

Estrogen Receptor (ER) β Modulates ER α Responses to Estrogens in the Developing Rat Ventromedial Nucleus of the Hypothalamus

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The mechanisms by which estradiol exerts specific actions on neural function are unclear. In brain the actions of estrogen receptor (ER) α are well documented, whereas the functions of ER β are not yet fully elucidated. Here, we report that ER β inhibits the activity of ER α in an anatomically specific manner within the neonatal (postnatal d 7) brain. Using selective agonists we demonstrate that the selective activation of ER α in the relative absence of ER β activation induces progesterone receptor expression to a greater extent than estradiol

alone in the ventromedial nucleus, but not the medial preoptic nucleus, despite high ER α expression. Selective activation of ER β attenuates the ER α -mediated increase in progesterone receptor expression in the ventromedial nucleus but has no effect in medial preoptic nucleus. These results suggest that ER α /ER β interactions may regulate the effects of estrogens on neural development and reveal the neonatal brain as a unique model in which to study the specificity of steroid-induced gene expression. (Endocrinology 149: 4615–4621, 2008)

STEROID HORMONE RECEPTORS, as nuclear transcription factors, exert powerful effects on a broad range of neural functions (1, 2). Estradiol, although best known for its role in neuroendocrine function and reproductive behaviors in rodents (for review, see Ref. 1), has recently been associated with a number of neurological disorders, including Parkinson's disease (3), Alzheimer's disease (3), and stroke (4). Estrogens and selective estrogen receptor modulators (SERMs) are commonly used clinically, despite a poor understanding of how these treatments might influence brain function (5, 6). A growing literature clearly demonstrates that the actions of estradiol are tissue specific and developmentally dependent, creating the possibility for unwanted and potentially dangerous side effects of estrogen treatment (7, 8). Therefore, it becomes essential to elucidate the mechanisms by which estradiol and estrogen receptors (ERs) exert their specific actions within the brain.

To date, two nuclear ERs, ER α and ER β , have been identified (9). These two receptors share a highly conserved DNA binding domain but poor to moderate homology in their N-terminal domain (containing AF-1) and ligand binding domain (containing AF-2). Despite the moderate homology at the ligand binding domain, both ER α and ER β bind to estradiol with a similar affinity (9). However, due to differ-

ences in the AF-1 and AF-2 domains, the intracellular actions of ER α and ER β may differ significantly (10). ER α and ER β are expressed at high levels within specific brain regions and are often colocalized within cells (11–15). Although a role for ER α in a variety of neural and behavioral functions has been well documented (for review, see Ref. 1), a clear and direct function of ER β at the cellular level in brain has remained elusive (for review, see Ref. 16). In contrast, *in vitro* studies consistently reveal an inhibitory action of ER β on ER α transcriptional activity when coexpressed in the same cells (17–19). Such an interaction between ER α and ER β has not been demonstrated at the cellular level *in vivo*, within developing brain. Although several reports have demonstrated a role for ER β activity in behavioral outcomes (16, 20–22), the cellular mechanisms underlying the actions of ER β on ER α activity have yet to be demonstrated *in vivo*.

Within specific regions of the brain, a highly robust bioassay for ER α activity is the induction of progesterone receptor (PR) gene expression (14, 23–27). In adult female rats, estradiol dramatically increases PR within both the medial preoptic nucleus (MPN) and the ventromedial nucleus (VMN) of the hypothalamus. However, previous studies from our laboratory demonstrate that the regulation of PR expression by estradiol is anatomically and developmentally specific (25). For example, in neonatal females, estradiol increases PR levels in the MPN at least 50-fold but does not significantly alter PR levels in the VMN of the same animals. PR expression within the VMN becomes increasingly more responsive to estradiol as development ensues. ER α protein is expressed at high levels throughout development in both regions (28, 29), suggesting that ER α activity is transiently inhibited in the VMN, but not the MPN, during development.

Interestingly, ER β expression is high in the neonatal female VMN but is relatively low in the MPN (13, 28). In addition, ER β expression in the VMN gradually decreases as

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Abbreviations: DMSO, Dimethyl sulfoxide; DPN, diethylpropionitrile; EB, estradiol benzoate; ER, estrogen receptor; ir, immunoreactivity; KO, knockout; MPN, medial preoptic nucleus; PB, phosphate buffer; P5, postnatal d 5; P6, postnatal d 6; P7, postnatal d 7; PPT, 1,3,5 Tris (4-hydroxyphenyl)-4-propyl-1H-pyrazole; PR, progesterone receptor; SERM, selective estrogen receptor modulator; TBS, Tris-buffered saline; VMN, ventromedial nucleus; VMNvl, ventrolateral ventromedial nucleus.

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the animal ages. These findings, together with *in vitro* studies implicating ER β in the inhibition of ER α , suggest that ER β function may be responsible for the reduced sensitivity of the VMN to estradiol during development. To test this hypothesis, the actions of the ER subtypes were dissociated using the *in vivo* administration of ER α and ER β selective agonists. Results suggest that activation of ER β inhibits the ER α dependent induction of PR expression within the developing brain in a regionally specific manner. These results implicate ER β in the specificity of estrogen signaling *in vivo* and, more specifically, in the proper development of steroid-sensitive brain areas. In addition, these findings introduce the neonatal female VMN as a unique biologically relevant model in which to examine the mechanisms underlying the regulation and specificity of steroid-induced gene expression.

Materials and Methods

Animals

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

All animals were treated on postnatal d 5 and 6 (P5 and P6, respectively), and tissue was collected on postnatal d 7 (P7). All treatment groups were assigned to at least three different litters to control for litter effects. In addition, all animals were housed in a mixed sex litter until tissue was collected. The time period for tissue collection was chosen based on previous studies demonstrating that a single dosage of estradiol benzoate (EB) at this age does not induce PR within 48 h in the VMN but strongly induces PR within the MPN (25).

Treatments

Experiment 1: selective ER α -mediated transactivation of PR gene in the brain. On P5 and P6, female pups received either EB (20 μ g/kg, $n = 8$), the ER α -selective agonist, 1,3,5 Tris(4-hydroxyphenyl)-4-propyl-1H-pyrazole (PPT) (1, 2, or 3 mg/kg sc, $n = 9, 10, 12$), or an equal volume of the vehicle [0.01 cc/g 10% dimethyl sulfoxide (DMSO) in sesame oil, sc, $n = 10$]. PPT selectively binds to ER α with a 400-fold preference over ER β and has been a potent activator of ER α transcriptional activity, but not ER β , in transfected cell lines (30, 31). The range of PPT dosages was based on previous studies reporting that PPT in the range of 1–3 mg/kg can induce PR within the rodent brain similarly to 17 β -estradiol during neonatal life (24).

Experiment 2: selective activation of ER α and ER β . On P5 and P6, female pups received either EB (20 μ g/kg, $n = 8$), PPT (2 mg/kg, $n = 8$) alone, or PPT in combination with the ER β selective agonist diarylpropionitrile (DPN) (0.5, 3.5, or 5 mg/kg, $n = 10, 9, 7$), or an equal volume of the vehicle (0.01 cc/g 10% DMSO in sesame oil, $n = 11$). DPN demonstrates a 40-fold higher binding preference for ER β and has been 30-fold more potent in activating transcription using multiple reporter genes *in vitro* (31, 32). The dosages of DPN were chosen from previously published studies in which DPN was used to manipulate ER β activity in the brains of rodents (24, 33, 34). A range of dosages has been used previously (32) to study the role of ER β in many different behavioral paradigms. Due to the variability of dosages, we chose to use a broad range of dosages to find the most effective level of this selective agonist.

Experiment 3: selective activation of ER β alone. On P5 and P6, female pups received the most effective dosage of DPN from experiment 2 (3.5 mg/kg) or an equal volume of the vehicle (0.01 cc/g 10% DMSO in sesame oil). A total of 3.5 mg/kg DPN provided the strongest attenuation of PPT when compared with 0.5 and 5 mg/kg, and was therefore used within this experiment. Tissue collection and immunocytochemistry were performed as below.

Experiment 4: localization of ER α , ER β , and PR via immunocytochemistry. P7 females were allowed to reach P7 with no drug treatments. Brains were collected for ER α , ER β , and PR via immunocytochemistry as described below.

Tissue collection

For all experiments animals were anesthetized by hypothermia and killed by rapid decapitation on P7. Brains were removed from the skull and quickly immersion fixed in 5% acrolein in 0.1 M phosphate buffer (PB) (pH 7.6) for 6 h, cryoprotected in 30% sucrose in 0.1 M PB, and cut at 50 μ m in the coronal plane. Sections were stored in cryoprotectant (30% sucrose, 0.1% polyvinyl-pyrrolidone-40 in ethylene glycol and 0.1 M PB) at -20 C until processing for immunocytochemistry.

For experiment 4, animals processed for ER β immunocytochemistry were fixed as described previously using a 3% acrolein in 0.1 M PB solution for 6 h.

Immunocytochemistry

Immunocytochemistry was performed, as previously described (35, 36), 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 antibody detects both the A and B isoform of PR, and its specificity has been well documented (14, 35). All incubations were performed at room temperature unless otherwise stated. Sections were rinsed in Tris-buffered saline (TBS) (pH 7.6) (3×5 min), incubated in 1% sodium borohydride in TBS (10 min), rinsed in TBS (4×5 min), incubated in TBS containing 20% normal goat serum, 1% H₂O₂, and 1% BSA for 30 min. PR antiserum was diluted 1:1000 in TBS containing 0.3% Triton X-100, 2% normal goat serum (referred to as TTG) for 72 h at 4 C. Sections were rinsed in TTG (3×5 min), incubated in biotinylated goat antirabbit IgG (Vector Laboratories, Burlingame, CA) at a concentration of 5 μ g/ml in TTG for 90 min. Sections were rinsed in TTG (2×5 min), in TBS (2×5 min), then incubated in avidin-biotin complex reagent (Vectastain Elite Kit; Vector Laboratories) for 60 min. Sections were rinsed in TBS (3×5 min), then incubated in TBS containing 0.05% diaminobenzidine, 0.75 mM nickel ammonium sulfate, 0.15% β -D-glucose, 0.04% ammonium chloride, and 0.2% glucose oxidase for approximately 15 min. Sections were rinsed in TBS (3×5 min), mounted on gelatin-coated slides and coverslipped with Permount (Fisher Scientific, Pittsburgh, PA).

In experiment 4, immunocytochemistry was performed as described previously with the following changes: ER α was detected using polyclonal antisera (C1355; Upstate Signaling, Lake Placid, NY) directed against the last 15 amino acids in the rat ER α for 48 h at 1:2000.

ER β immunoreactivity (ir) was detected using mouse monoclonal human ER β (hER β) antibody (hER β NT-221.3; Ligand Pharmaceuticals, Inc., San Diego, CA) raised against a synthetic peptide corresponding to the 14-amino acid N-terminal sequence of hER β (1–485 form) at a dilution of 1 μ g/ml. The specificity of this antibody has been previously published (14). After primary incubation for 72 h, tissue was incubated in a biotinylated goat antimouse IgG (Vector Laboratories) at a concentration of 4 μ g/ml in TTG.

Analysis

For both experiments a representative, anatomically matched section through the rostral MPN and caudal VMN of each animal was selected for image analysis by an experimenter blind to the treatment group. Animals from which an anatomical match could not be found due to damaged sections were excluded from analysis. Microscope images of the PR-ir in the MPN and ventrolateral VMN (VMNvl) were captured with a Nikon Eclipse E600 microscope (Nikon Corp., Tokyo, Japan) fitted with a SPOT Insight camera (Diagnostic Instruments, Sterling Heights, MI) connected to a Dell Inspiron 8600 laptop (Dell Computer Corp., Round Rock, TX). National Institutes of Health Image software (W. Rasband, National Institutes of Health, Bethesda, MD) was used to analyze captured images. Briefly, the relative total amount of PR-ir above background levels was determined according to previously published methods (37–39). Only cells that had a gray level darker than a defined criteria above background were counted. The relative amount of PR-ir in the MPN and VMNvl was determined 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 over and above background OD (*i.e.* gray level), and was defined as the OD three times the SD higher than the mean background density. The mean background density was measured in a region devoid of PR-ir, immediately lateral to the analyzed region containing PR-ir. For experiments 1 and 2, statistical analyses were performed using a one-way ANOVA ($P < 0.05$), followed by preplanned, pairwise comparisons using Student-Newman-Keuls *post hoc* analysis ($P < 0.05$). For experiment 3, statistical analysis was performed using a Student's *t* test ($P < 0.05$).

Results

Experiment 1: selective activation of ER α in the relative absence of ER β activation

In the VMN the selective activation of ER α alone induced PR over and above EB treatment. In addition, PPT (2 mg/kg) induced PR above that of vehicle controls. In contrast, in the MPN, both EB and all doses of PPT dramatically increased PR levels above that of vehicle controls.

In the neonatal VMN, one-way ANOVA revealed a main effect of treatment [$F_{(4,48)} = 7.674$; $P < 0.001$; Fig. 1A]. *Post hoc* analysis revealed that PR-ir levels were significantly higher in the PPT group (2 mg/kg) compared with EB treatment ($P < 0.05$). In addition, PPT at 2 and 3 mg/kg increased PR levels compared with oil-treated controls ($P < 0.001$), whereas EB treatment did not significantly alter PR levels compared with controls ($P = 0.072$).

In the MPN, one-way ANOVA revealed a significant main effect of treatment [$F_{(4,51)} = 13.271$; $P < 0.001$; Fig. 1B]. *Post hoc* analysis revealed that EB and PPT at all doses significantly increased PR-ir levels compared with vehicle-treated controls ($P < 0.001$). EB and PPT treatments were not different from one another.

Experiment 2: selective activation of ER α and ER β

Consistent with experiment 1, PPT induced PR levels in the VMN significantly greater than EB treatment. Furthermore, the effect of PPT was attenuated with the addition of the ER β agonist, DPN. The combination of PPT and DPN significantly decreased PR levels compared with PPT alone but were similar to those of EB treatment.

In the VMN, one-way ANOVA revealed a main effect of treatment [$F_{(5,52)} = 25.483$; $P < 0.001$; Fig. 2A]. *Post hoc* analysis revealed that levels of PR-ir were higher in the EB treated group compared with the oil-treated group ($P < 0.05$). PPT significantly increased PR levels over and above that of EB ($P < 0.001$). Furthermore, the effect of PPT was significantly attenuated by the addition of DPN. All three doses of DPN in combination with PPT decreased PR levels compared with PPT alone ($P < 0.001$ for 3.5 mg/kg, and $P < 0.05$ for 0.5 and 5 mg/kg DPN). In contrast, in the MPN, all treatments significantly increased PR levels compared with vehicle controls, but there were no differences between the treatment groups. One-way ANOVA revealed a significant main effect of treatment [$F_{(5,56)} = 22.277$; $P < 0.001$; Fig. 2B]. *Post hoc* analysis revealed that all treatments (EB, PPT, and PPT plus DPN) significantly increased PR levels compared with vehicle treatment ($P < 0.001$). PR levels did not differ between the PPT plus DPN treated groups and the EB treated group.

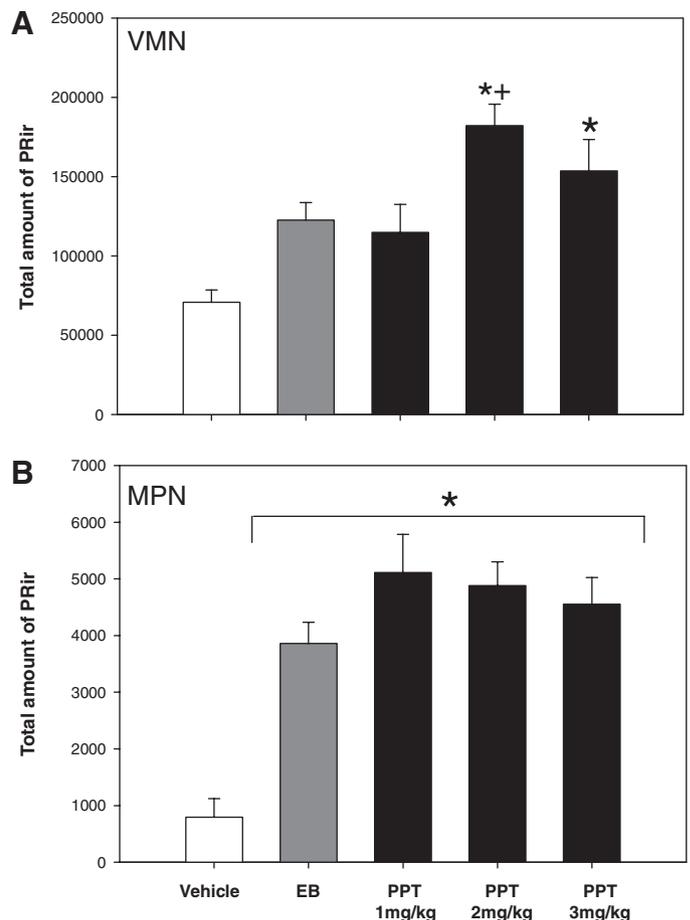


FIG. 1. Selective activation of ER α , in the relative absence of ER β activation, induces PR expression over and above estradiol in the VMN, but not the MPN. The mean (SEM) total amount of PR-ir within the VMNvl or the MPN of female rats on P7 treated with vehicle ($n = 10$), EB (20 $\mu\text{g}/\text{kg}$, $n = 8$), or the ER α selective agonist, PPT, at 1, 2, or 3 mg/kg ($n = 9, 10, 12$) on P5 and P6. A, VMNvl. PR-ir levels were significantly higher after PPT treatment (2 mg/kg) compared with EB treatment. PPT (2 and 3 mg/kg) significantly increased PR-ir levels compared with vehicle treatment. B, MPN. PPT treatment (all doses) and EB treatment significantly increased levels of PR-ir compared with vehicle treatment. PR-ir levels did not differ between EB and PPT treatments at any dose. *, Significantly different from vehicle ($P < 0.001$); +, significantly different from EB ($P < 0.05$).

Experiment 3: selective activation of ER β alone

Selective activation of ER β alone had no effect on PR-ir levels in the MPN, suggesting that DPN, at the dose used, had little effect on ER α activity. DPN alone increased PR-ir levels slightly in the VMN ($P < 0.05$; Fig. 3A). PR-ir levels did not significantly differ between the DPN treated group and vehicle-treated group in the MPN (Fig. 3B).

Experiment 4: ER β , ER α , and PR expression in the neonatal female VMN and MPN

ER β , ER α , and PR are all expressed at high levels within the VMNvl of P7 females (Fig. 4A). Furthermore, the anatomical distribution of immunoreactive nuclei fall within the same region of the VMNvl, suggesting that these three receptors overlap in their expression within the VMN.

In the MPN, although ER α is expressed at high levels, ER β

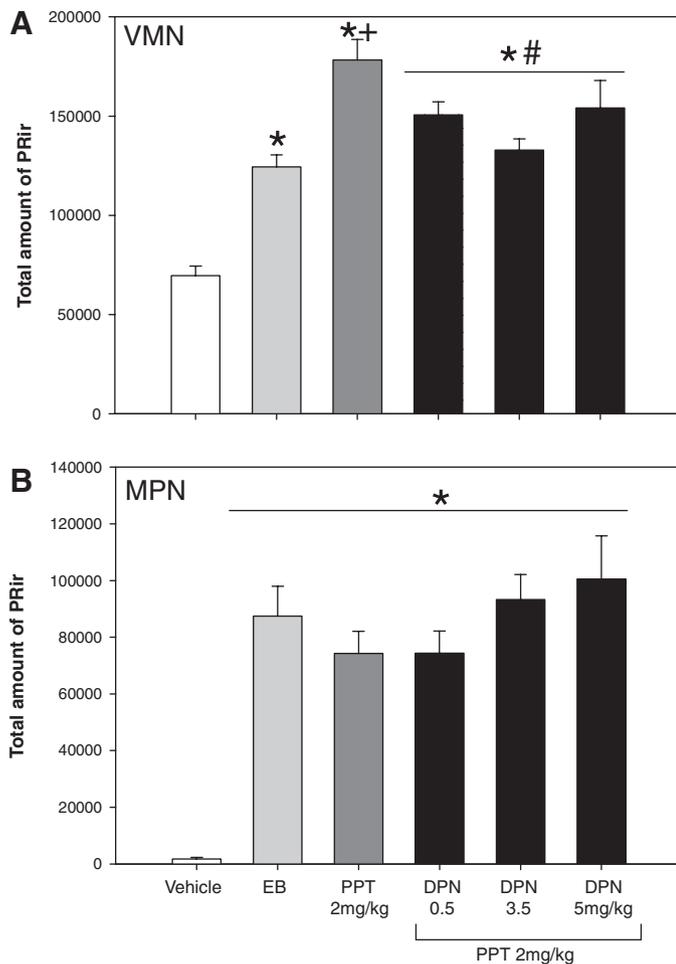


FIG. 2. Selective activation of ER β attenuates the induction of PR expression by ER α in the VMN, but not the MPN. The mean (SEM) total amount of PR-ir within the VMNvl or the MPN of female rats on P7 treated with vehicle ($n = 11$), EB ($20 \mu\text{g}/\text{kg}$, $n = 8$), or the ER α selective agonist, PPT ($2 \text{ mg}/\text{kg}$, $n = 8$) or PPT in combination with the ER β selective agonist, DPN, at 0.5, 3.5, or 5 mg/kg ($n = 10, 9, 7$) on P5 and P6. **A**, VMNvl. PR-ir levels were significantly higher after PPT treatment ($2 \text{ mg}/\text{kg}$) compared with EB treatment. The effect of PPT was significantly attenuated by the addition of DPN at all doses. PR-ir was significantly higher in all treatment groups compared with vehicle. **B**, MPN. PPT treatment alone, or PPT in combination with any dose of DPN, did not significantly alter PR-ir levels compared with EB treatment. PR-ir was significantly higher in all treatment groups compared with vehicle. *, Significantly different from vehicle ($P < 0.001$); +, significantly different from EB ($P < 0.01$); #, significantly different from PPT ($3.5 \text{ mg}/\text{kg}$ DPN $P < 0.001$; 0.5 and 5 mg/kg DPN, $P < 0.05$).

and PR expression is virtually absent (Fig. 4B). EB treatment dramatically alters PR-ir in the MPN but does little to PR-ir in the VMNvl. The ability of EB treatment to induce PR-ir in these regions coincides with the differential expression of ER β in MPN and VMN.

Discussion

The present results implicate ER β as an inhibitor of ER α transcriptional activity in the brain, representing a cellular level function for ER β *in vivo*. Selective activation of ER α by PPT, in the relative absence of ER β activation, induced PR expression in the neonatal VMN to a greater extent than

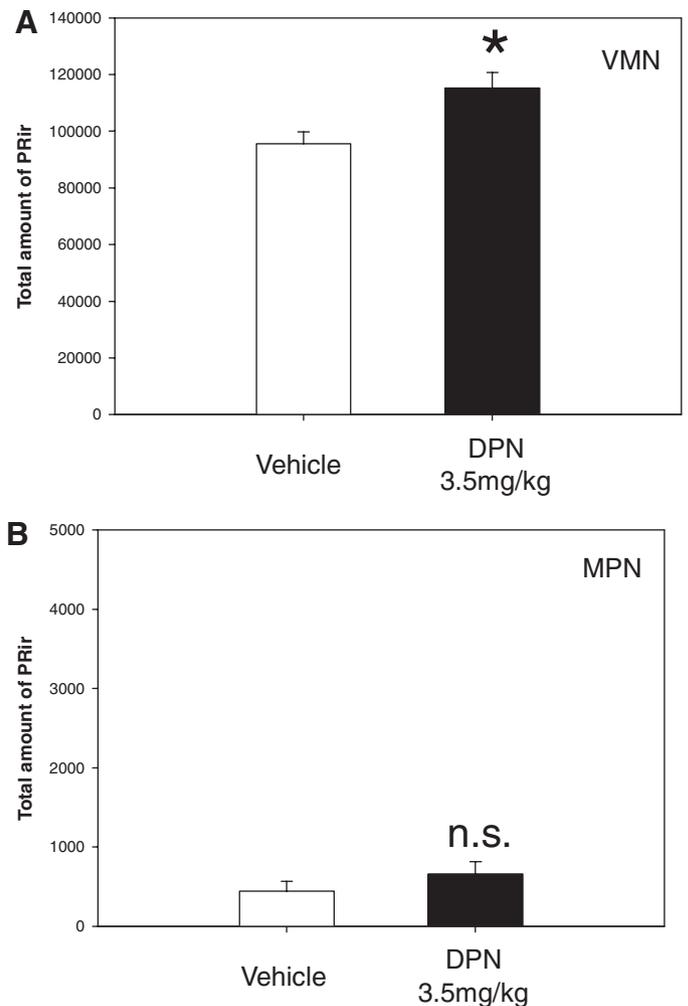


FIG. 3. DPN alone does not activate ER α in the MPN. The mean (SEM) total amount of PR-ir within the VMNvl or the MPN of female rats on P7 treated with vehicle ($n = 11$), or DPN at 3.5 mg/kg ($n = 10$) on P5 and P6. **A**, VMNvl. PR-ir levels were significantly higher after DPN treatment compared with vehicle. **B**, MPN. DPN treatment had no significant (n.s.) effect on PR-ir. *, Significantly different from vehicle ($P < 0.05$).

estradiol, which similarly activates both ER α and ER β . The PPT-induced increase was attenuated when ER β was activated with the selective agonist, DPN. These results suggest that activation of ER β by estradiol in the VMN inhibits ER α transcriptional activity, thereby suppressing ER α -mediated transactivation of the PR gene in this brain region. The inhibition of ER α transcriptional activity by ER β was specific to the VMN, which expresses both ER α and ER β . In the MPN, an area that expresses high levels of ER α but very low levels of ER β (28), PR was equally induced by EB and PPT, and the ER β agonist DPN had no effect, indicating an absence of an ER α /ER β interaction in this region. These data implicate ER β in the anatomically and developmentally specific effects of estradiol in the brain.

Estradiol can dramatically influence brain development as clearly demonstrated in the sexual differentiation of the rodent brain (22, 40). However, the actions of estradiol are often

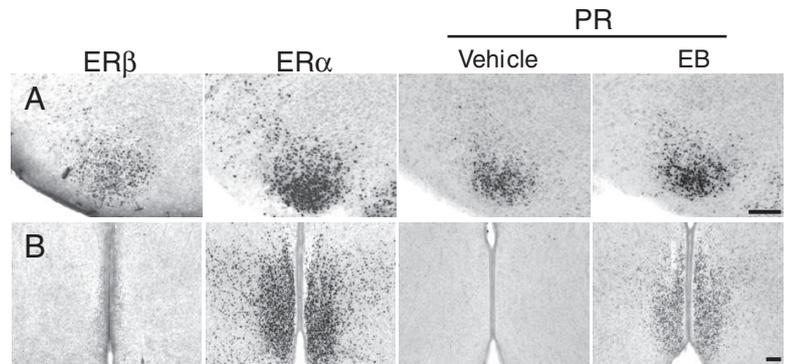


FIG. 4. Differential ER β expression coincides with the ability of estradiol to induce PR in the VMN and MPN. ER β , ER α , and PR immunoreactive nuclei within (A) the VMNvl or (B) the MPN of P7 female rats. PR-ir is shown in females treated with vehicle or with EB (20 μ g/kg) on P5 and P6. Magnification bars, 100 μ m.

anatomically specific, such that estradiol can exert strong effects in one area of the brain, whereas having little to no influence in other areas despite ample ER α expression (41, 42). For example, in the neonatal female rat brain, PR expression is increased 50-fold within the MPN after a single injection of estradiol 48 h before tissue collection (25). In stark contrast, PR expression is not altered in the VMN of the same animals and only becomes more responsive to estradiol later in postnatal development. Here, we demonstrate a potential mechanism underlying the regional specificity of estradiol action in developing brain.

Treatment with DPN alone (at the most effective dose from experiment 2) had no effect on PR-ir in the MPN in which ER β expression is very low, demonstrating that this dose of DPN was selective for ER β and did not significantly activate ER α . Interestingly, DPN alone increased PR levels slightly within the ER β -rich VMN of the same animals. This may be explained by findings from *in vitro* studies demonstrating that ER β , in the absence of ER α activity, can form homodimers capable of weakly inducing the transcription of ER α -driven reporter genes (7, 43–45). The present findings also document doses of PPT and DPN that when combined, mimic the effect of estradiol in our paradigm. It is noteworthy that DPN at the highest dose had a diminished inhibitory effect on PR-ir levels compared with lower doses in experiment 2. This is most likely due to the activation of ER α by high levels of DPN. Furthermore, we noticed a similar effect with the highest dose of PPT in experiment 1, suggesting that at high doses, the selectivity of these agonists may be diminished *in vivo*.

The differential regulation of estradiol signaling in the MPN and VMN can be attributed to the differential expression of ER β , and may be essential for the proper development of these two reproductively important brain regions as previously reported (36, 37, 39). The VMN is important for successful mating behavior in females (46, 47), whereas the MPN is essential for the complex repertoire of maternal behaviors after parturition (48). The present results are consistent with the idea that interactions between ER α and ER β within the developing VMN may be critical to protect the VMN from defeminization by estradiol during important periods of development, thus ensuring proper feminization of the VMN and subsequent adult female sexual behavior.

Results from Kudwa *et al.* (22) support this hypothesis by demonstrating that selective activation of ER α or ER β during development alters female sexual behavior in adulthood.

Additional work demonstrates that a role for ER β is not exclusive to sexual behavior but, rather, may mediate numerous neurological functions. ER β has been implicated in modulating behaviors such as spatial ability (21), anxiety (49, 50), and aggression [(51), and see Ref. 52 for an extensive review]. Furthermore, Bodo *et al.* (34) have suggested that ER β may be mediating the effects of estradiol through interactions with ER α . For example, Imwalle *et al.* (50) have demonstrated that ER β knockout (KO) mice have higher anxiety levels compared with ER α KO females and wild-type mice, suggesting that ER β may modulate the effects of estradiol through antagonistic effects on ER α activity. In addition, the ability of estradiol to induce PR expression in the medial preoptic area of adult ER α KO and ER β KO mice and ER α ER β KO mice suggests that ER α and ER β interact in complex ways to regulate the brain and behavior (27). Our results are consistent with many of these observations and provide a possible model to study the cellular mechanisms underlying the modulation of ER α by ER β within the brain.

The present results elucidating a novel role for ER β *in vivo* are consistent with *in vitro* work reporting that ER β inhibits the transcriptional activity of ER α by direct physical interactions between the two receptors, (*i.e.* heterodimerization and/or cofactor recruitment) (16, 17, 19). Present findings strongly suggest that ER α , ER β , and PR are expressed within overlapping cell populations within the VMN, but not the MPN, creating the possibility that ER α and ER β could have direct interactions with one another in PR expressing cells. However, the intriguing possibility also exists that indirect, transsynaptic regulation of ER α activity by ER β occurs in the developing brain. *In vitro* studies (17) as well as *in vivo* studies in adult brain (53, 54) demonstrate that estradiol increases the transcription of the PR gene. Therefore, changes in PR-ir levels in the present study are likely attributable to changes in transcription of the PR gene, but the possibility also exists that alterations in translation or turnover rate of PR protein may also occur within the developing brain.

The present studies elucidate mechanisms underlying estrogen signaling in brain, which becomes an increasingly important issue as the clinical use of estrogens and SERMs

increases. The present results implicate ER β in the inhibition of ER α transcriptional activity in the anatomically and developmentally specific actions of estradiol within the developing brain. Furthermore, we introduce the neonatal female VMN as a powerful *in vivo* model in which to study the mechanisms by which specificity of steroid-induced gene expression is achieved.

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