ESTRADIOL RECEPTOR LEVELS IN RAT HYPOTHALAMIC AND LIMBIC NUCLEI¹

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Abstract

The amount of cytoplasmic receptor for the steroid hormone, estradiol (E_2), was determined in 46 nuclei and subdivisions of rat brain. Individual nuclei were removed from 300- μ m frozen sections according to the punch-out method of Palkovits (Palkovits, M. (1973) *Brain Res.* 59: 449–450), and the content of E_2 receptor was measured with a sensitive radioligand binding method. Cytoplasmic receptors for E_2 were distributed heterogeneously throughout the rat brain. The highest level of receptor (40 fmol/mg of protein) was found in the periventricular nucleus of the preoptic area, while low (1 fmol/mg) but detectable levels of receptors were found in such limbic regions as the nucleus of the diagonal band, the olfactory tubercle, and the cingulate cortex. Regions that were devoid of detectable receptor included the medial septum, the parietal cortex, and the ventral thalamus. Our results support the notion that E_2 influences reproductive behavior and neuroendocrine function by binding to receptors in discrete areas of the brain and provide the first quantitative map of E_2 receptors in individual rat brain nuclei.

The brain, like peripheral organs, possesses specific intracellular receptors for the steroid hormone, 17 β -estradiol (E_2) (Eisenfeld and Axelrod, 1965; McEwen and Pfaff, 1970; McEwen, 1978). These are high molecular weight molecules that move from the cytoplasm of brain cells into nuclei after E_2 binds to them (Lieberburg et al., 1980). While it has been shown from gross biochemical studies that the highest concentration of E_2 receptors are found in the hypothalamus and amygdala (McEwen et al., 1975) and it has been shown by *in vivo* autoradiography that the receptors are distributed heterogeneously within these regions (Pfaff and Keiner, 1973; Stumpf, 1968), the amount of E_2 receptor within individual hypothalamic and limbic nuclei has never been determined by direct biochemical methods. It is important to establish both the location and relative concentration of E_2 receptors in the brain because there is compelling evidence that they mediate the actions of E_2 on behavior and neuroendocrine function. For example, the effects of E_2 on sexual behavior or on gonadotropin secretion are correlated with the degree and duration of occupation of E_2 on neural receptors (Parsons et al., 1981, 1982a; Krey

and Parsons, 1982; McGinnis et al., 1981), and steroid and nonsteroid antagonists of receptor binding will block the actions of E_2 on the brain (Arai and Gorski, 1968; Roy and Wade, 1977; Labhsetwar, 1970). In this study, we have used a sensitive radioligand binding assay to measure the amount of E_2 receptor in individual nuclei of rat brain. Cytoplasmic receptors for E_2 are found in a variety of brain regions in concentrations ranging from 1 to 40 fmol/mg of protein.

Materials and Methods

Animals. We assayed E_2 receptor levels in brain tissue from female Sprague-Dawley rats (180 to 220 gm; Charles River Laboratories, Wilmington, MA). The rats were ovariectomized 1 to 3 weeks before use. The animals were housed under a 12:12-hr light-dark cycle.

Microdissection of nuclei and subregions. The procedure of Palkovits (1973) was used to remove nuclei and subregions from 300- μ m-thick frozen sections of rat brain. Rats were perfused through the heart with 20 ml of ice cold 10% dimethyl sulfoxide (DMSO), a cryoprotective agent that has been shown to protect steroid receptors from damage caused by freezing (MacLusky et al., 1982). The brains were blocked on both ends with a razor blade and frozen onto cryostat chucks in powdered dry ice. Coronal sections were cut in the König and Klippel (1963) anatomical plane using an American Optical cryostat. The microtome temperature of the cryostat was -15°C .

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The sections were thaw-mounted briefly onto glass slides and were stored at -40°C for 24 to 72 hr before experimental use. Using a 500- or 1000- μm hollow stainless steel tube, brain regions were removed from portions of 27 separate 300- μm -thick sections. These sections were cut consecutively starting from the A8380 level of the König and Klippel atlas (1963). The microdissection was done with the aid of a stereomicroscope. During the dissection, the temperature of the sections was maintained at -15°C with a Cambion thermoelectrically cooled plate. The location of the nuclei and brain regions was determined from the stereotaxic atlas of König and Klippel (1963) and from the stereotaxic atlas of Palkovits (1980). The location of the microdissected regions within the 27 serially cut sections and the cannula sizes used for removal are given in Table I. In some cases, brain regions were removed with a small scalpel. In preliminary measurements on 32- μm cresyl violet-stained sections, the diameter of the punch cannula did not exceed the size of the removed nuclei.

Estrogen receptor assay. We pooled tissue from three rats to obtain material for one assay. After removal from the frozen sections, the tissue samples were blown into ice cold plastic tubes containing 24 μl of TEGD buffer (10 mM Tris-HCl, 5 mM EDTA, 10% glycerol, and 1 mM dithiothreitol, pH 7.4). The tissue was homogenized at moderate speed with a motor-driven homogenizer (10 strokes, up and down), and the cytosol fraction of the homogenate was obtained by centrifugation in a Beckman Airfuge at $100,000 \times g$ for 20 min. One hundred microliters of the cytosol was added to 50 μl of TEGD buffer that contained either sufficient [^3H]estradiol ([2-, 4, 6, 7- ^3H (N)]estradiol, 115 or 140 Ci/mmol, New England Nuclear) to make a final concentration of 1 nM [^3H]E₂ or 1 nM [^3H]E₂ along with 1 μM moxestrol to determine nonspecific binding. Aliquots of the cytosol were incubated with [^3H]E₂ for 4 hr at 4°C in plastic microcentrifuge tubes. The [^3H]E₂ bound to receptors was separated from free ligand by gel filtration at 4°C on Sephadex LH-20 columns. The columns were made from 1-ml plastic disposable micropipette tips, which were filled to 5% of the total volume with Sephadex LH-20 (Pharmacia) equilibrated with 10 mM Tris-HCl, 5 mM EDTA, and 10% glycerol, pH 7.4 (TEGD buffer). The columns were stoppered with a glass bead. The columns were first washed with 200 μl of TEGD. At 15 min after the TEGD wash, 100- μl aliquots of the incubates were applied to the columns and were washed onto the column bed with an additional 100 μl of TEGD. At 30 min after the samples were applied, the [^3H]E₂ bound to receptors was eluted from the columns with 400 μl of TEG buffer. The radioactivity in the eluate was measured by liquid scintillation counting using Liquiscint scintillation fluid (National Diagnostics, Somerville, NJ) at 32% efficiency. The protein content of the incubate was determined by the dye-binding method of Bradford (1976). All results were expressed as femtomoles of E₂ specifically bound to receptors per mg of protein.

To evaluate any loss of receptors caused by freezing, we used Scatchard analysis to compare E₂ receptors from DMSO-perfused and frozen samples of mediobasal hypothalamus-preoptic area (MBH-POA) to unperfused

tissue assayed immediately after sacrifice. The MBH-POA samples were removed as described (McEwen et al., 1975). The tissue was either assayed immediately for receptor levels or was frozen on dry ice and then kept at -15°C for 2 hr to approximate the treatment that sectioned tissue received. The frozen tissue then was stored overnight at -40°C . Tissue from three or four rats was pooled for analysis and was homogenized in 2.8 or 3.5 ml of TEGD. The cytosol fraction of the homogenate was obtained in a standard ultracentrifuge ($100,000 \times g$ for 30 min), and the levels of E₂ receptors were determined as described. We also used pooled MBH-POA cytosol to determine the protein concentrations over which the amount of E₂ receptor varied linearly with the amount of protein. This was done by incubating 1 nM [^3H]E₂ with varying amounts of cytosol from DMSO-perfused and frozen tissue.

Results

Scatchard analysis and linearity. Scatchard analysis revealed that there was a moderate loss in the number of E₂ receptors in frozen, DMSO-perfused MBH-POA as compared to unfrozen tissue (B_{max} for unfrozen tissue, 23.6 ± 4.3 ; B_{max} for frozen tissue, 15.1 ± 2.6), but there was no change in the affinity of receptors after freezing (fresh K_d , 0.44 ± 0.01 nM; frozen K_d , $0.48 \pm .03$ nM, mean $\pm$ SEM, $n = 3$). This indicated that substantial amounts of E₂ receptor was preserved during the freezing and thawing process. In preliminary studies, we observed only slightly higher levels (10 to 20%) of E₂ receptor in samples microdissected from DMSO-perfused tissue compared to samples from unperfused frozen tissue. This indicated that the DMSO perfusion had only a slightly protective effect on freezing-induced loss in our samples in contrast to its larger cryoprotective action on pituitary E₂ receptors and neural progesterin receptors (MacLusky et al., 1982; Parsons et al., 1982).

The amount of E₂ receptor was proportional to the amount of cytosol protein over concentrations from 5 to 200 μg . The protein content of all nuclei and subregions was within this range (Table I). In areas with moderate amounts of receptor, about 40 μg of cytosol protein (120 μg of homogenate protein) was needed to obtain reliable measurements of E₂ binding (20 μg for total binding and 20 μg for nonspecific binding). It was sufficient to use only 10 μg of cytosol protein in regions with a high density of receptor. Depending on the density of receptor, the amount of specific binding for [^3H]E₂ ranged from 20 to 80% of the total binding.

Distribution of E₂ receptor in brain regions. There were significant levels of cytoplasmic E₂ receptors in most of the 46 brain regions that we examined (Table II). In agreement with previous biochemical and autoradiographic studies (McEwen and Pfaff, 1970; Pfaff and Keiner, 1973), the highest levels of receptor were found in nuclei of the mediobasal hypothalamus and preoptic area. There were low to moderate levels of E₂ receptors in certain regions of the limbic system and diencephalon, and there were also regions of the rat brain, such as the caudate-putamen, that lacked measurable amounts of E₂ receptor.

TABLE I

Location and protein content of nuclei and subregions in 300- μ m frozen sections

The location and protein content of nuclei and subregions within frozen sections and the size of punch cannula used for removal are given. Three hundred-micrometer-thick coronal sections of rat brain were cut with a cryostat and thaw-mounted onto glass slides. Nuclei and subregions were removed according to the procedure of Palkovits (1973) using a 500- or 1000- μ m stainless steel cannula or with a small scalpel. Sections were cut in consecutive 300- μ m intervals. Section 1 corresponded to König and Klippel's (1963) level A8380. Section 2 is 300- μ m posterior to section 1, section 3 is 300 μ m posterior to section 2, etc. The protein content of the assay samples was measured by the method of Bradford (1976). The values given in the table are the amounts of protein applied to LH-20 columns and represent four to six experiments; three rats were used per experiment.

Region	Section	Punch Size	Protein per Assay Sample
			μ g
Diagonal band of Broca	1-3	Medial, 1000 μ m; bilateral, 500 μ m	27 $\pm$ 4
Medial septum	1-3	Single, 1000 μ m	17 $\pm$ 2
Lateral septum	1-3	Bilateral, 1000 μ m	14 $\pm$ 1
Olfactory tubercle ^a	1-3	Bilateral, scalpel	105 $\pm$ 11
Nucleus stria terminalis	3, 4	Bilateral, 500 μ m above and below anterior commissure (3); bilateral, 1000 μ m (4)	23 $\pm$ 4
Medial preoptic nucleus	4, 5	Bilateral, 1000 μ m	24 $\pm$ 4
Suprachiasmatic preoptic nucleus	4, 5	Bilateral, 500 μ m	8 $\pm$ 2
Periventricular preoptic nucleus	4, 5	Scalpel, 250 μ m around ventricle	12 $\pm$ 1
Lateral preoptic nucleus	4, 5	Bilateral, 1000 μ m	32 $\pm$ 3
Caudate-putamen	4-6	Bilateral, 1000 μ m	35 $\pm$ 3
Parietal cortex	4-7	Bilateral, scalpel	173 $\pm$ 11
Cingulate cortex	4-7	Scalpel	170 $\pm$ 3
Periventricular anterior hypothalamus	6-9	Scalpel or single, 1000 μ m	17 $\pm$ 2
Anterior hypothalamus	6-9	Bilateral, 1000 μ m	37 $\pm$ 5
Supraoptic nucleus	6-8	Bilateral, 500 μ m	7 $\pm$ 1
Paraventricular nucleus	8-10	Single, 1000 μ m	19 $\pm$ 2
Ventromedial nucleus	10-14	Bilateral, 1000 μ m	60 $\pm$ 2
Dorsomedial nucleus	11-14	Bilateral 1000 μ m	53 $\pm$ 2
Arcuate-median eminence ^b	11-14	Scalpel	51 $\pm$ 2
Lateral hypothalamus	11-14	Bilateral, 1000 μ m	46 $\pm$ 6
Medial amygdala	11-14	Bilateral, 1000 μ m	60 $\pm$ 4
Cortical amygdala	11-14	Bilateral, 1000 μ m	60 $\pm$ 2
Ventral thalamus	11-14	Bilateral, 1000 μ m	44 $\pm$ 5
Reuniens nucleus	11-14	Single, 1000 μ m	24 $\pm$ 4
Rhomboid nucleus	11-14	Single, 500 μ m	12 $\pm$ 3
Dentate gyrus	12-15	Bilateral, 2 $\times$ 500 μ m	28 $\pm$ 3
CA ₁	12-15	Bilateral, 2 $\times$ 500 μ m	27 $\pm$ 2
CA ₃	12-15	Bilateral, 1000 μ m	43 $\pm$ 3
Dorsal subiculum	12-15	Bilateral, 500 μ m	15 $\pm$ 3
Habenula	15-18	Bilateral, 1000 μ m	35 $\pm$ 1
Ventral premammillary nucleus	15, 16	Bilateral, 1000 μ m	30 $\pm$ 1
Ventral subiculum	22-25	Bilateral, 1000 μ m	56 $\pm$ 7
Central grey	22-27	Single, 1000 μ m	43 $\pm$ 4

^a The sample of the olfactory tubercle included all three cell layers (plexiform, pyramidal, and polymorphic (Krieger, 1981)).

^b The arcuate nucleus could not be removed consistently without taking some of the median eminence; therefore, this sample must be considered as a pool of the two regions.

TABLE II

Estrogen (E₂) receptor levels in nuclei and subregions

The values are measurements of cytoplasmic E₂ receptor levels in nuclei and subregions of rat brain. The amount of E₂ receptor in microdissected regions of rat brain was measured as described in the text. Tissue from three rat brains was pooled for one assay. *N* is the number of assays. Results are expressed as femtomoles per mg of E₂ specifically bound per mg of cytosol protein $\pm$ SEM. The total counts per min per sample ranged from 50 to 100 cpm above background in regions with a high concentration of E₂ receptors (Fig. 1) and 20 to 50 cpm in areas with zero to moderate amount of E₂ receptor. The nonspecific counts per min ranged from 10 to 35 cpm. The background was 7 cpm. All samples were counted for a minimum of 10 min. The amount of cytosol protein used in one assay is given in Table I. Statistical significance was assessed with a two-tailed Student's *t* test. All receptor levels are significantly (*p* < 0.05) different from zero unless footnoted.

Region	Amount	<i>N</i>
	fmol/mg protein	
Diagonal band of Broca	2.8 $\pm$ 0.7	4
Medial septum	N.S. ^a	6
Lateral septum	1.8 $\pm$ 0.8	6
Olfactory tubercle	2.2 $\pm$ 0.4	4
Nucleus stria terminalis	6.1 $\pm$ 1.3	6
Medial preoptic nucleus	21.6 $\pm$ 4.9	6
Suprachiasmatic preoptic nucleus	13.2 $\pm$ 2.9	4
Periventricular preoptic area	32.0 $\pm$ 1.0	6
Lateral preoptic nucleus	1.9 $\pm$ 0.8	5
Caudate-putamen	N.S. ^a	4
Parietal cortex	N.S. ^a	6
Cingulate cortex	1.4 $\pm$ 0.2	4
Periventricular anterior hypothalamus	18.5 $\pm$ 1.3	6
Anterior hypothalamus	6.7 $\pm$ 2.5	5
Supraoptic nucleus	8.6 $\pm$ 1.7	6
Paraventricular nucleus	6.5 $\pm$ 1.0	5
Ventromedial nucleus	12.0 $\pm$ 1.7	6
Dorsomedial nucleus	4.9 $\pm$ 0.7	6
Arcuate-median eminence	13.4 $\pm$ 1.7	6
Lateral hypothalamus	4.2 $\pm$ 1.0	4
Medial amygdala	9.8 $\pm$ 1.7	6
Cortical amygdala	3.4 $\pm$ 0.5	6
Ventral thalamus	N.S. ^a	5
Reuniens nucleus	N.S. ^a	4
Rhomboid nucleus	2.4 $\pm$ 1.4	5
Dentate gyrus	3.0 $\pm$ 1.3	5
CA ₁	N.S. ^a	5
CA ₃	N.S. ^a	5
Dorsal subiculum	N.S. ^a	4
Habenula	1.3 $\pm$ 0.6 ^b	4
Ventral premammillary nucleus	5.4 $\pm$ 1.5	4
Ventral subiculum	2.4 $\pm$ 0.9	4
Central grey	N.S. ^a	4

^a N.S., not significantly different from a zero level of receptor.

^b *p* = 0.056 compared to 0 $\pm$ 0.

Mediobasal hypothalamus-preoptic area. The highest concentration of cytoplasmic E₂ receptors in the brain was found in the nuclei of the preoptic area. Within the POA, the amount of E₂ receptor was greatest in the periventricular nucleus (30 to 40 fmol/mg of protein), followed by the medial preoptic nucleus (20 fmol/mg) and the suprachiasmatic preoptic nucleus (13 fmol/mg). By contrast, the lateral preoptic nucleus had low levels of E₂ binding (2 fmol/mg). There were moderate (6 fmol/mg) levels of receptor dorsal to the POA, in the bed nucleus of the stria terminalis, while there was no de-

tectable receptor immediately lateral to the bed nucleus in the caudate-putamen. There were high levels of E₂ receptor (18 fmol/mg) immediately caudal to the POA in the periventricular nucleus of the anterior hypothalamus (PVAH) with moderate (6 fmol/mg) but significantly lower levels of receptor lateral to the PVAH in the anterior hypothalamic nucleus. There were moderate levels of E₂ binding lateral and dorsal to the anterior hypothalamus in the supraoptic and paraventricular nuclei. We also observed high levels (13 fmol/mg) of E₂ receptor immediately caudal to the PVAH in pooled tissue from the arcuate nucleus and median eminence. There were also high levels of E₂ receptor immediately lateral to the arcuate in the ventromedial nucleus (VMN). The dorsomedial nucleus, immediately above the VMN, had moderate levels of E₂ receptor (5 fmol/mg) as did the lateral nucleus of the hypothalamus. The ventral premammillary nucleus, immediately caudal to the VMN, also had moderate levels of receptor.

Limbic regions. There were low (1 to 3 fmol/mg) but detectable levels of E₂ receptor immediately rostral to the POA in the nucleus of the diagonal band of Broca. The olfactory tubercle, immediately lateral to the diagonal band, also had a low but significant amount of E₂ receptor (2 fmol/mg). There were detectable numbers of binding sites in the lateral septum but no measurable E₂ receptor in the medial septum. The cingulate cortex had low but significant amounts of E₂ receptor, while there was no apparent receptor in the neighboring parietal cortex. We observed relatively high amounts of E₂ receptor in the medial amygdaloid nucleus (10 fmol/mg), while moderate (3 fmol/mg) but significantly lower amounts of receptor were present in the cortical nucleus of the amygdala. In the hippocampus, there were low but significant amounts of receptor in the dentate gyrus and the ventral subiculum. There was no detectable E₂ receptor in the CA₁ and CA₃ hippocampal subfields nor in the dorsal subiculum.

Diencephalon and midbrain. The midline reuniens nucleus of the thalamus had a low level of E₂ binding, while there was no detectable receptor in the rhomboid nucleus immediately dorsal to the reuniens. The ventral thalamic nucleus also lacked measurable E₂ receptors. The level of E₂ receptor in the habenula approached statistical significance ($p = 0.056$), while no significant receptor was observed in the central grey, an area that shows moderate labeling in [³H]E₂ autoradiography (Pfaff and Keiner, 1973).

Subregions of MBH-POA nuclei. The high level of E₂ binding in particular MBH-POA nuclei enabled us to assay the amount of receptor in their anatomical subregions (Table III). The bed nucleus of the stria terminalis (Nst) extended over two, 300- μ m-thick coronal sections, corresponding roughly to König and Klippels (1963) levels A7190 and A6860. There was no significant difference between the amount of receptor in the rostral portion of the Nst (the first 300- μ m section) and the caudal portion of the nucleus (the second 300- μ m section). Similarly, the medial preoptic nucleus (MPOA) and the periventricular preoptic nucleus (PVPOA) also extended over two, 300- μ m sections. There was no difference in the amount of E₂ receptor in the rostral and caudal portions

TABLE III
Estrogen (E₂) receptor levels in subdivisions of mediobasal hypothalamic and preoptic nuclei

The nuclei in Table II with the highest concentration of E₂ receptor were subdivided into rostral and caudal portions or, in the case of the VMN, into medial and lateral portions as well ($N = 4$ for all regions). The rostral bed nucleus was removed from section 3 (Table I); its caudal portion was removed from section 4. The rostral medial preoptic nucleus and periventricular preoptic nucleus were removed from section 4; their caudal portions were removed from section 5. The rostral periventricular anterior hypothalamus was taken from sections 6 and 7; its caudal portion was removed from sections 8 and 9. The rostral VMN was taken from sections 10 to 12; the caudal VMN was removed from sections 13 and 14. The medial and lateral portions of the VMN were removed from sections 10 to 14. The sizes of the punch cannulas used were the same as in Table I. The medial and lateral VMN were removed bilaterally with a 500- μ m cannula. The amount of receptor in the caudal portion of the periventricular anterior hypothalamus is significantly different than the amount in the rostral portion ($p < 0.05$; paired t test). There were no additional significant differences.

Nucleus	Amount [fmol/mg protein]
Nucleus stria terminalis, rostral	8.1 $\pm$ 3.0
Nucleus stria terminalis, caudal	10.6 $\pm$ 1.5
Medial preoptic nucleus, rostral	10.9 $\pm$ 1.1
Medial preoptic nucleus, caudal	23.0 $\pm$ 5.2
Periventricular preoptic nucleus, rostral	41.8 $\pm$ 15.0
Periventricular preoptic nucleus, caudal	58.0 $\pm$ 22.0
Periventricular anterior hypothalamus, rostral	27.0 $\pm$ 3.4
Periventricular anterior hypothalamus, caudal	9.7 $\pm$ 1.4
Ventromedial nucleus, rostral	15.0 $\pm$ 3.0
Ventromedial nucleus, caudal	13.5 $\pm$ 1.2
Ventromedial nucleus, lateral	11.6 $\pm$ 4.1
Ventromedial nucleus, medial	N.S. ^a

^a N.S., not significantly different from a zero level of receptor.

of the PVPOA, and although it appeared that there was more receptor in the caudal portion of the MPOA than in the rostral portion, this difference did not reach statistical significance. The PVAH and the VMN are large nuclei that extend over four and five 300- μ m sections, respectively. There was no difference in the amount of E₂ receptor in the first three sections of the VMN, compared to the last two sections. However, the rostral two sections of the PVAH had significantly more receptor than the caudal sections. It is also possible to divide the VMN into medial and lateral portions. In agreement with [³H]E₂ autoradiography (Pfaff and Keiner, 1973), there were high levels of E₂ receptor in the lateral portion of the VMN and no significant receptor in its medial portion.

Discussion

Our biochemical measurements of the amount of E₂ receptor in rat brain nuclei and subregions are in good agreement with the general pattern of labeling obtained with [³H]E₂ autoradiography (Stumpf, 1968; Pfaff and Keiner, 1973). Pfaff and Keiner (1973) observed large numbers of labeled cells in the periventricular region extending rostrally through the POA and caudally through the arcuate nucleus. They found less uptake of [³H]E₂ in nuclei lateral to the periventricular region, and

there were also many labeled cells in the ventrolateral portion of the VMN and medial nucleus of the amygdala, while less intense but consistent labeling was seen in the diagonal band of Broca, lateral septum, and olfactory tubercle. These observations agree with our measurements (Tables II and III; Fig. 1).

There are also several discrepancies between our results and the autoradiographic studies. There is a moderate (8 fmol/mg) level of soluble E_2 receptor in the supraoptic nucleus (SON), while no E_2 labeling was observed in the SON with autoradiography (Pfaff and Keiner, 1973). Similarly, there was only a moderate amount of receptor in the caudal portion of the Nst, which, in [3H] E_2 autoradiograms, appears as intensely labeled as the PVPOA. In addition, there was no detectable E_2 receptor in the central grey, a region which shows moderate but reliable labeling of some cells in autoradiography.

There are several possible explanations for the discrepancies between the autoradiographic and biochemical measurements. First, we are measuring only cytoplasmic receptors for E_2 , while the autoradiographic studies are visualizing both cytoplasmic receptors and nuclear receptors translocated from the cytoplasm by the *in vivo* administration of [3H] E_2 (Pfaff and Keiner, 1973). Since only half of the cytoplasmic receptors in pooled MBH-POA tissue move into nuclei in response to E_2 (Lieberburg et al., 1980), it is possible that regional differences

in the ability of E_2 receptors to translocate into nuclei might account for some of the differences between the biochemical and autoradiographic measurements. For example, it is conceivable that the E_2 receptors in the SON do not translocate as extensively into nuclei in response to the steroid as in the VMN. It is also possible that the amount of cytoplasmic E_2 receptor varies considerably within individual cells so that a biochemically small level of receptor might be concentrated in a few cells, which appear labeled in autoradiography. This could explain why scattered labeled cells appear in the central grey, while no detectable receptor is seen by biochemical assay. We also observed a moderate loss in E_2 receptors from large samples of MBH-POA during freezing and cannot exclude the possibility that the extent of the loss differs among nuclei and subregions.

The high levels of E_2 receptor in particular MBH-POA nuclei, such as the MPOA or the VMN, supports the notion that these regions are important target sites for the actions of E_2 on behavior and neuroendocrine function. It is known that direct implants of E_2 into the nuclei of the MBH-POA with the highest levels of receptor (MPOA and VMN) will duplicate many of the actions of peripherally administered steroid on reproductive or non-reproductive behaviors (Davis et al., 1979; Rubin and Barfield, 1980; Wade and Zucker, 1970) or on gonadotropin secretion (Goodman, 1978). The ablation of these nuclei (Pfaff and Sakuma, 1979; Taleisnik and McCann,

Figure 1. Concentration of E_2 receptors in selected nuclei and subregions. The data are from Table II. Results are expressed as femtomoles per mg of E_2 specifically bound per mg of protein. The abbreviations used are: PVPOA, periventricular preoptic area; MPOA, medial preoptic area; PVAH, periventricular anterior hypothalamus; SCPOA, suprachiasmatic preoptic nucleus; ARC-ME, arcuate nucleus-median eminence; VMN, ventromedial nucleus; MA, medial amygdaloid nucleus; SO, supraoptic nucleus; PVN, paraventricular nucleus; NsT, bed nucleus of the stria terminalis; VPV, ventral premammillary nucleus; DMN, dorsomedial nucleus; DG, dentate gyrus; DB, nucleus of the diagonal band of Broca; OT, olfactory tubercle; LS, lateral septum.

1961) or disruption of their function with pharmacological inhibitors (Jackson, 1972; Rainbow et al., 1980) also will impair the same behavioral and neuroendocrine effects of E_2 . The functional significance of the moderate and low levels of E_2 receptors in particular brain regions is more obscure. The presence of moderate levels of E_2 receptor in the supraoptic and paraventricular nuclei might provide an explanation for the actions of E_2 on the secretion of oxytocin and vasopressin (Skowsky et al., 1979; Yamaguchi et al., 1979), the peptide hormones synthesized by these nuclei. The presence of a low level of E_2 receptor in subregions of the hippocampus is consistent with the observed changes in hippocampal electrical activity after direct application of E_2 to hippocampal slices maintained *in vitro* (Teyler et al., 1980). These receptors also might mediate a sexually divergent effect of E_2 that could explain the sexually dimorphic pattern of axonal sprouting observed in the hippocampus (Loy and Milner, 1980). The possible functions of the low and moderate levels of E_2 receptors in such regions as the ventral premammillary nucleus or the olfactory tubercle are less apparent. It may be that these receptors mediate currently unknown actions of E_2 on the brain.

An important aspect of our study is that it provides a method for the relatively rapid quantification of E_2 receptors in individual brain nuclei and subregions. Thus, alterations in whole hypothalamic or limbic E_2 receptor levels (Roy and Wilson, 1981; Camp-Grossman and Wise, 1981; Phelps et al., 1981) now can be localized to particular nuclei or subregions. It is also feasible to make quantitative comparisons in the amount of E_2 receptor in nuclei of male or female brains (Lieberburg et al., 1980; Nordeen and Yahr, 1981) or between different species. We have used the same methodology to measure the levels of cytoplasmic progesterin receptors in nuclei and subregions of rat brain (Parsons et al., 1982b), and it should be possible to extend the method to quantitate receptors for other steroid hormones in neural tissue. It also may be feasible to measure nuclear receptors for steroid hormones in frozen punch-out samples. It is likely that the further biochemical analysis of the receptors for E_2 and other steroid hormones at or below the level of individual nuclei will clarify further how steroid hormones affect the brain.

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