior. *Brain Res*. 1979; 169:481–497. [PubMed: 571753]
15. Maclusky N, McEwen B. Oestrogen modulates progesterin receptor concentrations in some rat brain regions but not in others. *Nature*. 1978; 274:276–278. [PubMed: 683307]
16. Quadros PS, Pfau JL, Goldstein AYN, De Vries GJ, Wagner CK. Sex differences in progesterone receptor expression: A potential mechanism for estradiol-mediated sexual differentiation. *Endocrinology*. 2002; 143:3727–3739. [PubMed: 12239082]
17. Wagner CK, Nakayama AY, De Vries GJ. Potential role of maternal progesterone in the sexual differentiation of the brain. *Endocrinology*. 1998; 139:3658–3661. [PubMed: 9681521]
18. Hojo Y, Hattori T-A, Enami T, Furukawa A, Suzuki K, Ishii H-T, Mukai H, Morrison JH, Janssen WGM, Kominami S, Harada N, Kimoto T, Kawato S. Adult male rat hippocampus synthesizes estradiol from pregnenolone by cytochromes p45017 α and p450 aromatase localized in neurons. *PNAS*. 2004; 101:865–870. [PubMed: 14694190]
19. Amateau SK, Alt JJ, Stamps CL, McCarthy MM. Brain estradiol content in newborn rats: Sex differences, regional heterogeneity, and possible de novo synthesis by the female telencephalon. *Endocrinology*. 2004; 145:2906. [PubMed: 14988386]
20. Lauber ME, Lichtensteiger W. Pre- and postnatal ontogeny of aromatase cytochrome p450 messenger ribonucleic acid expression in the male rat brain studied by in situ hybridization. *Endocrinology*. 1994; 135:1661–1668. [PubMed: 7925130]
21. Olesen KM, Jessen HM, Auger CJ, Auger AP. Dopaminergic activation of estrogen receptors in neonatal brain alters progesterin receptor expression and juvenile social play behavior. *Endocrinology*. 2005; 146:3705–3712. [PubMed: 15919740]

22. Robertson JA, Zhang Y, Nancy H. ICI 182,780 acts as a partial agonist and antagonist of estradiol effects in specific cells of the sheep uterus. *J Steroid Biochem Mol Biol*. 2001; 77:281–287. [PubMed: 11457666]
23. Dudley MW, Sheeler CQ, Wang H, Khan S. Activation of the human estrogen receptor by the antiestrogens ICI 182,780 and tamoxifen in yeast genetic systems: Implications for their mechanism of action. *PNAS*. 2000; 97:3696. [PubMed: 10725345]
24. Hilton G, Ndubuizu A, Mccarthy M. Neuroprotective effects of estradiol in newborn female rat hippocampus. *Developmental Brain Res*. 2004; 150:191–198.
25. Alfinito PD, Chen X, Atherton J, Cosmi S, Deecher DC. ICI 182,780 penetrates brain and hypothalamic tissue and has functional effects in the brain after systemic dosing. *Endocrinology*. 2008; 149:5219–5226. [PubMed: 18599545]
26. Quadros PS, Goldstein AYN, De Vries GJ, Wagner CK. Regulation of sex differences in progesterone receptor expression in the medial preoptic nucleus of postnatal rats. *J Neuroendocrinol*. 2002; 14:761–767. [PubMed: 12372000]
27. Quadros PS, Pfau JL, Wagner CK. Distribution of progesterone receptor immunoreactivity in the fetal and neonatal rat forebrain. *J Comp Neurol*. 2007; 504:42–56. [PubMed: 17614295]
28. López V, Wagner CK. Progestin receptor is transiently expressed perinatally in neurons of the rat isocortex. *J Comp Neurol*. 2008; 512:124–139.
29. Wagner CK, Xu J, Pfau JL, Quadros PS, De Vries GJ, Arnold AP. Neonatal mice possessing an sry transgene show a masculinized pattern of progesterone receptor expression in the brain independent of sex chromosome status. *Endocrinology*. 2004; 145:1046–1049. [PubMed: 14645115]
30. Sun J, Huang YR, Harrington WR, Sheng S, Katzenellenbogen JA, Katzenellenbogen BS. Antagonists selective for estrogen receptor {alpha}. *Endocrinology*. 2002; 143:941–947. [PubMed: 11861516]
31. Rao, M.; Jacobson, M. *Developmental Neurobiology*. Kluwer Academic/Plenum; 2005.
32. Webb P, Lopez G, Uht R, Kushner P. Tamoxifen activation of the estrogen receptor/AP-1 pathway: Potential origin for the cell-specific estrogen-like effects of antiestrogens. *Mol Endocrinol*. 1995; 9:443–456. [PubMed: 7659088]
33. Klinge CM, Jernigan SC, Mattingly KA, Risinger KE, Zhang J. Estrogen response element-dependent regulation of transcriptional activation of estrogen receptors alpha and beta by coactivators and corepressors. *J Mol Endocrinol*. 2004; 33:387–410. [PubMed: 15525597]
34. Katzenellenbogen BS, Katzenellenbogen JA. Biomedicine. Defining the “S” in SERMs. *Science*. 2002; 295:2380–2381. [PubMed: 11923515]
35. Karmakar S, Gao T, Pace MC, Oesterreich S, Smith CL. Cooperative activation of cyclin d1 and progesterone receptor gene expression by the src-3 coactivator and smrt corepressor. *Mol Endocrinol*. 2010; 24:1187–1202. [PubMed: 20392877]
36. Tetel M, Auger A, Charlier T. Who’s in charge? Nuclear receptor coactivator and corepressor function in brain and behavior. *Front Neuroendocrinol*. 2009; 30:328–342. [PubMed: 19401208]
37. Molenda HA, Griffin AL, Auger AP, Mccarthy MM, Tetel MJ. Nuclear receptor coactivators modulate hormone-dependent gene expression in brain and female reproductive behavior in rats. *Endocrinology*. 2002; 143(2):436–434. [PubMed: 11796496]
38. Mccarthy MM, Wright CL, Schwarz JM. New tricks by an old dogma: Mechanisms of the organizational/activational hypothesis of steroid-mediated sexual differentiation of brain and behavior. *Horm Behav*. 2009; 55:655–665. [PubMed: 19682425]
39. Mccarthy MM. Estradiol and the developing brain. *Physiol Rev*. 2008; 88:91–134. [PubMed: 18195084]
40. Schwarz JM, Mccarthy MM. Cellular mechanisms of estradiol-mediated masculinization of the brain. *J Steroid Biochem Mol Biol*. 2008; 109:300–306. [PubMed: 18430566]
41. Mccarthy MM, Schwarz JM, Wright CL, Dean SL. Mechanisms mediating oestradiol modulation of the developing brain. *J Neuroendocrinol*. 2008; 20:777. [PubMed: 18601700]
42. Patisaul HB, Adewale HB, Mickens JA. Neonatal agonism of er [alpha] masculinizes serotonergic (5-HT) projections to the female rat ventromedial nucleus of the hypothalamus (vmn) but does not impair lordosis. *Behav Brain Res*. 2009; 196:317–322. [PubMed: 18950659]

43. Pfaff DW, Kow LM, Loose MD, Flanagan-Cato LM. Reverse engineering the lordosis behavior circuit. *Horm Behav.* 2008; 54:347–354. [PubMed: 18539279]
44. Ogawa S, Eng V, Taylor J, Lubahn DB, Korach KS, Pfaff DW. Roles of estrogen receptor- α gene expression in reproduction-related behaviors in female mice. *Endocrinology.* 1998; 139:5070. [PubMed: 9832446]
45. Davis PG, Krieger MS, Barfield RJ, McEwen BS, Pfaff DW. The site of action of intrahypothalamic estrogen implants in feminine sexual behavior: An autoradiographic analysis. *Endocrinology.* 1982; 111:1581–1586. [PubMed: 7128527]
46. Pfaff DW, Sakuma Y. Deficit in the lordosis reflex of female rats caused by lesions in the ventromedial nucleus of the hypothalamus. *J Physiol.* 1979; 288:203–210. [PubMed: 469716]
47. Kuhnemann S, Brown TJ, Hochberg RB, Maclusky NJ. Sex differences in the development of estrogen receptors in the rat brain. *Horm Behav.* 1994; 28:483–491. [PubMed: 7729817]
48. McCarthy MM, Schlenker EH, Pfaff DW. Enduring consequences of neonatal treatment with antisense oligodeoxynucleotides to estrogen receptor messenger ribonucleic acid on sexual differentiation of rat brain. *Endocrinology.* 1993; 133:433–439. [PubMed: 8344188]

Figure 1. Progestin receptor immunoreactivity (PRir) within the medial preoptic nucleus (MPN) and ventromedial nucleus (VMN) of postnatal day 4 (P4) females treated with oil and vehicle (Oil/Veh), estradiol benzoate (EB) and vehicle (EB/Veh), EB and ICI 182,7280 (EB/ICI) or EB and tamoxifen (EB/Tam). Bar = 100 μ m.

Figure 2.

The relative amount of progesterin receptor immunoreactivity (PRir) in the medial preoptic nucleus (MPN) of postnatal day 4 (P4) female rats that were treated with either estradiol benzoate (EB) or the oil vehicle (Oil) and either tamoxifen, ICI 182,780 (ICI) or the vehicle alone from the day of birth (P1) through P4. * Significantly different from the vehicle group within the same hormone treatment ($p < 0.05$); + significantly different from the Oil/Vehicle group ($p < 0.05$).

Figure 3.

The relative amount of progesterin receptor immunoreactivity (PRir) in the ventromedial nucleus (VMN) or VMN? of postnatal day 4 (P4) female rats that were treated with either estradiol benzoate (EB) or the oil vehicle (Oil) and either tamoxifen, ICI 182,780 (ICI) or the vehicle alone from the day of birth (P1) through P4. * Significantly different from the vehicle group within the same hormone treatment ($p < 0.05$).

Figure 4. Progestin receptor immunoreactivity (PRir) within the medial preoptic nucleus (MPN) and ventromedial nucleus (VMN) of postnatal day 4 (P4) females treated with oil and vehicle (Oil/Veh) or oil and tamoxifen (Oil/Tam). Bar = 100 μ m.