MOLECULAR AND CELLULAR NEUROSCIENCES 5, 283–290 (1994)

# Progesterone Modulation of Estrogen Receptors in Microdissected Regions of the Rat Hypothalamus[1]

THEODORE J. BROWN*,† AND NEIL J. MACLUSKY*,‡

*Division of Reproductive Science, The Toronto Hospital, and *Departments of Obstetrics and Gynecology, †Zoology, and ‡Physiology, The University of Toronto, Toronto, Ontario, Canada M5G 1L7*

Received for publication October 19, 1993

**One mechanism whereby progesterone opposes the regulatory actions of estrogen in the brain may include the down-regulation of estrogen receptors. A previous study has shown a small decrease in estrogen binding in the combined preoptic area–hypothalamic area in response to progesterone treatment. To determine if this effect is region specific, gonadectomized/adrenalectomized (GDX/ADX) estrogen-treated female rats were administered a single injection of progesterone (5.0 mg, sc) or a silastic capsule containing crystalline progesterone. Control animals were treated identically but without progesterone exposure. Animals were killed 24 or 72 h after initiating progesterone treatment and estrogen binding was measured in cytosol and cell nuclear extracts of the anterior pituitary, mediobasal hypothalamus (MBH), and preoptic area. A significant effect of progesterone injection on nuclear estrogen binding was observed in the MBH at 24 h. This effect had subsided by 72 h. No effect was observed when progesterone was administered in a continuous fashion. To further examine the regional specificity of the progesterone effect and to determine if males were similarly affected, nuclear-bound estrogen receptors were measured in microdissected brain regions from male and female estrogen-treated GDX/ADX rats treated with 5.0 mg progesterone or vehicle 24 h before sacrifice. A significant decrease in estrogen binding was found in the ventromedial nucleus of the female but not the male. A significant effect of progesterone treatment was found in the periventricular preoptic area of the male. These studies confirm that progesterone acts in the brain to down-regulate estrogen binding and demonstrate that this action is not evenly displayed in all areas of the brain containing high levels of both estrogen and progestin receptors.** 

[1] This work was supported by grants from MRC Canada to T.J.B. (MT-11235) and to N.J.M. (PG-11115; Program Grant in Birth and Perinatal Development).

## INTRODUCTION

Progesterone is an important modulator of estrogen action in the brain, where it both enhances and opposes the regulatory actions of estrogen on reproductive function (1, 2). This dual action of progesterone is perhaps best illustrated by the hormonal regulation of female sex behavior (2). During the rodent estrous cycle, the gradually increasing release of estradiol during diestrus and proestrus, followed by a preovulatory surge of progesterone on the afternoon of proestrus, triggers the onset of a discrete period of sexual receptivity (3, 4). The action of progesterone on this behavior is initially facilitatory to the actions of estradiol; but subsequently inhibits further activation of sexual behavior (5). Thus, after termination of a receptive period activated by sequential estradiol and progesterone treatment, a second exposure to progesterone is unable to activate receptivity. Likewise, if progesterone is administered concurrently with estradiol, the activation of a receptive period by a subsequent progesterone injection is prevented (6, 7).

Both the facilitatory and desensitization effects of progesterone on sex behavior have been correlated with effects on progestin receptor availability in the hypothalamus (5, 8, 9). Estrogen treatment increases progestin receptor levels in the brain, and subsequent progesterone treatment results in a transient increase in occupied nuclear-bound progestin receptors, the duration of which correlates with the duration of the receptive period (10–15). However, as the concentration of occupied nuclear bound progestin receptors returns to baseline, the receptors are not made available for binding additional progesterone (11, 12). Thus, subsequent progesterone administration appears to be ineffective due to the lack of sufficient progestin receptor. This progesterone-induced down-regulation of progestin receptors may play a key role in the termination of the sexually receptive period (12–15) and could have important implications for the action of progesterone on the duration of the LH surge (1).

The mechanism by which progesterone induces down-regulation of the progestin receptor may involve effects



1044-7431/94 $6.00

on estrogen binding. Progestin receptor down-regulation resulting from progesterone action occurs even when estradiol is administered in a continuous manner, thus raising the possibility that progesterone interferes with estrogen-induced progestin receptor synthesis (8, 9). Leavitt and co-workers (16–19) have demonstrated that progesterone action has a marked effect on nuclear estrogen binding in the uterus. Progesterone induces a factor that selectively decreases occupied cell nuclear estrogen receptors, thereby disrupting the estrogen response. A similar effect has been reported in the hypothalamus preoptic area (20). Progesterone exposure resulted in a small but consistent decrease in unoccupied (cytosolic) estrogen receptors by 6 h after progesterone administration followed at 24 h by a decrease in occupied (nuclear) estrogen receptors (20). One explanation for the small size of this response might be that progesterone modulation of estrogen receptors occurs in only select regions or cell groups within the hypothalamus/preoptic area. Reports that estrogen receptor occupation is differentially modulated in the preoptic area and mediobasal hypothalamus during pregnancy are consistent with this idea (21, 22). Nuclear estrogen receptor concentrations in the preoptic area increase gradually over the course of pregnancy, whereas receptor levels in the mediobasal hypothalamus remain low until near parturition, when progesterone levels decline (21).

In the present study, we examined the effects of progesterone treatment on nuclear estrogen binding in discrete areas of both the male and female rat brain using a microdissection procedure. Our results confirm the previous finding of a progesterone-induced decrease in hypothalamic estrogen binding (20) and indicate that the effect is most pronounced in the ventromedial nucleus of the hypothalamus, the principal site for the hormonal activation of female sex behavior (23, 24).

## MATERIALS AND METHODS

### *Animals*

Sprague–Dawley CD-strain rats (Charles River Breeding Laboratories, Wilmington, MA) 70–75 days of age were group-housed in plastic cages with food and water freely available. A 12-h light/12-h dark photoperiod cycle was maintained with lights on beginning at 1900 h. Animals were bilaterally gonadectomized (GDX) and adrenalectomized (ADX) under ether anesthesia 8 days before beginning estrogen treatment. Normal saline was substituted for drinking water after adrenalectomy to help maintain electrolyte balance.

### *Hormonal Treatment*

Estradiol-17$\beta$ was administered by subcutaneous placement of a 1.0-cm capsule containing crystalline estradiol-17$\beta$ diluted to 10% with cholesterol. Capsules were constructed from Silastic medical grade tubing as described previously (25). Progesterone was administered either as a single subcutaneous injection in sesame oil (5 mg/rat/0.1 ml) or as a subcutaneously placed 2.0-cm capsule containing crystalline progesterone. All capsules were incubated in phosphate-buffered saline containing 0.1% BSA for 24 h at 37°C immediately preceding placement in the nape of the neck (estradiol capsules) or in the axillary region (progesterone capsules). Capsule implantation was performed under light ether anesthesia.

### *Brain Dissection*

For experiments involving grossly dissected brain regions, animals were killed by decapitation and their brains were rapidly removed and placed on a chilled glass plate. The mediobasal hypothalamus (MBH) and preoptic area (POA) were dissected with a chilled razor blade and placed in 1.0-ml cold buffer TEGT (10 m*M* Tris–HCl, 1.5 m*M* $Na_2EDTA$, 10% (v/v) glycerol, 12 m*M* monothioglycerol, pH 7.4). The anterior pituitary gland was also removed and placed in buffer. The wedge-shaped MBH sample was bounded rostrally by the caudal edge of the optic chiasm and caudally by the posterior edge of the mammillary bodies. Two cuts made from the top of the third ventricle to the lateral hypothalamic sulci formed the lateral boundaries. The POA sample extended approximately 2-mm rostral to the hypothalamic sample. A cut made in a line continuous with each of the lateral ventricles formed the lateral boundaries, and a horizontal cut at the level of the anterior commissure formed the dorsal boundary.

For the measurement of estrogen receptors in microdissected brain regions, animals were killed by intracardial perfusion of ice-cold 10% dimethyl sulfoxide (DMSO) followed by decapitation. DMSO serves as a cryoprotectant and minimizes any possible loss of estrogen receptors due to tissue freezing and thawing (26). Brains were rapidly removed, frozen onto cryostat chucks using powdered dry ice, and stored at −80°C. The brains were transferred to an IEC cryostat maintained at −15°C and allowed to equilibrate for at least 20 min before sectioning. Twelve serial 300-$\mu$m-thick sections were cut and adhered onto glass slides, with the first section corresponding to section A7020 of the atlas of Konig and Klippel (27). The mounted sections were transferred to a freezing microscope stage (Cambion, Cambridge, MA) maintained at −15°C and the periventricular preoptic area (PVP), medial preoptic area (mPO), bed nucleus of the stria terminalis, arcuate–median eminence region, ventromedial nucleus (VMN), and corticomedial amygdala were dissected by the punch method of Palkovits (28) using 500 and 1000 $\mu$m diameter stainless steel punches as illustrated previously (29).

### *Combined Cytosol and Nuclear Estrogen Receptor-Binding Assay*

Tissues were homogenized in 1.0 ml TEGT buffer with a Teflon pestle with adequate clearance for cell nuclei.

Homogenates were transferred to 12 × 75-mm polycarbonate tubes and centrifuged at 1000*g* for 10 min. The supernatant was transferred to ultracentrifugation tubes and centrifuged at 105,000*g* for 45 min to obtain cytosol. The pellet from the first centrifugation was resuspended in 1.0 ml buffer BI (1.0 m*M* $KH_2PO_4$, 3.0 m*M* $MgCl_2$, 0.32 *M* sucrose, pH 6.5) and 1.0 ml buffer BII (1.0 m*M* $KH_2PO_4$, 1.0 m*M* $MgCl_2$, 2.4 *M* sucrose, 0.25% Triton X-100, pH 6.5) was added. To this, 1.5 ml buffer BIII (1.0 m*M* $KH_2PO_4$, 1.0 m*M* $MgCl_2$, 1.8 *M* sucrose, pH 7.0) was carefully added to displace the BI/BII mixture upward. Following centrifugation for 35 min at 20,000*g* (Sorvall RC3B, HB-4 rotor), the supernatant was discarded and nuclear-bound estrogen receptors were salt extracted from the pellet (cell nuclear material) as described by Roy and McEwen (30). The pellet was resuspended in 100 μl buffer TEGTB (TEGT + 0.5 m*M* bacitracin). A salt extract of the nuclear preparation was obtained by adding an equal volume (100 μl) of buffer $TEGTBK_{0.8}$ (TEGTB + 0.8 *M* KCl), resulting in a final KCl concentration of 0.4 *M*. After allowing 30 min for full extraction, the cell nuclear extracts were centrifuged at 20,000*g* for 10 min to pellet the DNA. The supernatant was transferred to conical tubes and the pellets were digested in 0.3 *N* KOH for DNA measurement by the method of Burton (31).

Cytosol estrogen receptors were measured by incubating 200 μl cytosol for 16 h at 0–4°C with 100 μl buffer TEGT containing 2.5 n*M* [$^3$H]estradiol (2.5 n*M* final concentration, sp act 96.4 Ci/mmol; New England Nuclear, Boston, MA) with or without a 100-fold molar excess of unlabeled diethylstilbestrol (DES). Bound [$^3$H]estradiol was separated from free by gel filtration on 5 × 60-mm Sephadex LH-20 columns (32). An aliquot (250 μl) of incubate was loaded onto the columns and washed into the column bed with 150 μl buffer TEGT. The macromolecular-bound fraction was eluted into scintillation vials 30 min later by adding 700 μl buffer TEGT. Radioactivity was extracted overnight into 5 ml Betacount scintillation fluid (ICN) and counted for 10 min in a Taurus Micromedic 400 scintillation counter at 50% efficiency. An aliquot of cytosol was taken for cytosol protein quantification by the dye-binding method of Bradford, with BSA as the standard (33). Specific binding was calculated by subtracting binding measured in the presence of unlabeled DES (nonspecific binding) from that measured in the absence of unlabeled DES (total binding). Results are expressed as fmol [$^3$H]estradiol specifically bound per milligram cytosol protein.

Cell nuclear estrogen receptors were measured by incubating 75 μl of the nuclear extract at 25°C for 4.5 h with 50 μl buffer TEGT containing [$^3$H]estradiol (2.5 n*M* final concentration) with or without a 100-fold molar excess of unlabeled DES. Bound [$^3$H]estradiol was separated from free by gel filtration on 5 × 60-mm Sephadex LH-20 columns as described for cytosol receptors except that 100 μl of incubate was loaded onto each column and washed into the column bed with 100 μl buffer $TEGTBK_{0.4}$. The macromolecular-bound fraction was eluted into scintillation vials 30 min later by adding 400 μl buffer $TEGTBK_{0.4}$. Results are expressed as fmol [$^3$H]estradiol specifically bound per milligram DNA.

*Cell Nuclear Estrogen Receptor Assay for Microdissected Brain Regions*

Cell nuclear estrogen receptors were measured by *in vitro* exchange assay. Cell nuclei from microdissected brain regions were prepared as described by MacLusky *et al.* (34). Briefly, tissues were homogenized in 200 μl TEGT buffer and centrifuged at 8700*g* for 1 min. The resulting pellets were washed twice by resuspension in 200 μl TEGT followed by centrifugation at 8700*g* for 1 min. The pellets were then suspended in 100 μl TEGTB and an equal volume of $TEGTBK_{0.8}$ was added, resulting in a final KCl concentration of 0.4 *M*. After a 30-min extraction period, the tubes were centrifuged at 8700*g* for 1 min. Aliquots (75 μl) of the supernatant were incubated for 4.5 h at 25°C with 50 μl TEGT buffer containing 2.0 n*M* (final concentration) [$^3$H]estradiol with or without a 100-fold molar excess of unlabeled DES. Bound [$^3$H]estradiol was separated from free by gel filtration as described above. The DNA content of the nuclear pellets was determined by the ethidium bromide fluorescence method of Boer (35). Results are expressed as fmol [$^3$H]estradiol specifically bound per milligram DNA.

*Statistical Analysis*

Initial data analysis was accomplished by two-way analysis of variance (ANOVA) using Statgraphics software (Statistical Graphics Corp., Rockville, MD) for the IBM PC. Further comparisons were made by Student's *t* test or by Duncan's multiple range test if a significant gender by brain region effect was found. Differences were considered statistically significant at the $P < 0.05$ (two-tailed) level.

## RESULTS

*Progesterone Modulation of Estrogen Receptors in the POA and MBH: Comparison between Progesterone Injection and Progesterone Capsules*

Previous work in estrogen-treated rats has shown that 6 h after a single injection of 5 mg progesterone, cytosol estrogen receptor levels in the combined MBH–POA are reduced (20). A subsequent decrease in occupied nuclear estrogen receptors at 24 h indicated that this decrease is reflected in functional nuclear receptor binding. To confirm and extend this initial finding, the effect of progesterone treatment on estrogen receptors in the preoptic area and in the mediobasal hypothalamus was compared to determine if the regulation occurs selectively in either

brain region. In addition, the effect of a single progesterone injection (5 mg) and the effect of a subcutaneously placed progesterone capsule were compared to determine if the magnitude of the effect could be increased by delivering progesterone continuously. GDX/ADX female rats received a subcutaneously placed 10% estradiol capsule that was designed to produce circulating estradiol levels similar to those measured during proestrus (25). Seventy-two hours after estradiol capsule insertion, all animals were anesthetized and were administered either a single progesterone injection (5 mg) or a 2.0-cm subcutaneously placed capsule containing progesterone. Animals in the control group that had received estrogen capsules were also anesthetized and received a 2.0-cm empty capsule. A fourth group of animals treated with sham estrogen and progesterone capsules was included as a second control group to indicate receptor levels in the absence of hormone. All animals were killed 24 or 72 h after progesterone/sham capsule insertion or progesterone injection. Estrogen receptor levels were measured in cytosol and cell nuclear extracts from the anterior pituitary gland, mediobasal hypothalamus, and preoptic area.

As expected, estrogen treatment caused a marked decrease in estrogen receptors measured in the cytosol fractions and an increase in receptors measured in cell nuclear extracts. In both the preoptic area and mediobasal hypothalamus, but not the anterior pituitary gland, nuclear estrogen binding declined from 24 to 72 h in the absence of progesterone treatment. In the mediobasal hypothalamus this decline was very slight and did not attain statistical significance, whereas the decline was more than 25% in the preoptic area (Fig. 1).

Injection of 5 mg progesterone had no apparent effect on progestin receptors measured in cytosol fractions from either the mediobasal hypothalamus or the preoptic area; however, a 20% decrease in nuclear estrogen receptors was detected in the mediobasal hypothalamus at 24 h (Fig. 1). This decrease in nuclear binding was not present at 72 h after injection. Although a similar trend was present in the preoptic area, this effect did not attain statistical significance. Progesterone injection resulted in an increase in estrogen binding in cytosol extracts from the anterior pituitary gland at 24 h. However, this was not reflected in changes in nuclear binding at either 24 or 72 h after injection.

No significant effects of progesterone capsule insertion on estrogen binding were observed in either cytosol or cell nuclear extracts of any of the tissues examined.

*Progesterone Modulation of Estrogen Receptors in Microdissected Brain Regions*

To further examine the regional specificity of the progesterone-induced decrease in estrogen binding, a microdissection procedure was used which allowed the sampling of discrete areas of the hypothalamus–preoptic area. Male rats were also included in this experiment to determine if progesterone similarly affects estrogen binding in the male brain. GDX/ADX male and female rats received a subcutaneously placed 10% estradiol capsule. All animals received a subcutaneous injection of 5 mg progesterone or vehicle 72 h after estrogen capsule insertion and 24 h before sacrifice.

Results of this experiment are shown in Fig. 2. ANOVA indicated an overall effect of progesterone on nuclear estrogen binding ($F_{(1,236)} = 8.914$, $P < 0.005$). Duncan's multiple range test revealed a significant decrease in estrogen binding in the VMN of the female and in the PVP of the male as a result of progesterone exposure. Consistent with our previous studies (25, 29, 36), a significant sex by region interaction was apparent ($F_{(5,236)} = 6.122$, $P < 0.0001$), reflecting greater estrogen binding in the PVP, mPO, and VMN of the female as compared to that of the male (Fig. 2).

## DISCUSSION

Progesterone acts as a potent modulator of estrogen binding in the uterus, causing a rapid down-regulation of the occupied nuclear receptor followed by a reduction of unoccupied or "available" receptor measured in cytosol extracts. Attempts to show a similar mechanism in the brain have yielded mixed results. Lisk and Reuter (37) reported that progesterone acts in the hypothalamus to enhance the retention of nuclear bound occupied estrogen receptors. In contrast, Smanik et al. (38) and Attardi (39) reported that progesterone had no effect on estrogen binding in either the anterior pituitary gland or the brain. Blaustein and Brown (20) provided the first evidence that progesterone acts to down-regulate neural estrogen receptors, but with a mechanism that is distinct from that reported for the uterus. A slight but consistent effect of progesterone on estrogen binding in the combined mediobasal hypothalamus–preoptic area was observed; however, in contrast to the uterus, the effect of progesterone in the brain was directed initially at unoccupied receptors, with a decrease apparent 6 h after progesterone injection. A subsequent decrease in occupied receptors was observed 24 h after progesterone injection. The magnitude of the down-regulation as well as the difficulties experienced by others in observing a similar effect suggested the effect might be limited to specific regions of the hypothalamus–preoptic area.

The results of the present study confirm the finding that progesterone can act to decrease estrogen binding in the female rat brain and indicate that the effect occurs primarily in the mediobasal hypothalamus, particularly the ventromedial nucleus of the hypothalamus. This brain region has been demonstrated by hormonal implant studies (23, 24) to be the principal neural site for the activation of female sex behavior by both estradiol and progesterone. Moreover, Rubin and Barfield (40) have demonstrated that the inhibitory actions of progesterone (i.e., desen-

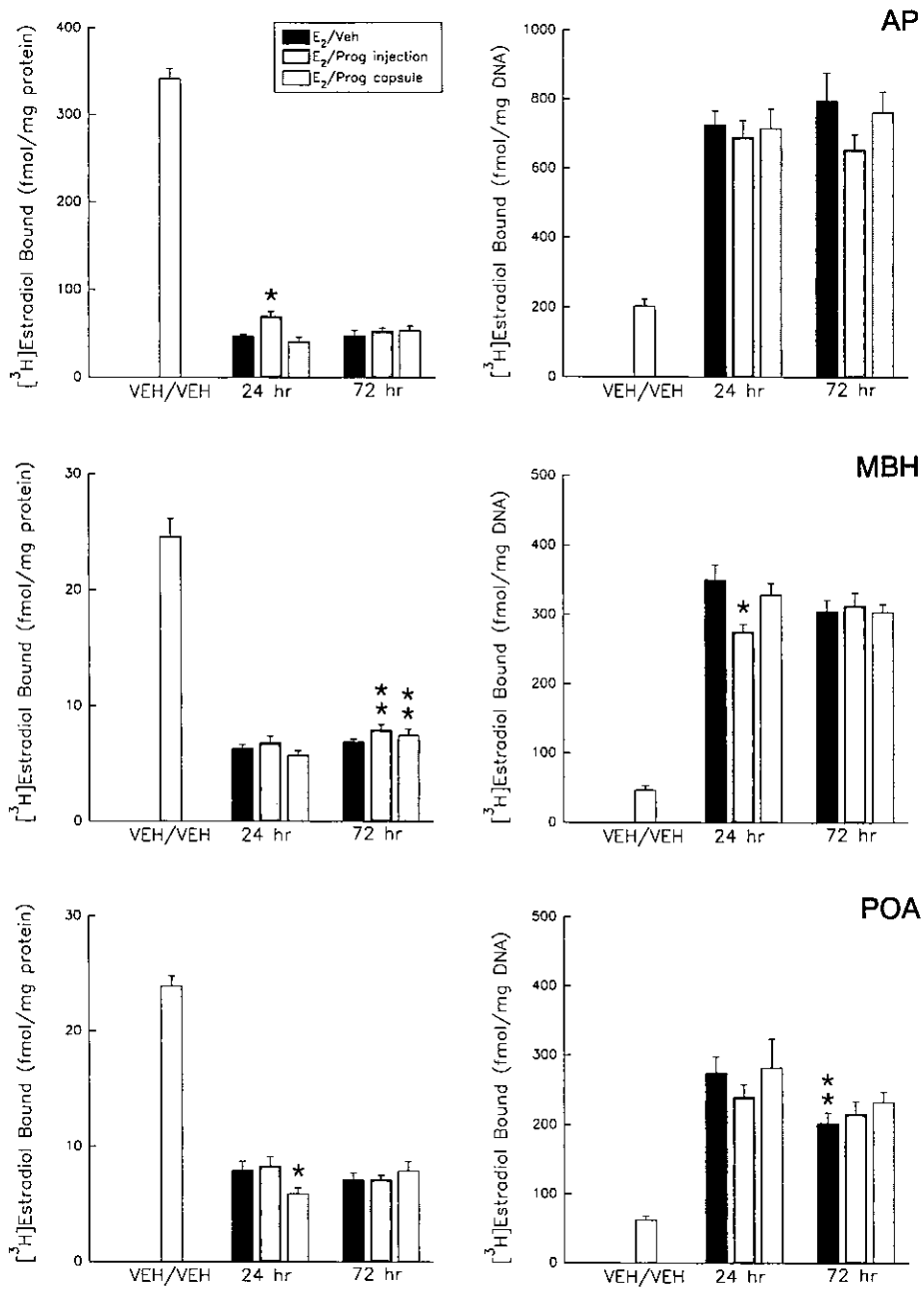


**FIG. 1.** Estrogen receptor measurements in cytosol (left) and cell nuclear (right) extracts of anterior pituitary (top), mediobasal hypothalamus (middle) and preoptic area (bottom) from GDX/ADX female rats treated with an estradiol capsule for 3 days and then a single injection of 5 mg progesterone ($E_2$/prog injection), a 2.0-cm progesterone capsule ($E_2$/prog capsule), or a vehicle injection and sham capsule ($E_2$/veh). Receptors were measured 24 and 72 h after progesterone injection or progesterone capsule insertion. Nonestrogen- and -progesterone-treated rats were included for comparison (veh/veh; open bars). Bars indicate the mean ± SEM for five or six observations per group. *Significant progesterone effect (group differs from $E_2$/veh group within the same time point). **Significant time effect (group differs from the same group at 24 h).

sitization effects) on female sex behavior occur in this brain region as well.

The mechanism whereby progesterone causes a down-regulation of estrogen binding in the brain is currently unknown. Scatchard analysis has demonstrated that the decrease in binding reflects a decrease in the number of estrogen-binding sites and not a decrease in receptor affinity (20); thus the decreased binding could reflect either increased receptor degradation and/or decreased receptor synthesis. Moreover, it is likely that the effect is not exerted equally upon all estrogen-sensitive cells. Although the anatomical resolution made possible by the adaptation of the microdissection procedure has allowed us to examine individual brain nuclei, it does not allow us to look

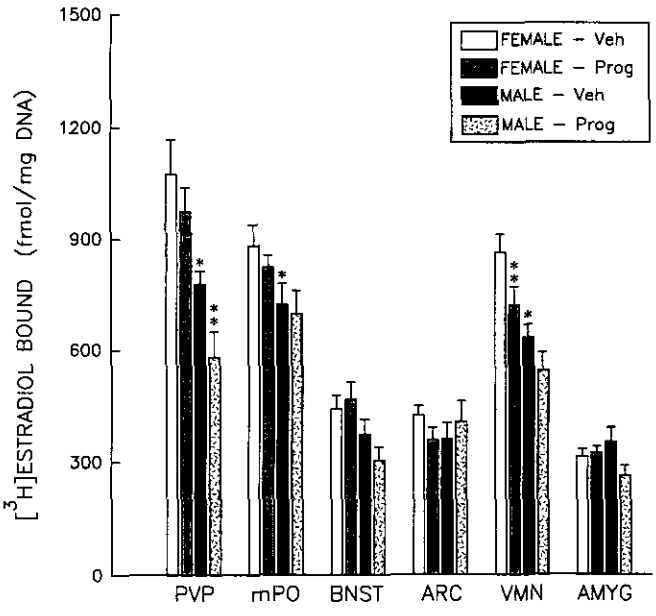


**FIG. 2.** Estrogen receptors measured in cell nuclear extracts from microdissected brain regions from GDX/ADX male and female rats killed 24 h after a single injection of either progesterone (prog) or vehicle (veh) and 96 h after placement of a 1-cm 10% estradiol capsule. Bars represent the mean ± SEM of 7–13 observations per group. *Significant sex difference in animals treated with a vehicle injection. **Significant progesterone effect.

*within* target structures on a cell-by-cell basis. It is possible that the changes we have observed in microdissected samples may reflect much larger changes restricted to a particular phenotype of estrogen target neuron. Quantitative autoradiographic or immunocytochemical studies may be necessary to resolve this issue.

Consistent with previous studies from our laboratory (25, 29, 36), higher concentrations of estrogen binding were measured in the female PVP, mPO, and VMN than in the male. These sex differences have been shown to be reflected in the ability of estradiol to increase intracellular progestin receptors (25, 41, 42). Whereas the fact that progesterone administered to the male did not affect estrogen binding in the VMN can be explained by a sex difference in progestin receptor content, the progesterone-induced decrease observed in the PVP was unexpected. Although this brain region plays an important role in the regulation of gonadotropin secretion, it is not known what role progesterone normally plays in male reproductive physiology; thus the significance of this effect is difficult to assess.

In a recent studies, Giordano *et al.* (21) reported that the pattern of nuclear estrogen receptor retention in the MBH and POA differs markedly during pregnancy. In the POA, nuclear estrogen receptors increased on Day 10 and remained elevated for the duration of pregnancy. In contrast, nuclear estrogen receptor levels in the MBH remained low throughout pregnancy, until around Day 22 when levels were found to be elevated. This suppression of nuclear estrogen receptor concentrations was most evident in the VMN (43). This may provide an explanation for the lack of female sex behavior displayed during pregnancy despite the presence of circulating estradiol and progesterone. Because the release from this suppression parallels the decline in circulating progesterone levels toward the end of pregnancy, one possibility is that progesterone acts selectively in the MBH to suppress estrogen receptor levels. While our data demonstrate such an action of progesterone, they also indicate that other contributory processes may be involved. First, progesterone administered in a continuous fashion failed to affect nuclear estrogen-binding levels. Second, our data indicate a slight, albeit statistically nonsignificant, decrease in estrogen binding in the POA resulting from progesterone injection. Lastly, Giordano *et al.* (43) report a strong suppression of nuclear estrogen binding in the arcuate and medial amygdala during pregnancy, areas in which progesterone did not significantly decrease estrogen binding in the present study. It is possible that more potent effects of progesterone would be manifest under conditions that more closely mimic the hormonal conditions of pregnancy. The capsules used in the present study were designed to reproduce and maintain blood levels of progesterone approximating those measured on proestrus (~20 ng/ml), but below the peak level measured on the afternoon of proestrus (30–40 ng/ml) (44–46). Whereas injection of 5 mg progesterone produces a much higher blood level of progesterone, in the range measured during midpregnancy or above (47), the transient nature of this increase does not allow a direct comparison with the conditions of pregnancy. Circulating levels of estradiol may also play a key role in determining the magnitude of the progesterone effect (20). It is also possible that physiological changes in other factors, such as afferent neurotransmitter or neuropeptide input to the estrogen target cells (48) may significantly modulate the relationship between estrogen/progesterone levels and estrogen receptor biosynthesis. Additional studies on regional changes in estrogen receptor concentrations within the brain under different physiological conditions of estrogen and progesterone secretion will be required to resolve this issue.

## REFERENCES

1. Barraclough, C. A., P. Camp, N. Wieland, and A. Akabori (1986). Stimulatory versus inhibitory effects of progesterone on estrogen-induced phasic LH and prolactin secretion correlated with estrogen nuclear and progestin cytosol receptor concentrations in brain and pituitary gland. *Neuroendocrinology* **42:** 6–14.
2. Blaustein, J. D., and D. H. Olster (1989). Gonadal steroid hormone receptors and social behaviors. In *Advances in Environmental and Comparative Physiology: Cellular Basis of Social Behavior in Vertebrates.* (J. Balthazart, Ed.), pp. 31–104. Springer-Verlag, Berlin.
3. Barfield, R. J., B. S. Rubin, J. H. Glaser, and P. G. Davis (1974). Relative contribution of ovarian and adrenal progesterone to the timing of heat in the 4-day cyclic rat. *Endocrinology* **94:** 571–575.

4. Powers, J. B. (1970). Hormonal control of sexual receptivity during the estrous cycle of the rat. *Physiol. Behav.* **5:** 831–835.
5. Blaustein, J. D. (1982). Alteration of sensitivity to progesterone facilitation of lordosis in guinea pigs by modulation of hypothalamic progestin receptors. *Brain Res.* **243:** 287–300.
6. Lisk R. D. (1969). Progesterone: Biphasic effects on the lordosis response in adult or neonatally gonadectomized rats. *Neuroendocrinology* **51:** 49–160.
7. Wallen, K., R. W. Goy, and C. H. Phoenix (1975). Inhibitory actions of progesterone on hormonal induction of estrus in female guinea pigs. *Horm. Behav.* **6:** 127–138.
8. Moguilewsky, M., and J. P. Raynaud (1979). The relevance of hypothalamic and hypophyseal progestin receptor regulation in the induction and inhibition of sexual behavior in the female rat. *Endocrinology* **105:** 516–522.
9. Blaustein, J. D., and H. H. Feder (1979). Cytoplasmic progestin receptors in female guinea pig brain and their relationship to refractoriness in expression of female sexual behavior. *Brain Res.* **177:** 489–498.
10. MacLusky, N. J., and B. S. McEwen (1978). Oestrogen modulates progestin receptor concentrations in some rat brain regions but not in others. *Nature* **274:** 276–278.
11. Blaustein, J. D., and H. H. Feder (1980). Nuclear progestin receptors in guinea pig brain measured by an in vitro exchange assay after hormonal treatments that affect lordosis. *Endocrinology* **106:** 1061–1069.
12. Brown, T. J., and J. D. Blaustein (1985). Loss of hypothalamic nuclear bound progestin receptors: Factors involved and the relationship to heat termination in female guinea pigs. *Brain Res.* **358:** 180–190.
13. Brown, T. J., and J. D. Blaustein (1984). Supplemental progesterone delays heat termination and loss of progestin receptors from hypothalamic cell nuclei in female guinea pigs. *Neuroendocrinology* **39:** 384–391.
14. Brown, T. J., and J. D. Blaustein (1986). Abbreviation of the period of sexual behavior in female guinea pigs by the progesterone antagonist, RU 486. *Brain Res.* **373:** 103–113.
15. Brown, T. J., M. J. Moore, and J. D. Blaustein (1987). Maintenance of progesterone-facilitated sexual behavior in female rats requires continued hypothalamic protein synthesis and nuclear progestin receptor occupation. *Endocrinology* **121:** 298–304.
16. Okulicz, W. C., R. W. Evans, and W. W. Leavitt (1981). Progesterone regulation of the occupied form of nuclear estrogen receptor. *Science* **213:** 1503–1505.
17. Evans, R. W., and W. W. Leavitt (1980). Progesterone inhibition of uterine nuclear estrogen receptor-dependence on RNA and protein synthesis. *Proc. Natl. Acad. Sci. USA* **77:** 5856–5860.
18. MacDonald, R. G., S. P. Rosenberg, and W. W. Leavitt (1983). Localization of estrogen receptor regulatory factor in the uterine nucleus. *Mol. Cell. Endocrinol.* **32:** 301–313.
19. Leavitt, W. W., W. C. Okulicz, J. A. McCracken, W. Schramm, and W. F. Robidoux (1985). Rapid recovery of nuclear estrogen receptor and oxytocin receptor in the ovine uterus following progesterone withdrawal. *J. Steroid Biochem.* **22:** 687–691.
20. Blaustein, J. D., and T. J. Brown (1984). Progesterone decreases the concentration of hypothalamic and anterior pituitary estrogen receptors in ovariectomized rats. *Brain Res.* **304:** 225–236.
21. Giordano, A. L., H. B. Ahdieh, A. D. Mayer, H. I. Siegel, and J. S. Rosenblatt (1990). Cytosol and nuclear estrogen receptor binding in the preoptic area and hypothalamus of female rats during pregnancy and ovariectomized, nulliparous rats after steroid priming: Correlation with maternal behavior. *Horm. Behav.* **24:** 232–255.
22. Giordano, A. L., H. I. Siegel, and J. S. Rosenblatt (1989). Nuclear estrogen receptor binding in the preoptic area and hypothalamus of pregnancy-terminated rats: Correlation with the onset of maternal behavior. *Neuroendocrinology* **50:** 248–258.
23. Rubin, B. S., and R. J. Barfield (1980). Priming of estrous responsiveness by implants of 17$\beta$-estradiol in the ventromedial hypothalamic nucleus of female rats. *Endocrinology* **106:** 504–509.
24. Rubin, B. S., and R. J. Barfield (1983). Progesterone in the ventromedial hypothalamus facilitates estrous behavior in ovariectomized estrogen-primed rats. *Endocrinology* **113:** 797–804.
25. Brown, T. J., N. J. MacLusky, M. Shanabrough, and F. Naftolin (1990). Comparison of age- and sex-related changes in cell nuclear estrogen-binding capacity and progestin receptor induction in the rat brain. *Endocrinology* **126:** 2965–2972.
26. MacLusky, N. J., E. J. Roy, M. Shanabrough, and A. Eisenfeld (1986). Preservation of steroid receptors in frozen brain and pituitary tissue: Use of the cryoprotective agent, dimethylsulfoxide. *J. Neurosci. Methods* **16:** 131–140.
27. Konig, J. F. R., and R. A. Klippel (1963). *A Stereotaxic Atlas of the Forebrain and Lower Parts of the Brainstem.* Williams and Wilkins, Baltimore.
28. Palkovits, M. (1983). Isolated removal of hypothalamic or other brain nuclei of the rat. *Brain Res.* **59:** 449–450.
29. Brown, T. J., R. B. Hochberg, J. E. Zielinski, and N. J. MacLusky (1988). Regional sex differences in cell nuclear estrogen-binding capacity in the rat hypothalamus and preoptic area. *Endocrinology* **123:** 1761–1770.
30. Roy, E. J., and B. S. McEwen (1977). An exchange assay for estrogen receptors in cell nuclei of the adult rat brain. *Steroids* **30:** 657–669.
31. Burton, K. (1956). A study of the conditions and mechanism of diphenylamine reaction for the colorimentrc estimation of deoxyribonucleic acid. *Biochem. J.* **62:** 315–323.
32. Ginsburg, M., B. D. Greenstein, N. J. MacLusky, I. D. Morris, and P. J. Thomas (1974). An improved method for the study of high-affinity steroid binding: Oestradiol binding in brain and pituitary. *Steroids* **23:** 773–792.
33. Bradford, M. M. (1976). A rapid and sensitive method for quantification of microgram quantities of protein utilizing the principle of protein dye-binding. *Anal. Biochem.* **72:** 248–254.
34. MacLusky, N. J., T. J. Brown, E. E. Jones, C. Leranth, and R. B. Hochberg (1990). Autoradiographic and microchemical methods for quantitation of steroid receptors. In *Methods in Neuroscience* (P. M. Conn, Ed.), Vol. 3, pp. 3–35. Academic Press, San Diego, CA.
35. Boer, G. J. (1975). A simplified microassay of DNA and RNA using ethidium bromide. *Anal. Biochem.* **65:** 225–231.
36. Brown, T. J., F. Naftolin, and N. J. MacLusky (1992). Sex differences in estrogen receptor binding in the rat hypothalamus: Effects of subsaturating pulses of estradiol. *Brain Res.* **578:** 129–134.
37. Lisk, R. D., and L. A. Reuter (1977). In vivo progesterone treatment enhances [$^3$H]estradiol retention by neural tissues of the female rat. *Endocrinology* **100:** 1652–1658.
38. Smanik, E. J., H. K. Young, T. G. Muldoon, and V. B. Mahesh (1983). Analysis of the effect of progesterone in vivo on estrogen receptor distribution in the rat anterior pituitary and hypothalamus. *Endocrinology* **113:** 15–22.
39. Attardi, B. (1981). Facilitation and inhibition of the estrogen-induced luteinizing hormone surge in the rat by progesterone: Effects on cytoplasmic and nuclear estrogen receptors in the hypothalamus–preoptic area, pituitary and uterus. *Endocrinology* **108:** 1487–1496.
40. Rubin, B. S., and R. J. Barfield (1984). Progesterone in the ventromedial hypothalamus of ovariectomized, estrogen-primed rats inhibits subsequent facilitation of estrous behavior by systemic progesterone. *Brain Res.* **294:** 1–8.

41. Rainbow, T. C., B. Parsons, and B. S. McEwen (1982). Sex differences in rat brain oestrogen and progestin receptors. *Nature* **300:** 648–649.

42. Brown, T. J., A. S. Clark, and N. J. MacLusky (1987). Regional sex differences in progestin receptor induction in the rat hypothalamus: Effects of various doses of estradiol benzoate. *J. Neurosci.* **7:** 2529–2536.

43. Giordano, A. L., H. I. Siegel, and J. S. Rosenblatt (1991). Nuclear estrogen receptor binding in microdissected brain regions of female rats during pregnancy: Implications for maternal and sexual behavior. *Physiol. Behav.* **50:** 1263–1267.

44. Biegon, A., A. Reches, L. Snyder, and B. S. McEwen (1983). Serotonergic and noradrenergic receptors in rat brain: Modulation by chronic exposure to ovarian hormones. *Life Sci.* **32:** 2015–2021.

45. Wise, P. M. (1982). Alterations in proestrous pattern of median eminence LHRH, serum LH, FSH, estradiol, and progesterone concentrations in middle-aged rats. *Life Sci.* **31:** 165–173.

46. Goodman, R. L. (1978). A quantitative analysis of the physiological role of estradiol and progesterone in the control of tonic and surge secretion of luteinizing hormone in the rat. *Endocrinology* **102:** 142–150.

47. Blaustein, J. D., and H. H. Feder (1979). Progesterone at plasma levels lower than those of mid-pregnancy decreases sexual behavior in ovariectomized rats. *Physiol. Behav.* **23:** 1099–1104.

48. Tetel, M. J., and J. D. Blaustein (1991). Immunocytochemical evidence for noradrenergic regulation of estrogen receptor concentrations in the guinea pig hypothalamus. *Brain Res.* **565:** 321–329.