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Oxytocin, But Not Oxytocin Receptor, is Regulated by Oestrogen Receptor β in the Female Mouse Hypothalamus

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Abstract

In the female rat, oestrogen receptor (ER) β is colocalized with both oxytocin- and vasopressin-producing neurones in the paraventricular nucleus of the hypothalamus (PVN). In this study, we demonstrate that the same pattern of colocalization between ERβ and oxytocin exists in the female mouse. Because this nucleus contains only a negligible quantity of ERα, it is likely that the oestrogen-dependent regulation of oxytocin and vasopressin synthesis in the PVN is mediated by ERβ. Thus, we compared the effect of ovarian hormones on oxytocin and vasopressin mRNA expression in the PVN of wild-type (WT) and ERβ knockout (βERKO) mice. We also compared the effects of ovarian hormones on oxytocin receptor (OTR) expression in the medial amygdala (MeA) and ventromedial nucleus of the hypothalamus (VMN) in female WT and βERKO mice. Ovariectomized mice underwent long-term treatment with oestradiol or oil. Progesterone was given concurrently on the final 7 days of treatment, and all mice were killed 48 h after the final progesterone injection. In the PVN, hormone treatment increased oxytocin mRNA expression in WT but not βERKO females. These results suggest that ERβ is necessary for the regulation of the expression of oxytocin in the PVN. Hormone treatment had no effect on vasopressin mRNA expression in the PVN, but significantly increased OTR binding in both the VMN and the MeA in both genotypes. Collectively, our data show region and peptide specific regulation by ERα and ERβ in the mouse hypothalamus.

Following its initial discovery (1), the distribution of oestrogen receptor (ER) β across the rodent brain has been well characterized (2–5), but little is known about its functional role. Although the distributions of the two oestrogen receptor isoforms, ERα and ERβ overlap throughout much of the rodent brain, a few areas contain primarily one isoform, including the paraventricular nucleus (PVN), and the ventromedial nucleus of the hypothalamus (VMN). Because they contain only one ER subtype, these regions provide a unique window of opportunity to independently examine how and what each of these two receptor subtypes may be doing within the brain.

While little or no ERα has been found in rat PVN (6, 7), ERβ expressing cells are abundant, particularly the posterior magnocellular subdivision of the caudal region. ERβ expressing cells are also plentiful in the parvocellular subdivision of the ventral and dorsal zones and, to a lesser extent, the lateral and periventricular zones (7–9). In this study, we sought to determine whether this pattern of ERβ expression also exists in the female mouse.

Both the PVN and the supraoptic nucleus (SON) are major sites of oxytocin and vasopressin synthesis in the brain. The majority of oxytocin and vasopressin is produced in the magnocellular neurones of these two nuclei, then transported to and released from the neurohypophysis into systemic circulation (10). Systemic oxytocin is important for the milk-ejection reflex and parturition, while systemic vasopressin is necessary for proper water balance. In the PVN, a smaller quantity of oxytocin and vasopressin is synthesized in parvocellular neurones with projections to numerous brain areas, including the amygdala and the hippocampus (10). The oxytocin and vasopressin of parvocellular origin is thought to be important for many behavioural and cognitive functions, including social behaviour, parental behaviour, aggression, learning and memory (11–15). In the rat PVN, oxytocin is coexpressed with ERβ in both magnocellular and parvocellular neurones across the nucleus, but coexpression is most abundant in the caudal region (7, 9). By contrast, ERβ is very rarely coexpressed with vasopressin anywhere in the PVN.

While the expression and regulation of ERβ and oxytocin in the brain have been well characterized in the rat, fewer studies have been conducted in the mouse. ERβ mRNA is not as abundant in the PVN and nearly absent from the SON in the mouse brain (16). Here, we employed dual-labelling immunocytochemistry to characterize the coexpression of ERβ and oxytocin in the PVN of the female mouse brain.

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Early evidence demonstrating that puberty, castration, and the oestrus cycle alter the expression of oxytocin mRNA in the PVN suggests that oestrogen regulates the expression of oxytocin (17, 18). In rats, sequential administration of oestrogen and progesterone, followed by the withdrawal of progesterone, can reliably increase oxytocin expression in the PVN (19). The colocalization of ERβ with oxytocin expressing neurones in the PVN strongly suggests a role for ERβ in the oestrogen-dependent regulation of oxytocin in this nucleus. To test this hypothesis, we used ovariectomized wild-type (WT) and ERβ- knockout (βERKO) mice to examine the effects of oestrogen on both oxytocin and vasopressin production in the PVN in both the presence and absence of ERβ. If ERβ is necessary for the oestrogen-dependent regulation of oxytocin production in the PVN, hormone treatment should increase oxytocin mRNA in WT but not βERKO mice.

Finally, we examined the oestrogen-dependent regulation of oxytocin receptor (OTR) expression. Regulation of OTR expression by oestrogen in the VMN has been well characterized in the rat (20–22), but has not yet been shown in the mouse. We hypothesize that ERβ is not necessary for the oestrogen-dependent regulation of OTR mRNA expression in the VMN because, although ERα expressing cells are abundant in this region, ERβ is expressed in very few neurones in only in the most caudal ventral region of this nucleus (3). We have already demonstrated that ERα is essential for the oestrogen-dependent regulation of OTR in the medial amygdala (MeA) and, in this study, we sought to determine what role, if any ERβ, has in this mechanism (23). If our hypothesis that ERβ plays only a negligible role in these mechanisms is correct, the effect of hormone treatment on OTR regulation in the VMN and the MeA should be the same regardless of genotype.

Materials and methods

Animals

All mice were born, housed and treated at the University of Virginia, USA. For the immunocytochemistry study, female C57BL/6J mice (n = 5) were ordered from Jackson Laboratory (Bar Harbor, ME, USA) and housed in groups of two upon arrival in a temperature-controlled (23 ± 1 °C) and light-controlled 12 : 12 h light/dark cycle vivarium. For the *in situ* hybridization study, ERβKO and WT female mice (n = 34) were generated by mating mice heterozygous for the disrupted ERβ gene (24). The mice were of a mixed SV127 and C57BL/6J background, approximately five generations back-crossed into C57BL/6J. At all times, mice received Purina mouse chow (#5001) (Purina Mills, Richmond, IN, USA) and water *ad libitum*. All animal maintenance and procedures were in accordance with the NIH Guide for the Care and Use of Laboratory Animals.

Surgery and hormone treatment

For the immunocytochemistry study, 5 C57BL/6J females were ovariectomized (OVX) and administered oestrogen for 29 days via a subcutaneous 5 mm capsule made from Dow Corning Silastic® Laboratory Tubing (1.02 mm inner diameter × 2.16 mm outer diameter) (Dow Corning Corp., Midland, MI, USA) packed with 17β-oestradiol. Progesterone (100 μg/0.03 cc sesame oil) was then injected subcutaneously daily for 7 days. The oestrogen implant remained in place during the progesterone treatment and the animals were sacrificed 48 h after the final progesterone injection, resulting in a total of 34 days of hormone treatment. The animals were then deeply anaesthetized with a lethal dose of sodium pentobarbital perfused with heparinized-saline solution (approximately 35 ml) followed by 4% paraformaldehyde (180–200 ml). After perfusions, brains were removed and placed in 30% sucrose overnight. Cryoprotected brains were frozen in 2-methylbutane and stored at −70 °C until sectioning. Tissue was cut in a coronal plane at 30 μm on a cryostat. Sections were collected into a series of three vials and stored at −20 °C in antifreeze (TBS containing 30% sucrose; 30% ethylene glycol; and 1% PVP-40) until processing.

For the *in situ* hybridization, both WT and βERKO female (n = 34) mice were divided into four treatment groups: WT, no hormone treatment (n = 10), WT, hormone treatment (n = 10), βERKO, no hormone treatment (n = 7), and βERKO, hormone treatment (n = 7). Hormone treatment was administered as described above and animals receiving no hormone treatment received empty Silastic capsules. As with the animals for the immunocytochemical study, 48 h following the last progesterone injection, the mice were deeply anaesthetized with a lethal dose of sodium pentobarbital and sacrificed by decapitation. The brains were removed immediately, frozen on crushed dry ice and shipped to Emory University, USA. Upon arrival, brains were stored at −80 °C until use. The brains were cut on a cyrostat into sections 20-μm thick and thaw mounted on Superfrost plus microscope slides (Fisher, Pittsburgh, PA, USA). Four sets of serial sections were taken from the lateral septum to the caudal end of the VMN.

Immunocytochemistry

One of the three vials of tissue was employed for immunocytochemistry with antiserum against ERβ followed by incubation with oxytocin antiserum 48 h later. Sections were rinsed in TBS and treated with sodium borohydride (1% NaBH in cold TBS). Tissue was then incubated for 48 h at 4 °C in polyclonal rabbit anti-ERβ (Zymed Cat no. 51–7900, San Francisco, CA, USA; Z8P 1 : 1000) in TBS carrier solution containing 0.25% λ-carrageenan and 0.1% sodium azide, 0.5% bovine serum albumin (BSA) and 1.5% Triton-X. After 48 h, brain sections were rinsed in TBS and incubated in biotinylated goat antirabbit immunoglobulin (Ig) G (1 : 500, Vector Laboratories, Burlingame, CA, USA) for 1 h. Carrier solution for secondary antiserum was the same as described above except no azide was used. Tissue was rinsed and incubated in avidin-biotin-peroxidase complex (ABC 1 : 1000, Vector

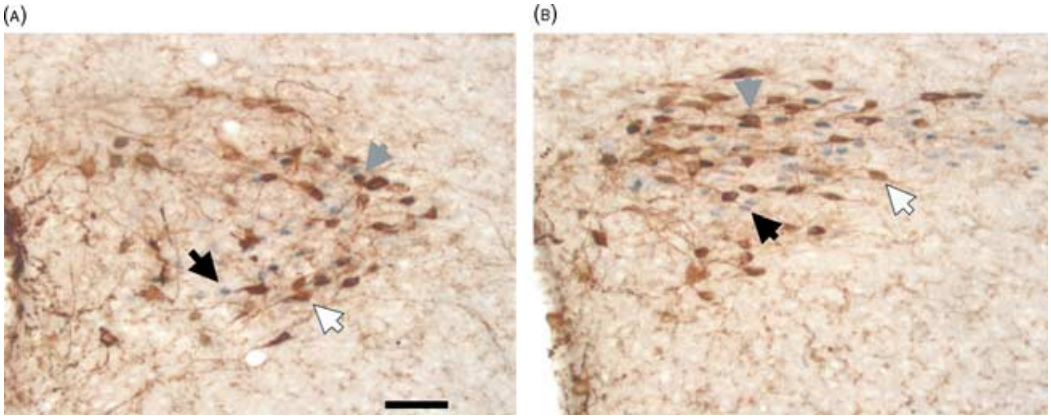

FIG. 1. Photograph of oxytocin-immunoreactive (OT-ir) (brown cytoplasmic; white arrow), oestrogen receptor (ER)β-ir (black nuclear; black arrow) and OT-ir/ERβ-ir (grey arrow) dual-labelled cells in the (A) anterior and (B) posterior portions of the paraventricular nucleus. Note that OT-ir/dval-labelled cells increase in number from anterior to posterior sections. Scale bar = 100 μm.

Elite Kit, Vector Laboratories) for 1 h. ABC carrier was the same as the secondary carrier with the omission of BSA. Immunoreactivity was visualized for ERβ using nickel-intensified (3% of 0.05 M $NiNH_4$) diaminobenzidine (0.015% DAB) with 0.002% hydrogen peroxide as the chromogen. After extensive rinses, tissue processed for oxytocin-immunoreactivity was incubated in monoclonal antisera (PS-36 oxytocin at 1 : 1000; generously provided by Dr Hal Gainer, NINDS). Then tissue was incubated in biotinylated horse antimouse IgG (1 : 250, Vector Laboratories) and staining was visualized using 0.04% DAB (without nickel) with hydrogen peroxide. All tissue was developed in DAB for the identical length of time. All antiserum had been previously validated for use in rodent brains (1, 25, 26).

Image analysis and counting

We selected the regions for quantification based on the Franklin and Paxinos mouse brain atlas (27). The paraventricular nucleus (PVN) was divided into anterior and posterior sections. The anterior PVN corresponds to panels 38 and 39 of the atlas (27) and includes the dorsal capsule, lateral magnocellular, medial magnocellular, and medial parvocellular portions of the PVN. Ventral landmarks were the position of the fornix, the shape of an optic tract and the supra-optic nucleus. The posterior PVN corresponds to panels 40 and 41 of the atlas (27) and includes the medial parvocellular nucleus. Ventral landmarks were the shape of the optic tract as well as the presence of the anterior medial amygdala.

Metamorph Image Analysis software was used to quantify immunoreactivity (Universal Imaging West Chester, PA, USA). Best-matched, uni-lateral sections containing the anterior and posterior sections of the entire PVN were captured at low magnification (27). Manual counts were conducted with the aid of Metamorph, which eliminated the possibility of counting cells more than once. Dual- and single-labelled oxytocin-immunoreactive (ir) cells were included in the counts only if a proximal fibre was visibly extending from the cell. We counted dual-labelled cells in which the nickel-stained nucleus was completely surrounded by brown cytoplasmic staining at a higher magnification within the same region specified above. These cells were selected only if the cell body and nucleus were in the same focal plane. The unit of area for the quantification was defined by the boundaries of the PVN. After quantification, several brains were restained with neutral red to verify all stained cells were within the PVN.

TABLE 1. Mean (±SEM) Oestrogen Receptor (ER)β-ir Nuclear, Oxytocin-Immunoreactive (ir), Dual-Labelled Cell (DLC) Numbers and Percentage of DLC in the PVN of C57BL/6J Female Mouse Brain.

	ERβ-ir	Oxytocin-ir	DLC	%DLC
Anterior	26.0 ± 5.6	27.7 ± 2.4	3.4 ± 0.8*	11.3 ± 2.8†
Posterior	38.4 ± 11.2	32.0 ± 3.8	6.7 ± 0.9	22.8 ± 4.6

*Significantly different from posterior section ($P < 0.03$). †Trend for a decrease from posterior section ($P = 0.064$).

In situ *hybridization*

Oxytocin *in situ* hybridization was performed using a single, 41 base, ^{35}S-labled oligonucleotide probe (GGG CTC AGC GCT CGG AGA AGG CAG ACT CAG GGT CGC AGG CG) complementary to nucleotides 906–946 of the rat oxytocin mRNA (GenBank Accession Number K01701). Vasopressin *in situ* hybridization was performed using a single, 48 base, ^{35}S-labled oligonucleotide probe (CAG AGC AAC GCC ACG CAG CTG GAC GGG CCA GCC CGG GAG CTG CTG CTT) complimentary to the region encoding amino acids 129–144 of vasopressin precursor peptide (GenBank Accession Numbers NM_016992, and NP_058688).

In situ hybridizations were performed using a well established protocol in our laboratory (28), except that the prehybridization step was omitted. Following *in situ* hybridization, the rinsed and dried sections were exposed to Kodak BioMax MR

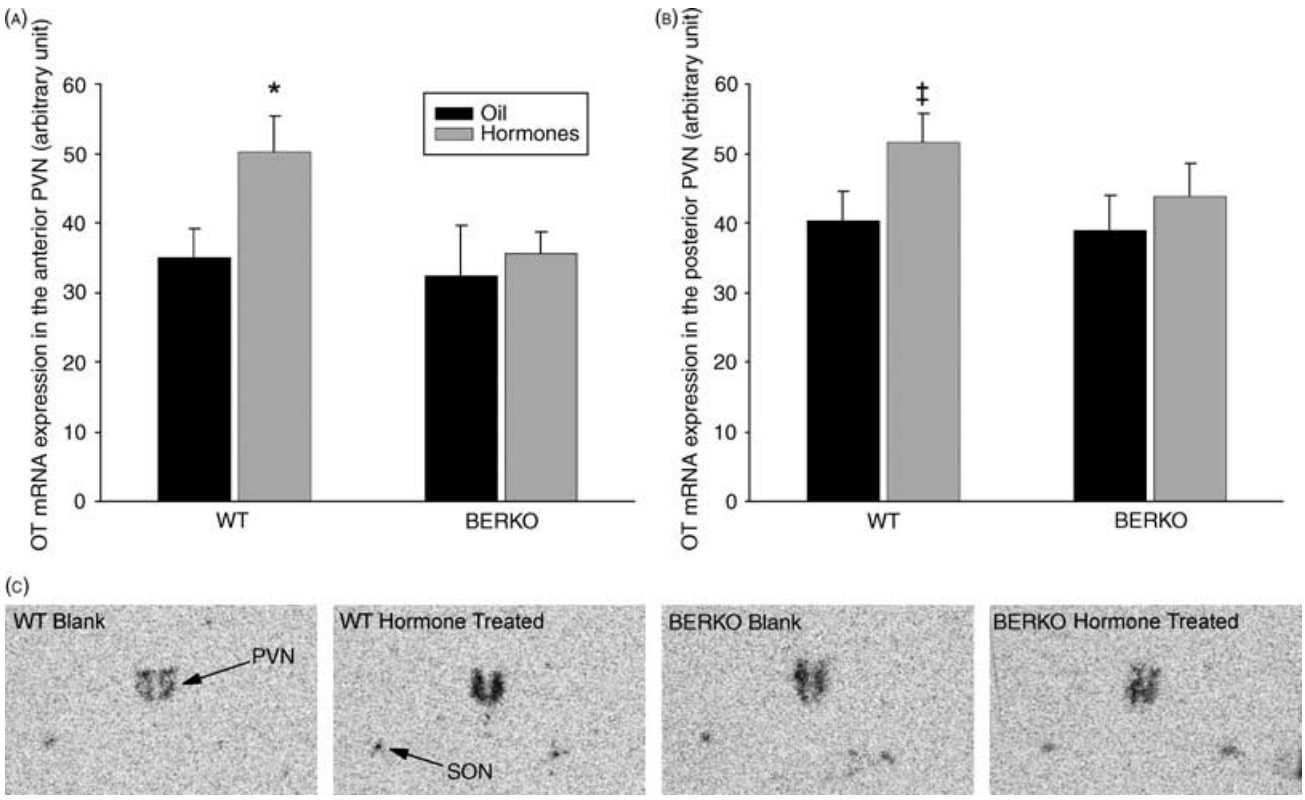

FIG. 2. Hormone treatment increased oxytocin (OT) transcripts in the paraventricular nucleus (PVN) of wild-type (WT) but not oestrogen receptor β knockout (βERKO) female mice. Concurrent oestrogen and progesterone treatment followed by progesterone withdrawal increased OT mRNA expression in the anterior (A) (*$P < 0.035$) PVN of WT but not βERKO female mice compared to their respective controls (Oil). Only a trend for increased OT mRNA expression in the WT mice was seen in the posterior PVN (B) (‡$P = 0.068$). This effect in the anterior PVN is clearly visible on the *in situ* films (C). Optical density is higher in the PVN of hormone treated WT females compared to the WT controls (Blank). Optical density in the PVN of the βERKO females is not statistically different regardless of treatment.

film (Eastman Kodak Co., Rochester, NY, USA) with ^{14}C-labled autoradiographic standards (Amersham Pharmacia Biotech, Arlington Heights, IL, USA) to produce autoradiograms for quantitative analysis. Oxytocin slides were exposed for 3 h and vasopressin slides were exposed for 9 days.

OTR autoradiography

OTR autoradiography was performed using ^{125}I-d $(CH_2)_5$[Tyr $(Me)_2$,Tyr-NH_2^9]OVT (NEN) as described previously (23). After air drying, the slides were exposed to BioMax MR film (Kodak) for 48 h. ^{125}I-Labled autoradiographic standards (Amersham Pharmacia Biotech) were included in the cassette for quantification.

Data acquisition and analysis

Image analysis of the *in situ* hybridization film was conducted on a PC using AIS imaging software (Imaging Research Inc., St Catharines, ON, Canada). The OTR autoradiograms were analysed on a Macintosh computer using the public domain Scion Image (available from http://rsb.info.nih.gov/nih-image/). Anatomically matched sections (two for vasopressin, three for OTR, and four for oxytocin) per subject were measured bilaterally and care was taken to ensure that the area of the regions selected for measurement did not differ by more than 5% between sections and subjects. Oxytocin mRNA was quantified in both the anterior (slightly rostral to Bregma −0.70 to −0.82) and posterior (Bregma −0.82 to −0.94) regions of the PVN, vasopressin was quantified across the entire PVN (Bregma −0.58 to −0.70) and OTR mRNA was quantified in the VMN and MeA. Vasopressin mRNA *in situ* optical densities were converted to nCi/g tissue equivalents using ^{14}C standards and OTR autoradiograms optical densities were converted to d.p.m./mg tissue equivalents using ^{125}I standards. Anterior and posterior oxytocin optical densities were quantified using arbitrary density measurements using ^{14}C standards exposed for 2 days.

Statistical analysis

The immunocytochemical data were analysed using one-way ANOVAs to detect differences between anterior (n = 5) and posterior (n = 5) PVN oxytocin-ir cytoplasmic cell counts, ERβ nuclear cell counts, the number of dual-labelled cells, and the proportion of dual-labelled cells. The number of female brains analysed are denoted by 'n'.

Data from the *in situ* hybridizations were analysed by two-way ANOVA with genotype and hormone treatment as factors. For the oxytocin mRNA *in situ* hybridization, this analysis was followed up with a Student's t-test comparing hormone treatment to control treatment within each genotype across the entire PVN, and then within the anterior and posterior regions. The effect of hormone treatment on vasopressin mRNA expression in the PVN was also compared with Student's t-test within each genotype. The OTR mRNA autoradioagraphs were analysed by two-way ANOVA with genotype and hormone treatment as factors, followed by post-hoc one-way ANOVAs for each treatment and genotype.

Results

Immunocytochemistry

The number of dual-labelled cells [oxytocin-ir/ERβ-ir; $F(1,9) = 7.58$, $P < 0.03$] increased from anterior to posterior sections (Fig. 1). However, oxytocin-ir cell counts [$F(1,9) = 0.89$] and ERβ-ir nuclear counts [$F(1,9) = 0.99$] were not significantly different between the anterior and posterior regions. A trend for a significant increase [$F(1,9) = 4.60$, $P < 0.064$] from anterior to posterior sections was noted for the proportion of dual-labelled cells (oxytocin-ir/ERβ-ir) (Table 1).

In situ *hybridization*

Hormone treatment increased oxytocin mRNA expression in the PVN of WT ($t = -3.058$, $P < 0.004$) but not βERKO females ($t = 0.785$, $P < 0.442$) compared to the ovariectomized control

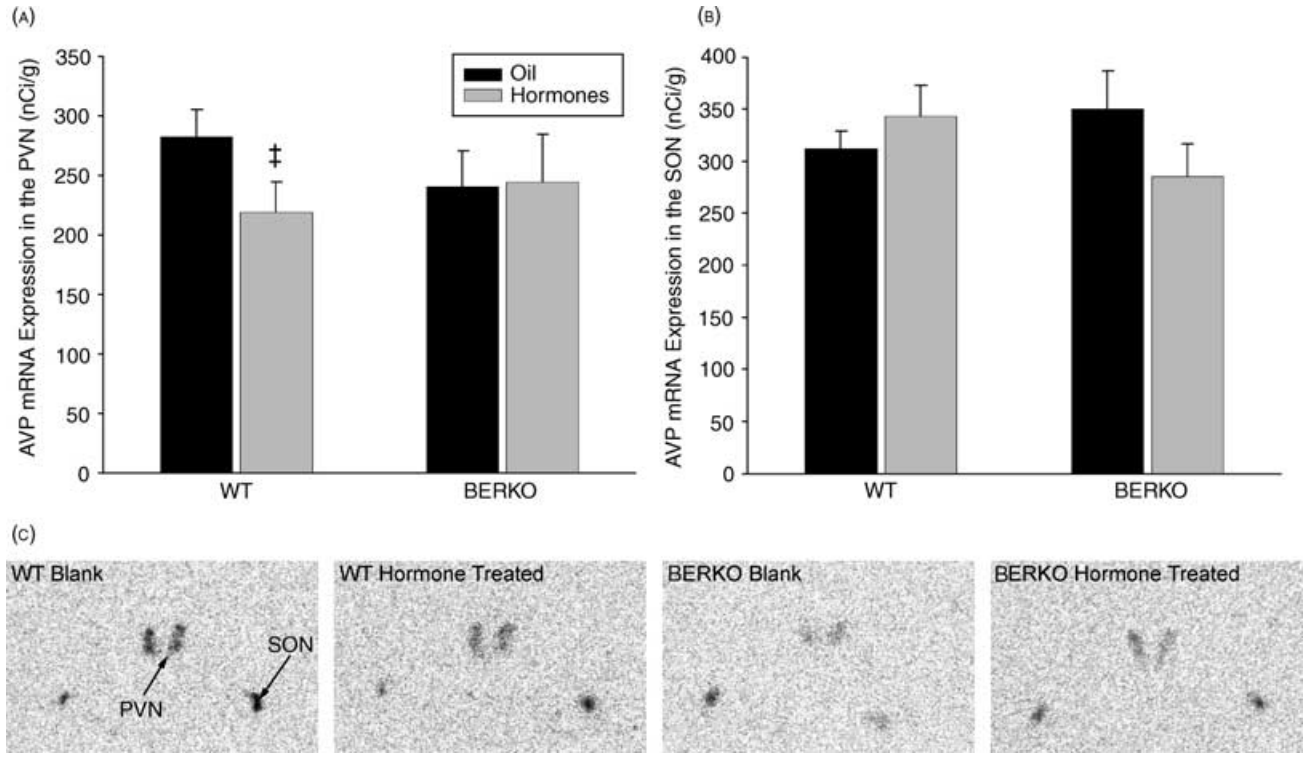

FIG. 3. Hormone treatment did not significantly affect vasopressin (AVP) transcripts in either the paraventricular nucleus (PVN) or the supraoptic nucleus (SON) of wild-type (WT) or oestrogen receptor β knockout (βERKO) female mice. Concurrent oestrogen and progesterone treatment followed by progesterone withdrawal only produced a trend for decreased AVP mRNA expression in the PVN (A) (‡P = 0.08) of WT female mice compared to their respective controls (Oil). Hormone treatment had no effect on the βERKO females in either the PVN or the SON (A,B). The *in situ* films show that optical density is not significantly lower in the PVN of the hormone treated WT females compared to the WT controls (Blank; C).

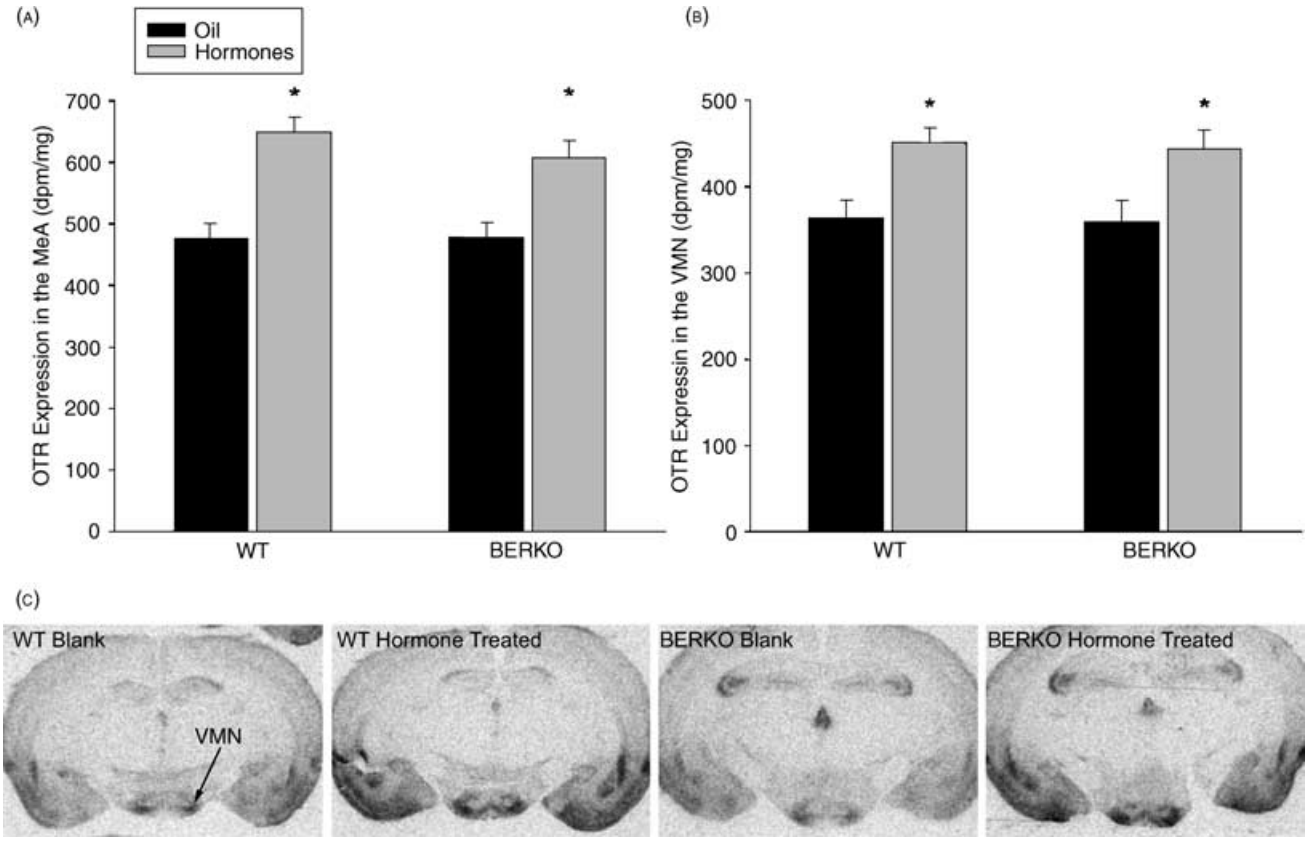

FIG. 4. Hormone treatment increased oxytocin receptor (OTR) binding in the medial amygdala (MeA) and the ventromedial nucleus of the hypothalamus (VMN) of both wild-type (WT) and oestrogen receptor β knockout (βERKO) females. Concurrent oestrogen and progesterone treatment followed by progesterone withdrawal significantly increased OTR binding in both the MeA (A) ($P < 0.0001$) and the VMN (B) ($P < 0.001$) of both WT and βERKO females. The effect of hormone treatment on OTR binding in the VMN is clearly visible on the *in situ* films (C). Optical density is greater in the VMN of both the hormone treated WT and βERKO females compared to their respective controls (Blank).

females. Oxytocin transcripts were 43.4% higher in the anterior region of the WT animals ($t = -2.302$, $P < 0.035$) but only 28% higher in the posterior region ($t = -1.939$, $P < 0.068$) of the WT animals compared to the controls (Fig. 2). By contrast, oxytocin mRNA expression was unaffected by hormone treatment in either the anterior or posterior regions of the PVN in the βERKO animals compared to controls.

In the initial analysis, there was no overall effect of either genotype ($F = 0.012$) or hormone treatment ($F = 0.186$) on vasopressin mRNA expression in the PVN. However, analysis with a t-test revealed a trend for decreased vasopressin mRNA expression by hormone treatment in the PVN of the WT animals (Fig. 3) ($t = 1.859$, $P < 0.08$) but not the βERKO animals compared to their respective controls.

Autoradiography

There was a significant effect of treatment, but not genotype on OTR binding in both the VMN ($F = 14.136$, $P < 0.001$) and the MeA ($F = 61.577$, $P < 0.0001$) in both genotypes (Fig. 4). Hormone treatment increased OTR binding in the MeA by 37% in the WT animals ($P < 0.0001$) and 27% in the βERKO animals ($P < 0.0001$). OTR binding was also increased by hormone treatment in the VMN of both the WT (24%; $P < 0.011$) and βERKO (23%; $P < 0.015$) animals.

Discussion

Concurrent oestrogen and progesterone treatment, followed by the withdrawal of progesterone significantly increased oxytocin transcripts in the PVN of ovariectomized female WT but not βERKO mice suggesting that ERβ is required for the hormone-dependent regulation of oxytocin. The effect was most pronounced in the anterior portion of the PVN, which is surprising since colocalization of ERβ-ir and oxytocin-ir cells was found to be most abundant in the posterior portion of the PVN. Basal oxytocin mRNA levels were comparable across the PVN in mice of both genotypes, but oxytocin mRNA expression was unchanged in the βERKO females following hormone treatment. These results suggest that ERβ plays a significant role in the oestrogen-dependent regulation of oxytocin in the PVN.

These results are consonant with numerous studies in the rat, demonstrating that concurrent treatment with oestrogen and progesterone, followed by the withdrawal of progesterone increases oxytocin mRNA levels in the PVN (19, 29). However, in rats, treatment with oestrogen alone is also sufficient to increase oxytocin mRNA expression in the hypothalamus (30), suggesting that, although progesterone may enhance the effect, oestrogen is the most essential ligand. Our results are also consistent with a recent report demonstrating that long-term oestrogen treatment alters oxytocin mRNA expression the PVN in male mice (31).

Oestrogen treatment increased oxytocin transcripts in both the middle and posterior regions of the PVN of male WT mice but not βERKO mice (31). Our study reveals that ERβ is also required for the hormone-dependent regulation of oxytocin in the PVN of female mice.

Hormone treatment only marginally decreased vasopressin transcripts in the PVN of WT females and, as expected, had no effect on vasopressin mRNA expression in the βERKO females. This result was surprising given that Nomura *et al*. (31) recently demonstrated that long-term oestrogen treatment significantly decreased vasopressin mRNA levels in the PVN of WT but not βERKO males. Based on these data, we hypothesized that the same pattern would be present in females. We may not have been able to replicate this effect in female mice for several reasons. First, because vasopressin transcripts in the PVN are so abundant, even relatively big changes in gene expression may be masked by the large pool of existing mRNA. It is also possible that the progesterone used in our hormone treatment attenuated the effect of oestrogen on vasopressin expression, thus diminishing the effect. Although progesterone appears to enhance the effect of oestrogen on oxytocin mRNA expression in the rat PVN (19), the same may not be true for vasopressin mRNA expression in the mouse. However, the most intriguing hypothesis is that the mechanisms governing the regulation of vasopressin mRNA levels in the PVN are more sensitive to hormone treatment in male mice than female mice, resulting in a sex difference in the regulation of vasopressin in the PVN. The distribution of vasopressin immunoreactive neurones is already known to be sexually dimorphic in several brain regions in the rat, including the medial amygdala and the bed nucleus of the stria terminalis (32, 33), and the same pattern of sexual differentiation may exist in mice (34). The PVN may be another sexually dimorphic brain region in the mouse.

Finally, we examined OTR binding in both the VMN and MeA of female WT and βERKO mice, regions where ERα is abundant but ERβ is scarce or absent (2–5). Although our previous work showed that ERα is essential for the oestrogen-dependent regulation of OTR binding in the mouse MeA (23), it was unclear what role, if any, ERβ played in this mechanism. Here, we demonstrated that OTR binding was significantly increased by hormone treatment in both the MeA and VMN of both WT and βERKO females. Collectively, these results suggest that while ERα is essential for the oestrogen-dependent regulation of OTR binding in both the MeA and the VMN, ERβ plays little to no role in this process. Our results are also consistent with earlier work demonstrating that oestrogen substantially increases OTR binding in the VMN of the female rat (20).

The relationship between oestrogen and oxytocin regulation is complex and still relatively unclear. Although oxytocin levels increase dramatically during pregnancy and lactation, in rats, hypothalamic oxytocin mRNA levels are unaffected by oestrogen unless it is accompanied by a progesterone administration and withdrawal (19). A functional hormone response element lies 160 bases upstream from the transcription start sight and is conserved across all species investigated, suggesting that it is the principle mediator of the influence of hormones on oxytocin production. However, this response element is not specific for either ER and has a much higher affinity for other receptors in the steroid hormone superfamily, including many orphan receptors (35). Although the rat PVN contains little to no ERα, ERβ is colocalized with both oxytocin and vasopressin-producing neurones throughout the PVN (7, 9). Here, we have demonstrated that, although this colocalization is present in the mouse, it is far less prevalent than it is in the rat. Our finding that ERβ is essential for the regulation of oxytocin and vasopressin transcripts by ovarian hormones is consistent with that pattern of colocalization in the PVN. However, given the complex relationship between oestrogen and oxytocin, it is unlikely that ERβ is the only receptor regulating the effects of oestrogen on oxytocin production. It is likely that indirect signalling from afferent neurones in other brain regions are also important. ERβ-containing nuclei including the bed nucleus of the stria terminalis, and the medial preoptic area (2, 3) project to the PVN and may also play a role in the regulation of both oxytocin and vasopressin transcripts in the PVN.

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