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PROGESTIN RECEPTOR LEVELS IN RAT HYPOTHALAMIC AND LIMBIC NUCLEI[1]

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Abstract

We have utilized a method to minimize cytosol progestin receptor loss during freezing in order to localize and quantify estrogen-inducible progestin receptors in individual nuclei of the female rat brain.

Ovariectomized females received estradiol benzoate (20 μg for 3 days) or vehicle prior to sacrifice. All animals were perfused with cold distilled H_2O containing the cryoprotective compound, dimethyl sulfoxide (DMSO; 10% (v/v)). Thirty-one nuclei or brain regions were removed from frozen sections (300 μm) according to the method of Palkovits (Palkovits, M. (1973) Brain Res. *59:* 449–450) and were assayed *in vitro* using a synthetic radioligand, [^{3}H]R5020.

In ovariectomized animals perfused with DMSO, a basal level (1 to 8 fmol/mg of protein) of progestin receptors was observed in a variety of preoptic, hypothalamic, and limbic structures. Moreover, estrogen treatment induced high levels (24 to 49 fmol/mg of protein) of progestin receptors in regions of the preoptic area of hypothalamus which contain high levels of estrogen receptors. These regions included the medial, periventricular, and superchiasmatic nuclei of the preoptic area, the periventricular anterior hypothalamus, the ventromedial nucleus, and the arcuate-median eminence. Moderate levels (2 to 8 fmol/mg of protein) of progestin receptors were induced by estrogen in other hypothalamic and limbic structures, including the anterior and lateral hypothalamus, the bed nucleus of the stria terminalis, the cingulate cortex, the medial amygdaloid nucleus, and the CA_1 subfield of the hippocampus. By contrast, some areas, such as the caudate-putamen and the supraoptic nucleus, were devoid of both estrogen-inducible and uninduced progestin receptors.

These results support the hypothesis that progesterone action in the central nervous system is mediated by cytosol receptors in discrete brain regions and provide the first quantitative map of progestin binding in a vertebrate brain.

Progesterone (P) and estradiol (E_2) synergize to activate feminine reproductive behavior and to induce gonadotropin secretion in a number of vertebrate species, including the rat and guinea pig. Previous studies which have employed [^{3}H]progesterone as a radioligand usually have failed to identify specific progestin-binding components which could mediate the central actions of P in the vertebrate brain (for references, see Feder and Marrone, 1978; McEwen, 1978). One notable exception was the autoradiographic study by Sar and Stumpf (1973), which identified some progestin-concentrating cells in the basal hypothalamus and pituitary of the estrogen-primed guinea pig. However, the recent availability of a high affinity synthetic progestin, [^{3}H]R5020, has facilitated the identification of specific progestin receptors in the brain and pituitary in ovariectomized and estrogen-stimulated rats (Moguilewsky and Raynaud, 1979; Kato and Onouchi, 1977; MacLusky and McEwen, 1978, 1980; Warembourg, 1978), guinea pigs (Blaustein and Feder, 1979, 1980), and the bonnet monkey (MacLusky et al., 1980).

One major feature of central and pituitary progestin receptor systems is the presence of a class of receptors which is unaffected by estrogenic stimulation as well as a class of receptors which is induced by E_2 (MacLusky and McEwen, 1978, 1980). Although the physiological significance of these two classes of progestin receptors is unclear, estrogen-inducible progestin receptors appear to be concentrated in those areas of the brain implicated in the control of sexual receptivity and gonadotropin secretion, the mediobasal hypothalamus (MBH) and preoptic

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TABLE I

Location of nuclei and subregions in 300-μm frozen sections

The location of nuclei and subregions within frozen (300-μm) sections of the female rat brain are given. Consecutive coronal sections (300 μm) were taken in König and Klippel's (1963) anatomical plane, beginning at the A8380μ level. Nuclei and brain regions were removed according to the method of Palkovits (1973) using 500- or 1000-μm hollow steel tubes or a small scalpel.

Region	Punch Size	Section	Protein per Assay Sample
			μg
Diagonal band of Broca	Medial, 1001 μm; bilateral, 500 μm	1–3	27 ± 3
Medial septum	Single, 1000 μm	1–3	18 ± 1
Lateral septum	Bilateral, 1000 μm	1–3	18 ± 2
Olfactory tubercle	Bilateral, scalpel	1–3	105 ± 11
Nucleus stria terminalis	Bilateral, 500 μm above and below anterior commissure (3); bilateral, 1000 μm (4)	3, 4	26 ± 3
Medial preoptic nucleus	Bilateral, 1000 μm	4, 5	24 ± 2
Suprachiasmatic preoptic nucleus	Bilateral, 500 μm	4, 5	5 ± 0.5
Periventricular preoptic nucleus	Scalpel, 250 μm around ventricle	4, 5	8 ± 1
Lateral preoptic nucleus	Bilateral, 1000 μm	4, 5	25 ± 1
Caudate-putamen	Bilateral, 1000 μm	3–6	44 ± 5
Parietal cortex	Bilateral, scalpel	4–7	91 ± 17
Cingulate cortex	Scalpel	4–7	73 ± 10
Periventricular anterior hypothalamus	Scalpel or single, 1000 μm	6–9	10 ± 1
Anterior hypothalamus	Bilateral, 1000 μm	6–9	43 ± 2
Supraoptic nucleus	Bilateral, 1000 μm	6–8	10 ± 1
Ventromedial nucleus	Bilateral, 1000 μm	10–14	48 ± 4
Medial ventromedial nucleus	Bilateral, 500 μm	10–14	13 ± 1
Lateral ventromedial nucleus	Bilateral, 500 μm	10–14	12 ± 1
Dorsomedial nucleus	Bilateral, 1000 μm	11–14	50 ± 5
Lateral hypothalamus	Bilateral, 1000 μm	11–14	54 ± 2
Arcuate-median eminence[a]	Scalpel	11–14	40 ± 3
Medial amygdala	Bilateral, 1000 μm	11–14	51 ± 8
Cortical amygdala	Bilateral, 1000 μm	11–14	48 ± 9
Ventral thalamus	Bilateral, 1000 μm	11–14	55 ± 5
Dentate gyrus	Bilateral, 2 × 500 μm	12–15	32 ± 4
CA_1	Bilateral, 2 × 500 μm	12–15	23 ± 4
CA_3	Bilateral, 1000 μm	12–15	37 ± 6
Ventral premammillary nucleus	Bilateral, 1000 μm	15, 16	32 ± 2
Habenula	Bilateral, 1000 μm	15–18	32 ± 2
Central grey	Single, 1000 μm	22–27	41 ± 3

[a] The arcuate nucleus could not be removed consistently without taking some of the median eminence; thus, this sample is a pool of these two regions.

area (POA) (Moguilewsky and Raynaud, 1979; Kato and Onouchi, 1977; MacLusky et al., 1980; MacLusky and McEwen, 1978, 1980; Warembourg, 1978; Blaustein and Feder, 1979). Moreover, the induction and decay of estrogen-inducible progestin receptors in the MBH-POA of the female rat (Parsons et al., 1980) and guinea pig (Blaustein and Feder, 1979) have been shown to be correlated temporally with the induction and decay of progesterone-facilitated sexual receptivity.

There have not been any definitive mapping studies of the localization of uninduced and estrogen-inducible progestin receptors in the vertebrate brain. Until such information becomes available, the interpretations of structure-activity studies, such as those based on the implantation of progesterone in discrete brain regions (for references, see McEwen et al., 1979), remain problematic. In this study, we have utilized a method to minimize cytosol progestin receptor loss during freezing (MacLusky et al., 1982) in order to localize and quantify cytosol progestin receptor (CPR) levels in individual nuclei of the rat brain.

Materials and Methods

Subjects. Sprague-Dawley female rats (180 to 220 gm) were obtained from Charles River Laboratories (Wilmington, MA) and were ovariectomized 1 to 3 weeks prior to experimentation. Female rats were housed in groups (three to five animals per cage) in temperature-controlled rooms with day-night lighting (lights on 7:30 A.M., lights off 7:30 P.M.). All animals were allowed Purina Rat Chow and water *ad libitum*.

Hormone administration. Estradiol benzoate (Sigma Chemical Co.) was dissolved in sesame oil by sonication (4 mg/20 ml). All animals received daily injections of estradiol benzoate (20 μm/0.1 ml) or oil (0.1 ml) for 3 days prior to sacrifice.

Brain preparation. Rats were anesthetized under methoxyflurane prior to sacrifice. The animals were perfused through the heart with 20 to 25 ml of cold (4° C) distilled water containing 10% (v/v) dimethylsufoxide (DMSO). DMSO is a cryoprotective compound, which has been shown to minimize damage to progestin receptors produced by rapid and severe freezing (MacLusky et al., 1982). Brains were blocked, mounted onto cryostat chucks with distilled water, and frozen in powdered dry ice. Brains then were transferred to an American Optical cryostat and were allowed to equilibrate to temperature (−20° to −15°C) for a minimum of 20 min. Consecutive coronal sections (300 μm) were taken in the König and Klippel (1963) anatomical plane, beginning at the A8380μ level. Sections were thaw-mounted briefly onto glass slides and were stored at −40°C for 24 to 72 hr prior to microdissection.

Microdissection and homogenization of brain nuclei and subregions. The locations and sizes of nuclei and brain regions were determined from the atlases of König and Klippel (1963) and Palkovits (1977) and were verified in preliminary experiments with cresyl violet staining of thin (32-μm) sections. On the day of microdissection, frozen sections were transferred to a Cambion plate, which had been cooled thermoelectrically to −15°C. Nuclei and brain regions were removed from frozen sections (300μm) according to the method of Palkovits (1973) (Table I). Briefly, nuclei or brain regions were removed with the aid of a stereomicroscope using 500- or 1000-μm hollow stainless steel tubes; in some instances, samples were removed using a small scalpel (Table I). Areas obtained by microdissection were blown into separate,

cold (4°C) plastic tubes, with each tube containing pools of tissue from three animals.

All brain tissues were homogenized in 240 μl of cold (4°C) TEGD buffer (10 mM Tris, 1.5 mM disodium EDTA, 10% (v/v) glycerol adjusted to pH 7.4 with hydrochloric acid, and 1mM dithiothreitol (Sigma) added on the day of use). The homogenates were centrifuged for 20 min at 100,000 × *g* in a Beckman Airfuge cooled to 4°C.

Determination of cytosol high affinity [^{3}H]R5020 binding. Cytosol progestin receptors were quantified using procedures developed by MacLusky and McEwen (1978, 1980). Aliquots (100 μl) of cytosol supernatants were incubated with TEGD buffer (50 μl) for 4 to 6 hr at 4°C in the presence of 0.4×10^{-9} M [^{3}H]R5020 (17,21 dimethyl-19-norpregna-4,9-diene-3,20-dione, New England Nuclear) and corrected for nonspecific binding by parallel incubations containing a 50-fold excess (2×10^{-8}M) of unlabeled R5020. This concentration of [^{3}H] R5020 was selected to bind specifically the high, but not low, affinity progestin binding site present in brain (MacLusky and McEwen, 1980). Bound [^{3}H]R5020 was measured by gel filtration on "miniature" Sephadex LH-20 (Pharmacia) columns as described in our companion paper (Rainbow et al., 1982b).

Protein concentrations were determined by the Bradford (1976) dye-binding method on duplicate 20-μl samples from each incubate.

Receptor levels are expressed as femtomoles of [^{3}H] R5020 bound per mg of cytosol protein.

Scatchard analysis and linearity determinations. In order to determine the potential loss of progestin receptors caused by freezing, we employed analysis by the method of Scatchard (1949) to compare receptor levels in fresh and frozen brain tissue. In these studies, brains were dissected according to the method of Luine et al. (1974). Samples consisted of pools of mediobasal hypothalamus-preoptic area (MBH-POA) tissue from three or four animals.

For frozen specimens, animals were perfused with 10% DMSO as described above. MBH-POA samples were frozen in powdered dry ice, transferred to an American Optical cryostat (−20° to −15°C) for 2 to 3 hr and stored overnight at −40°C. The purpose of such manipulations was to "mimic," insofar as possible, the procedures used to prepare tissue for microdissection. For fresh specimens, animals were sacrificed immediately prior to tissue homogenization.

Frozen MBH-POA samples (from four animals) were homogenized in a Teflon-glass homogenizer in 3.6 ml of TEGD; fresh MBH-POA samples (from three animals) were homogenized in 2.7 ml of TEGD. For Scatchard analysis, the aliquots (100 μl) of cytosol supernatants were incubated with TEGD buffer (50 μl) for 4 to 6 hr at 4°C in the presence of a range of [^{3}H]R5020 concentrations (0.28 to 2.0 nM) and corrected for nonspecific binding. Bound [^{3}H]R5020 was measured and expressed as described above.

In order to facilitate comparisons of receptor levels among tissues, we first determined the range over which [^{3}H]R5020 binding was related linearly to total protein. Varying amounts of cytosol supernatants obtained from frozen MBH-POA tissues were incubated for 4 to 6 hr at 4°C with TEGD (total volume, 150 μl) in the presence of 0.4×10^{-9} M [^{3}H]R5020 and corrected for nonspecific binding. Bound [^{3}H]R5020 was estimated and expressed as above.

Statistical tests. In order to determine if a significant level of uninduced receptor was present in the absence of E_2 treatment, CPR levels in a given region were compared to a theoretical group of equal size with a mean of zero using the Student's *t* test ($p \leq 0.05$ considered significant, two-tailed test) (Brunning and Klintz, 1968). In order to determine if E_2 treatment increased significantly CPR levels in a given region, induced and noninduced receptor levels were compared using the Student's *t* test for paired samples ($p \leq 0.05$ considered significant, one-tailed test). (Keppel, 1973).

Results

A series of preliminary experiments was performed in order to measure basal and estrogen-inducible CPRs in 12 hypothalmic and preoptic nuclei obtained from unperfused animals. We observed little detectable [^{3}H] R5020 binding in unperfused animals, irrespective of estrogen treatment. Therefore, in order to quantify CPR levels by microassay, we perfused animals with 10% DMSO prior to sacrifice (MacLusky et al., 1982; see "Materials and Methods").

Scatchard analysis and linearity measurements

Scatchard analysis indicated that freezing produced an insignificant loss of CPRs in the MBH-POA when animals were perfused with 10% DMSO (B_{max} for frozen tissue, 20.2 ± 1.2 fmol/mg of protein; B_{max} for fresh tissue, 23.3 ± 0.5 fmol/mg of protein; $p < 0.05$). Freezing did not alter the affinity of cytosol progestin binding sites in the MBH-POA for [^{3}H]R5020 (K_d for frozen tissue, 0.53 ± 0.03 nM; K_d for fresh tissue, 0.47 ± 0.03 nM; $p < 0.05$).

The amount of [^{3}H]R5020 specifically bound was related linearly to the amount of cytosol protein over concentrations from 5 to 200 μg. As shown in Table I, the protein content of all regions and nuclei assayed was within this linear range.

General distribution of estrogen-inducible and noninducible progestin receptors

Significant levels of uninduced progestin receptors were observed in some, but not all, preoptic and hypothalamic structures and in a few limbic regions (Table II). Many, but not all, preoptic and hypothalamic structures showed a 6- to 10-fold increase in progestin receptors after E_2 treatment. Estradiol also increased cytosol progestin receptors (CPRs) 2- to 3-fold in a few limbic regions (Table II). The following is a more detailed description of these results.

Specific brain regions which contain progestin receptors

Preoptic-hypothalamic structures. The greatest concentration of estrogen-inducible and uninduced CPRs in the female rat brain was observed in nuclei or subregions of the preoptic area and the mediobasal hypothalamus (Table II). Within the POA, a fairly high concentration of uninduced receptors (6.2 ± 2.2 fmol/mg of protein) was seen in the medial nucleus, whereas in the lateral,

TABLE II

Progestin receptor levels in brain nuclei and subregions

The values represent the progestin receptor levels in brain nuclei or subregions of the female rat brain. Cytosol progestin receptor levels were measured according to the methods of MacLusky and McEwen (1978, 1980). Results are expressed as femtomoles of [^{3}H]R5020 per mg of protein ± SEM. *N* represents number of experiments; in each experiment, tissue was pooled from three animals. Total counts per min per sample for regions with a high concentration of progestin receptors (Fig. 1) were from 50 to 150 cpm above background. Regions with a zero to moderate level of progestin receptor ranged from 20 to 50 cpm. Nonspecific counts per min were from 20 to 40 cpm. All samples were counted for at least 10 min.

Region	Amount		*N*
	$-E_2$	$+E_2$	
	fmol/mg protein		
Diagonal band of Broca	2.1 ± 1.4	1.9 ± 1.1	4
Medial septum	2.7 ± 1.6	0.9 ± 0.7	4
Lateral septum	1.9 ± 1.7	1.0 ± 0.6	5
Olfactory tubercle	1.4 ± 0.5[a]	1.7 ± 0.7	4
Nucleus stria terminalis	1.4 ± 0.9	4.6 ± 1.2[b]	5
Medial preoptic nucleus	6.2 ± 2.2[a]	41.8 ± 3.5[c]	6
Suprachiasmatic preoptic nucleus	5.1 ± 3.3	35.8 ± 7.8[b]	5
Periventricular preoptic nucleus	1.9 ± 1.5	48.8 ± 8.1[c]	5
Lateral preoptic nucleus	1.4 ± 0.7	4.9 ± 1.5[b]	6
Caudate-putamen	0.5 ± 0.4	1.3 ± 1.2	5
Parietal cortex	1.7 ± 0.5[a]	2.3 ± 0.8	5
Cingulate cortex	2.0 ± 1.0	4.9 ± 1.2[b]	5
Periventricular anterior hypothalamus	7.4 ± 3.3[a]	28.9 ± 4.9[c]	6
Anterior hypothalamus	2.2 ± 0.8	7.5 ± 2.1	4
Supraoptic nucleus	3.9 ± 3.9	3.3 ± 3.3	4
Ventromedial nucleus	4.5 ± 1.1[a]	24.7 ± 4.0[c]	6
Lateral ventromedial nucleus	8.9 ± 3.4[a]	24.4 ± 3.2[b]	4
Medial ventromedial nucleus	3.6 ± 3.6	12.7 ± 2.1	3
Dorsomedial nucleus	2.8 ± 1.1[a]	5.3 ± 0.9	5
Lateral hypothalamus	1.4 ± 0.7	4.9 ± 1.5[b]	6
Arcuate-median eminence	6.3 ± 1.0[a]	40.9 ± 5.3[c]	6
Medial amygdala	1.3 ± 0.7	2.8 ± 1.0[a]	5
Cortical amygdala	0.4 ± 0.2	3.5 ± 1.5	6
Ventral thalamus	3.2 ± 1.7	2.3 ± 1.3	4
Dentate gyrus	1.7 ± 0.9	0 ± 0	5
CA_1	1.0 ± 0.6	5.1 ± 1.1[b]	5
CA_3	0.8 ± 0.4	0.4 ± 0.4	5
Ventral premammillary nucleus	2.4 ± 1.5	3.3 ± 2.5	4
Habenula	1.5 ± 0.9	3.0 ± 1.8	4
Central grey	2.2 ± 1.0	3.8 ± 2.0	5

[a] Significantly different from no progestin receptor (Student's *t* test, two-tailed test; $p < 0.05$).

[b] Significant induction of cytosol progestin receptors after E_2 treatment (Student's paired *t* test, one-tailed test; $p < 0.05$).

[c] Significant induction of cytosol progestin receptors after E_2 treatment (Student's paired *t* test, one-tailed test; $p < 0.005$).

periventricular, or suprachiasmatic nuclei, receptors were not measurable in the absence of estrogen treatment. However, all four of these preoptic nuclei showed high levels of CPRs after E_2 treatment. In fact, the levels of inducible CPRs in the medial, periventricular, and superchiasmatic nuclei of the preoptic area were among the highest (36 to 49 fmol/mg of protein) in the female rat brain. By contrast, CPRs in the lateral nucleus of the POA were increased moderately (from neglible levels to 5 fmol/mg of protein) after E_2. No detectable progestin receptors were seen lateral to the POA in the caudate-putamen in the presence or absence of estrogen treatment.

A significant concentration of uninduced receptor was present caudal to the POA in the anterior hypothalamus (~2 fmol/mg of protein). E_2 treatment produced a moderate increase (to 8 fmol/mg of protein) in CPRs in this region. The highest concentration of uninduced CPRs in the female rat brain was seen in the periventricular anterior hypothalamus (PVAH; ~7 fmol/mg of protein). The PVAH also showed a large increase in CPRs (to ~29 fmol/mg of protein) after estrogenic stimulation. No detectable uninduced or inducible CPRs were found lateral and dorsal to the PVAH in the supraoptic and paraventricular nuclei.

Significant concentrations of uninduced progestin receptors were observed in regions caudal to the PVAH, specifically in the arcuate-median eminence (~6 fmol/mg of protein) and ventromedial nucleus (VMN; ~5 fmol/mg of protein). Each of these areas showed a 6- to 7-fold increase in CPRs after E_2 treatment. In the VMN, the greatest increase in CPRs occurred in the lateral portion of the nucleus to (~24 fmol/mg of protein). Significant levels of uninduced receptor were seen above the VMN in the dorsomedial nucleus (DMN; 2.8 ± 1.1 fmol/mg of protein), but E_2 treatment failed to increase significantly CPRs in the DMN. The lateral nucleus of the hypothalamus did not contain significant levels of estrogen-inducible or uninduced receptor. Similarly, no detectable uninduced CPRs were found caudal to the lateral nucleus in the ventral premammillary nucleus, and E_2 did not increase CPRs in this region.

Limbic structures. Neither the medial nor the lateral septum showed a significant concentration of uninduced or estrogen-inducible receptor. Similarly, no detectable uninduced receptor was seen below the septum in the diagonal band of Broca, and estrogen was without effect in this region. A significant concentration of uninduced receptor was observed lateral to the diagonal band in the olfactory tubercle; however, E_2 did not elevate CPRs in this region.

No detectable level of uninduced receptor was observed in the bed nucleus of the stria terminalis. However, moderately elevated CPRs (to ~5 fmol/mg of protein) were observed in the bed nucleus after E_2 treatment. The parietal cortex showed a significant concentration (~2 fmol/mg of protein) of uninduced receptor, although the cingulate cortex did not. Estrogen was without significant effect in the parietal cortex. However, E_2 significantly increased CPRs in the cingulate cortex (from negligible levels to ~5 fmol/mg of protein). No detectable level of uninduced receptor was observed in either the medial or cortical nucleus of the amygdala. After E_2 treatment, a low but significant induction of CPRs was observed in the medial (to ~3 fmol/mg of protein), but not cortical, amygdaloid nucleus.

In the hippocampus, uninduced receptor was not detected in the dentate gyrus or in the CA_1 and CA_3 subfields. E_2 treatment significantly elevated CPRs in

one region of the hippocampus, the CA_1 subfield (to ~5 fmol/mg of protein).

Mesencephalon. The central grey of the mesencephalon did not contain a significant concentration of uninduced or inducible CPRs.

Discussion

A significant concentration of uninduced CPRs was found in some, but not all, regions of the preoptic area, hypothalamus, and limbic system (Table II). Areas which contained a significant concentration of noninducible CPRs were the medial preoptic area, the anterior hypothalamus and periventricular anterior hypothalamus, the arcuate-median eminence, the ventromedial and dorsolmedial nuclei, the parietal cortex, and the olfactory tubercle. Whiel these findings do not exclude the possibility that other (or even all) brain regions contain some scattered cells which have uninduced progestin receptors, they do indicate that the distribution of uninduced receptor in the rat brain is not uniform. Thus, these findings support and extend the observations of MacLusky and McEwen (1980), who reported considerable variation in the amount of uninduced receptor in brain regions obtained by more conventional gross dissection.

A significant increase in estrogen-inducible CPRs was found in many, but not all, regions of the preoptic area and hypothalamus and in several limbic regions (Table II). After E_2 treatment, the greatest concentration of CPRs (24 to 49 fmol/mg of protein) was seen in structures which have the highest concentration of E_2 receptors; the medial, periventricular, and suprachiasmatic nuclei of the preoptic area, the periventricular anterior hypothalamus, the ventromedial nucleus, and the arcuate-median eminence (Fig. 1). Such high concentrations of inducible CPRs are consistent with autoradiographic studies, which have demonstrated that retention of radioactivity after [^{3}H]R5020 administration to E_2-primed rats is most pronounced in the mediobasal hypothalamus and medial preoptic area (Warembourg, 1978). In addition, estradiol treatment induced moderate levels of CPRs (2 to 8 fmol/mg of protein) in the lateral and anterior hypothalamus, the bed nucleus of the stria terminalis, the cingulate cortex, the medial amygdaloid nucleus, and the CA_1 subfield of the hippocampus (Fig 1). Previous autoradiographic (Warembourg, 1978) and physiochemical (MacLusky and McEwen, 1980) studies of the rat brain have failed to detect an induction of CPRs in limbic structures, such as the corticomedial region of the amygdala and the hippocampus. However, in some cases, measurement of steriod receptors by microassay may be more sensitive than analysis of steroid receptor distribution by autoradiography or quantification of receptors by crude brain dissection. For example, progestin receptors in cells of the amygdala and hippocampus may be induced sparsely or distributed nonuniformly. A sparse induction of CPRs could escape detection by autoradiographic analysis, while a nonuniform distribution of CPRs could be obscured by crude dissection.

Comparison of our present data with that presented for estrogen receptors in our companion paper (Rainbow et al., 1982b) suggests, but does not prove, that some brain regions which contain estrogen receptors lack the capacity to form progestin receptors in response to E_2. For example, both the medial and cortical amygdala contain estrogen receptors, but only the medial amydala shows a significant CPR induction following E_2 treatment. Nevertheless, it is conceivable that, in areas of low estrogen receptor concentration, such as the cortical amygdala, inducible CPRs are distributed too sparsely to be detected consistently by our present method of microassay.

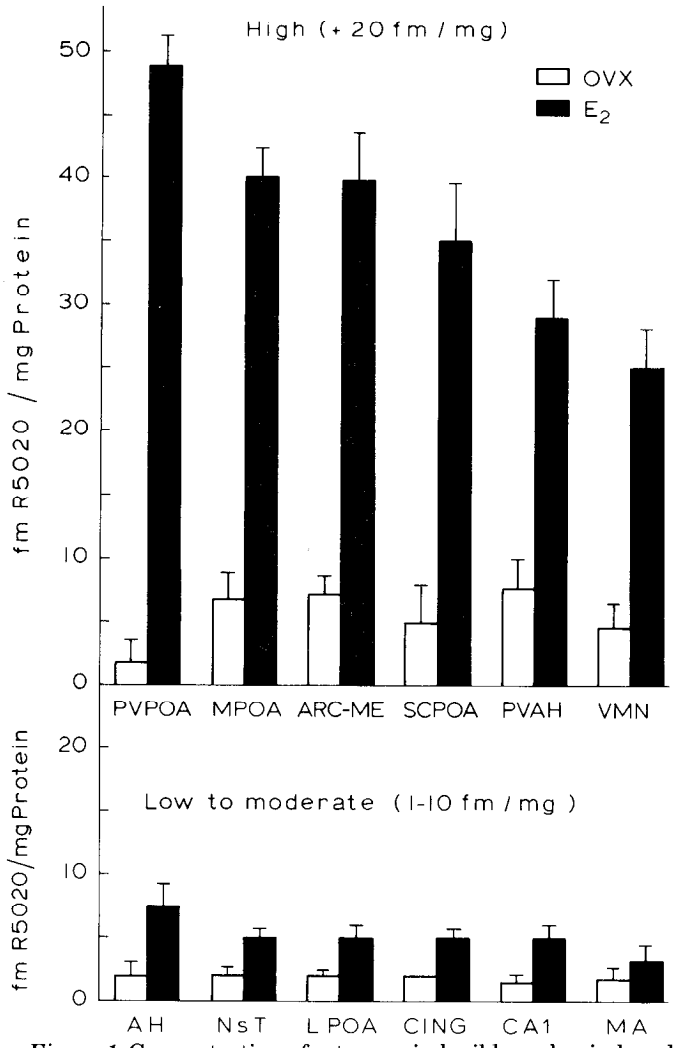

Figure 1. Concentration of estrogen-inducible and uninduced progestin receptors in selected nuclei and subregions of the female rat brain. The data are from Table II. Results are expressed as femtomoles of [^{3}H]R5020 specifically bound per mg of total protein (mean ± SEM). The abbreviations used are: *PVPOA*, periventricular preoptic area; *MPOA*, medial preoptic area; *ARC-ME*, arcuate-median eminence; *SCPOA*, superchiasmatic preoptic area; *PVAH*, periventricular anterior hypothalamus; *VMN*, ventromedial nuclei; *AH*, anterior hypothalamus; *NsT*, bed nucleus of the stria terminalis; *LPOA*, lateral preoptic area; *CING*, cingulate cortex; *CA1*, subfield of hippocampus; *MA*, medial amygdala; *OVX*, ovariectomized rats; E_2, E_2-treated rats.

Correlation of inducible CPRs with sites of progesterone action. The sites of peak CPR induction coincide with the sites at which progesterone implantations in the rat brain affect hormone-dependent functions. Although such studies must be interpreted carefully in light of

potential hormonal diffusion from the site of implantation, the balance of these studies suggests that regions of peak CPR induction contribute to such neuroendocrine events as the activation of feminine reproductive behavior.

Stereotaxic application of P to the mediobasal hypothalamus has been shown to facilitate reproductive behavior in the E_2- and P-primed rat (Powers, 1972). A recent study by Rubin and Barfield (B. S. Rubin and R. J. Barfield, unpublished results), which has employed refined techniques of P implantation to minimize steroid diffusion, indicates that a principal site of P action in the facilitation of feminine reproductive behavior is the ventromedial nucleus. These investigations found that stereotaxic application of progesterone to the VMN, but not the POA, facilitated sexual behavior in estrogen-primed rats. Rainbow and co-workers (1982a) have found that application of the protein synthesis inhibitor, anisomycin, to the VMN blocked the facilitation of reproductive behavior observed 4 to 6 hr after subcutaneous P administration. In this study, protein synthesis in the MBH was inhibited by 70% 4 hr after anisomycin application, but no inhibition was observed in the preoptic area, corticomedial amygdala, or pituitary. Taken collectively, these findings are consistent with, but not proof of, the hypothesis that genomic alterations in the VMN (but not the POA) are integral to progesterone's facilitation of feminine reproductive behavior in the rat. However, it should not be assumed from this information that other brain regions are not involved in progesterone's regulation of feminine reproductive behavior in the rat. In fact, several investigators have reported that implantation of P in the medial reticular formation facilitates sexual receptivity in the E_2-primed rat within 2 to 3 hr (Powers, 1972; Yanase and Gorski, 1976; for review, see McEwen et al., 1979).

Throughout much of the rat brain, CPR levels appear to be unaffected by estrogen treatment. The contribution of the uninduced receptor system to progesterone-mediated events is unclear. However, it should be noted that some actions of P, such as effects on cortical electroencephalograms, do not require prior estrogen priming (Arai et al., 1967). Thus, it is conceivable that inducible and uninduced CPRs are involved in different aspects of progesterone action.

Pathways connecting regions which contain progestin receptors. Previous anatomical and autoradiographic findings have indicated that estrogen-concentrating regions in the rodent brain are linked by neural pathways (for review, see Pfaff and Keiner, 1973; McEwen and Pfaff, 1973). Such fiber pathways could provide an anatomical substrate for coordinated and systematic physiological changes by E_2 (Pfaff and Keiner, 1973; McEwen and Pfaff, 1973). Because many estrogen-concentrating regions of the rat brain also contain progestin receptors, interactions between these areas also might coordinate the action of P in the central nervous system. For example, certain limbic structures project to progesterone-concentrating regions in the hypothalamus and preoptic area. The cortical and medial amygdala send projections to the medial preoptic area, the ventromedial nucleus, and the bed nucleus of the stria terminalis (Heimer and Nauta, 1969; Leonard and Scott, 1971). Cells in the ventral hippocampus project through the medial corticohypothalamic tract to the medial anterior hypothalamus and arcuate nucleus (Nauta, 1956; Valenstein and Nauta, 1959; Raisman et al., 1966). In addition, cells from the bed nucleus of the stria terminalis project to the medial POA (Millhouse, 1969).

Second, some progesterone-concentrating regions in the medial and cortical amygdala and the olfactory tubercle receive projections from the olfactory or accessory olfactory bulb (Powell et al., 1965; Heimer, 1968; Winans and Scalia, 1970).

Third, many hypothalamic, preoptic, and limbic regions which concentrate progesterone are interconnected. For example, the ventromedial nucleus receives projections from the medial POA and anterior hypothalamus (Millhouse, 1969; Chi, 1970). Neurons from the VMN project to the median eminence, to the bed nucleus of the stria terminalis, and to a specific portion of the cortical amygdaloid nucleus (Krieger et al., 1979). Fibers from the medial POA terminate in the medial amygdala, the median eminence, the dorsomedial nucleus, and the ventrolateral subdivision of the VMN (Conrad and Pfaff, 1977a). The medial anterior hypothalamus sends projections to the periventricular anterior hypothalamus, the bed nucleus of the stria terminalis, the dorsomedial nucleus, the medial amygdala, and the VMN (Conrad and Pfaff, 1977b). In addition, the arcuate nucleus sends projections to the bed nucleus of the stria terminalis and to the median eminence (Krieger et al., 1979).

Thus, progesterone receptor-containing nuclei and brain regions are interconnected by neural pathways. Such pathways may provide an anatomical substrate for integrated actions of progesterone and estrogen in the female rat brain.

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