0270-6474/82/0202-0199$02.00/0

The Journal of Neuroscience
Vol. 2, No. 2, pp. 199-205
February 1982

MODULATION BY ESTRADIOL OF SEROTONIN$_1$ RECEPTORS IN BRAIN[1]

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Received May 5, 1981; Revised September 28, 1981; Accepted October 7, 1981

Abstract

Estrogens were found to exert a biphasic effect on the density of serotonin$_1$ receptors in the female rat brain: an acute reduction in serotonin receptor density throughout the brain is followed 48 to 72 hr later by a selective increase in those brain regions known to contain estrogen receptors—hypothalamus, preoptic area, and amygdala. The acute reduction in serotonin receptor density can be mimicked by estradiol *in vitro*. We conclude that estradiol may have a fast, direct effect on brain membranes to modify serotonin receptor availability, while exerting a slow effect on the same receptors through an interaction with intracellular estrogen receptors in those brain regions that contain them. The observation that female sex hormones are involved in the regulation of serotonin$_1$ receptors may have important implications in the understanding of female sexual behavior in the female rat and in the understanding of hormone-linked emotional disturbances in women.

Female gonadal hormones have been shown to affect serotonin neurotransmission in several ways. The turnover and levels of brain serotonin change during the estrous cycle of the rat (Fludder and Tonge, 1975), during pregnancy, or following injection of ovarian sex steroids (Greengrass and Tonge, 1974). Estradiol and progesterone treatment modifies serotonin uptake in the hypothalamus (Endersby and Wilson, 1974) and other brain regions (Wirz-Justice et al., 1974). We have found that female rats are more sensitive than males to an elevation of brain serotonin, responding to lower doses of pargyline plus tryptophan (Biegon et al., 1979). Such a sex difference could be due to hormonal modulation of postsynaptic receptor affinity or density. A subsequent study showed that serotonin receptor density does indeed fluctuate in the basal forebrain during the estrous cycle of the rat (Biegon et al., 1980), resulting in a 50% lower density on proestrus than on diestrus.

Two questions raised by these results are: which hormone or hormones play a part in the modulation of serotonin receptors during the estrous cycle and through which mechanism or mechanisms are these effects exerted? Estradiol has been shown to affect several receptor systems in both the periphery and the brain (Roberts et al., 1978; MacLusky and McEwen, 1978; Rainbow et al., 1980a). Therefore, the present study was designed to test the effect of estradiol on serotonin$_1$ receptors in the brains of ovariectomized female rats *in vivo* and *in vitro* and to investigate the possible involvement of progesterone and pituitary hormones.

Materials and Methods

Sprague-Dawley female rats (150 to 200 gm, Charles River) were kept under standard light (14:10 light:dark cycle) and temperature (22.8°C) conditions. Food and water were available *ad libitum*. Bilateral ovariectomy was performed under ether anesthesia. Animals were allowed at least 7 days to recover. Hypophysectomized rats were purchased from Charles River and used 7 days after surgery.

Hormone treatment

Experiment 1. Pairs of ovariectomized females were injected with estradiol (E_2) in 50% ethanol/saline, s.c. Control animals were injected with the same volume (100 μl) of vehicle. One hour later, the animals were sacrificed by decapitation and the brains were removed quickly and dissected on ice using the method outlined by Luine et al. (1974). Estradiol doses were 2, 5, 10, 20, 50, and 100 μg/animal.

Experiment 2. Estradiol benzoate (EB) in sesame oil was injected subcutaneously and the animals were decapitated 2 hr later. Control animals received only vehicle

[1] This work was supported by National Institutes of Health Grant NS 07080 and by Institutional Grant RF 70095 from the Rockefeller Foundation for research in reproductive biology. Dr. Biegon is the recipient of a Weizmann Postdoctoral Fellowship. We would like to acknowledge the cooperation and assistance of Dr. Charles M. Paden in the experiments involving pituitary transplants under the kidney capsule.

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(100 μl). EB was given in doses of 2, 5, 10, and 25 μg/animal.

Experiment 3. Groups of six to eight animals were injected with 10 μg/animal of EB in oil at 0 and 24 hr and decapitated at 72 hr. In some cases, progesterone, 2.5 mg, was given 3 hr before sacrifice. Two groups of hypophysectomized animals were included in this experiment in order to establish the role of the pituitary in the response to estrogen treatment. The possible involvement of prolactin was investigated in animals implanted with ectopic pituitaries under the kidney capsule. Under these circumstances, the ectopic pituitary is believed to secrete high levels of prolactin (e.g., McNeilly et al., 1980).

Experiment 4. Several steroid hormones (final concentration, 10^{-10} to 10^{-4} M) were added directly to the assay tubes containing the labeled serotonin. All were dissolved in 10 μl of ethanol.

Experiment 5. A 10^{-4} M solution of EB in ethanol was diluted by a buffer containing 50 mM Tris-HCl and 3.5% polyvinylpyrrolidone (PVP), pH 7.6, to a final concentration of 10^{-8} M. Tissue from whole forebrain or cortex was preincubated in this steroid-containing buffer for 2 hr at room temperature prior to the receptor binding assay. Control tissue was incubated with Tris buffer alone or with Tris/PVP buffer.

Serotonin binding assay

The assay procedure is based on the method of Bennett and Snyder (1976) as modified by Nelson et al. (1978). For experiments 1 and 2, fresh tissue (weighed) was homogenized in an isotonic Tris-Na^+-K^+ buffer, pH 7.25, in a glass-Teflon homogenizer. The homogenate was centrifuged at 1,000 × *g* for 10 min. The pellet was washed by an additional portion of buffer and centrifuged again. The combined supernatant fractions (S_1) were homogenized by a Polytron (setting 7) and incubated for 10 min at 37°C to facilitate destruction of endogenous serotonin by endogenous monoamine oxidase. After the addition of 100 μM pargyline, aliquots from this fraction were added to the assay tubes. Alternatively, the S_1 fraction was spun down at 30,000 × *g* for 10 min, and the pellet was resuspended in 50 mM Tris-HCl buffer and incubated for 10 min at 37°C prior to the final resuspension in 100 μM pargyline and 0.02% ascorbate buffer and addition to the assay tubes.

Assay tubes contained 0.2 ml of the appropriate concentration of [^{3}H]serotonin (New England Nuclear, 30.2 Ci/mmol; 0.3 to 5 nM), 0.5 ml of brain homogenate (100 to 500 μg/sample), and 0.3 ml of Tris/pargyline/ascorbate buffer, with or without 10^{-6} M cold serotonin for estimating nonspecific binding. Tissue was incubated with the ligand for 10 min at 37°C and then transferred to an ice water bath and filtered through Whatman GF/B glass fiber filters using a Brandel cell harvester (Gaithersburg, MD). Filtration was followed by three 2-sec washes with ice cold buffer, 2 sec apart. This was found to be equivalent to 15 ml of wash buffer under the conditions of our assay. Filters were placed in plastic vials containing 5 ml of Liquiscint (National Diagnostics) scintillation fluid, incubated for 10 min at 50°C, and then cooled and counted in a Packard 3255 liquid scintillation counter at 33% efficiency.

For experiments 3 and 4, frozen crude membrane preparations were made up in isotonic buffer and kept at −70°C until used. Thawed membranes were washed in 50 mM Tris-HCl buffer, pH 7.6, and then processed in the same way as fresh membranes. In experiment 5, the 2-hr incubation at room temperature was taken to substitute for the 10-min incubation at 37°C and was followed by centrifugation at 30,000 × *g* for 10 min, resuspension in Tris/pargyline/ascorbate buffer, etc. Protein content was determined by the method of Bradford (1976).

Results

Validation of the binding assay. The characteristics of serotonin$_1$ binding, in terms of B_{max} and K_d, are similar to those reported in the literature by us and others (Biegon et al., 1980; Nelson et al., 1978; Bennett and Snyder, 1976), with K_d being 1 to 6 nM and B_{max} ranging from 200 to 1,000 fmol/mg of protein, depending on the brain region involved. The pharmacological specificity was validated through displacement experiments with several drugs. Using 2 nM [^{3}H]serotonin (5-HT) and several concentrations of each displacer, the following K_i values were obtained: lysergic acid diethylamide, 8.5 ± 1 nM; 5-HT, 3.5 ± 1 nM; methyltryptamine, 500 nM; mianserin, 1,000 ± 200 nM; haloperidol, 2,500 nM; spiroperidol, 7,500 nM; pimozide, 10,00 nM. These values, too, are in good agreement with the literature.

Acute in vivo treatment. Injection of estradiol in 50% ethanol/saline produces a significant and dose-dependent decrease in serotonin receptor binding. The reduction in serotonin binding appears in both hypothalamic and non-hypothalamic brain regions to the same extent. After observing this in preliminary experiments, we started using the whole forebrain to study the dose-response relationship. This decrease is due to a reduction in the maximal number of binding sites as revealed by Scatchard analysis of the binding data (Fig. 1). A trend toward increased affinity (lower K_d) is also apparent. Even the lowest dose, 2 μg/animal, produces a 35% reduction in B_{max}. However, the dose-response curve seems to level off at the higher doses, reaching 70 to 80% inhibition (Fig. 2).

An injection of 2 μg of E_2 ethanol/saline results in levels of E_2 in the circulation higher than those achieved under physiological conditions (Krey et al., 1979). Also, the presence of alcohol may conceivably complicate the interpretation of the data since alcohol itself may interfere with binding to membranes (e.g., Inaba and Kamata, 1979). Therefore, we repeated these experiments using estradiol benzoate (EB) dissolved in sesame oil. Under these conditions, a significant decrease in serotonergic binding is apparent following a 5-μg EB dose (Fig. 2). The effect is dose dependent and a dose of 25 μg of EB inhibits up to 70% of the binding. Thus, it is evident that, independently of solvent and whether it is administered as the free alcohol or as the benzoate ester, estradiol is capable of reducing the concentration of serotonin receptors within 1 to 2 hr of injection.

However, classical receptor induction or the development of subsensitivity usually takes longer than 1 to 2 hr (Nelson et al., 1978; Samanin et al., 1980). Therefore, we also studied the delayed effects of estradiol in animals

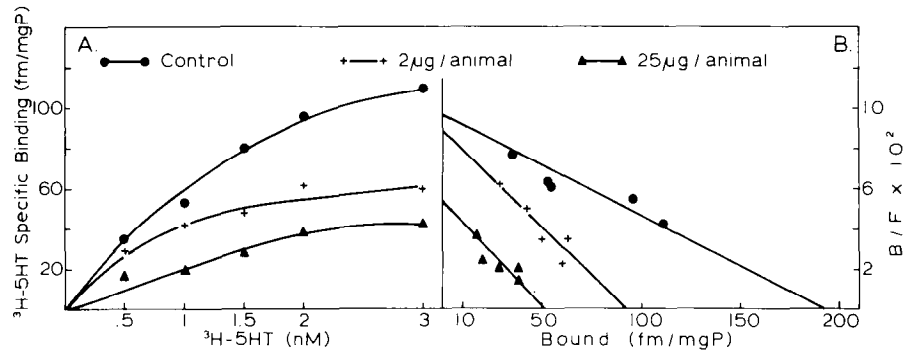

Figure 1. Acute effect of an estradiol injection on serotonin binding to the forebrains of ovariectomized female rats. *A*, Specific binding curves obtained in a single experiment; *B*, Scatchard (1949) analysis of the binding data in *A*. Control: K_d = 4.0, B_{max} = 190 fmol/mg of protein; 2 μg: K_d = 2.0, B_{max} = 90 fmol/mg of protein; 25 μg: K_d = 2.0, B_{max} = 52 fmol/mg of protein. *B/F*, bound/free.

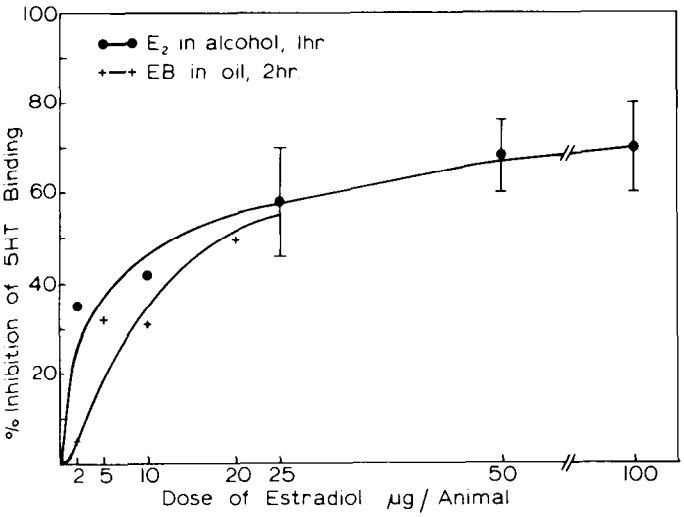

Figure 2. Reduction in serotonin receptor density as a result of increasing doses of estradiol. Estradiol in ethanol/saline or estradiol benzoate in oil were given as described under "Materials and Methods." Each *point* represents data from two to six complete binding curves, and the percentage of inhibition is 100 − (B_{max} experimental/B_{max} control). Tissue samples consisted of cortex, hypothalamus, and whole forebrain. The standard error did not exceed 8 to 15% of the mean in all of the points. The *bars* depict the range of results with the three highest doses of E_2.

sacrificed 72 hr after the first of two 10-μg injections of EB in oil. This paradigm results in an increase in serotonin receptor levels, which is, however, restricted to estrogen-concentrating areas in the brain (e.g., Pfaff and Keiner, 1973). The increase is due to a selective effect on B_{max}, with no significant changes in the affinity (Fig. 3; Table I). Increases of 30 to 40% occur in the amygdala, mediobasal hypothalamus, and preoptic area. No change is observed in the caudate nucleus or in the cerebral cortex, where the acute effects described above were studied (Table I). Progesterone (2.5 mg) had no additional effect when injected 3 hr before sacrifice in estrogen-primed rats (data not shown).

Hruska and co-workers (Hruska and Silbergeld, 1980; Hruska et al., 1980) have demonstrated the induction of dopamine receptors in the striatum following treatment with estradiol. In those studies, however, hypophysectomy abolished the estradiol-dependent increase and prolactin alone was able to produce such increases in the absence of estrogens. We found that hypophysectomy did not abolish the increase in serotonin receptors in steroid-concentrating areas. Moreover, no increase was observed in ovariectomized animals exposed for 2 weeks to elevated levels of prolactin (Table II) via pituitary grafts under the kidney capsule. Thus, pituitary hormones are not essential for the expression of estradiol's effect on neural serotonin receptors.

In vitro studies. The decreased binding of serotonin seen 1 or 2 hr after estradiol treatment might be due to either an extremely rapid estrogen receptor-mediated, genomic mechanism or a direct effect on the cell membrane. In an effort to identify the mechanism operative in this case, we studied the effect on serotonin binding of the incubation of membranes with steroids *in vitro*. The crude membrane preparation used for the binding studies does not contain cytoplasmic estrogen receptors so that any effect that we see is probably independent of those estrogen receptors.

Simultaneous incubation of steroids with serotonin has no effect at low concentrations. In and above the micromolar range, serotonin binding is decreased. At 10^{-4} M, 17β-estradiol and its metabolite, 2-hydroxy-17β-estradiol, reduce the apparent receptor number by 30 to 40% (Fig. 4). 17α-Estradiol, the inactive stereoisomer, is less active. Other steroid hormones, such as progesterone, corticosterone, and testosterone, showed no activity at all at concentrations up to 10^{-4} M. Varying [³H]5-HT concentrations in the presence of 15 mM 2-hydroxyestradiol followed by Scatchard analysis of the binding data reveals a 50% decrease in the B_{max} (McEwen et al., 1981). Thus, it seems that, even at these high concentrations of steroids, which are unlikely to occur *in vivo*, the steroids do not compete with serotonin for its receptor (an interaction that would result in an apparent decrease in affinity with no change in B_{max}) but rather interact with the membrane in a manner that reduces the number of available binding sites. A possible mechanism has been put forward recently by Heron et al. (1980). They suggest that, by changing the microviscosity in the membrane, the steroid (in their case, cholesterol) may increase or decrease the apparent number of serotonin receptors by exposing extra receptors or "submerging" them in the

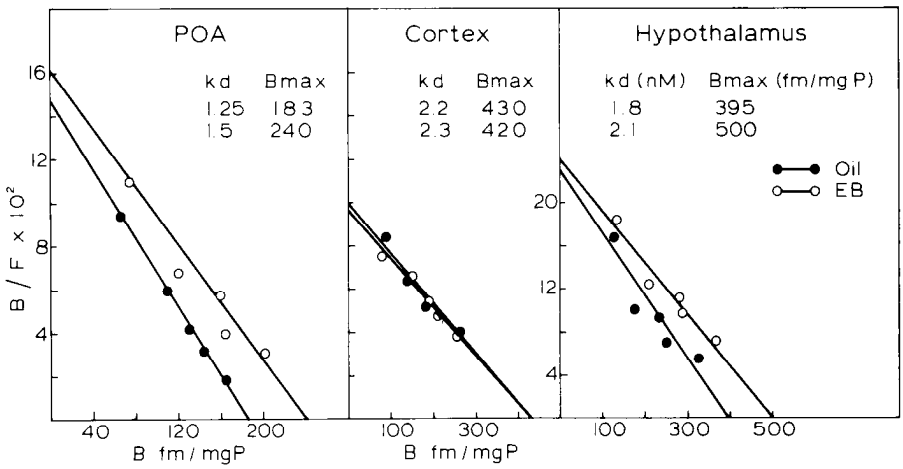

Figure 3. Selective increase in serotonin receptor binding 48 hr after an injection of estradiol benzoate. Two injections of 10 μg of EB were given as described under "Materials and Methods." The plots are Scatchard (1949) analyses of binding data from a single experiment in which brain regions were pooled from eight animals. *POA*, preoptic area; *B/F*, bound/free; *B fm/mg P*, bound in femtomoles per mg of protein.

TABLE I

Effect of estradiol benzoate on serotonin binding in several brain regions

The results are the means ± SEM of three to five repeated experiments, representing six to eight animals each. The binding capacity (B_{max}) and dissociation constant (K_d) were estimated by the method of Scatchard (1949). EB rats were killed 48 hr after the second of two 10-μg injections of estradiol benzoate in sesame oil.

Brain Region	B_{max}		K_d	
	Oil	EB	Oil	EB
	fmol/mg protein		*nM*	
Preoptic area	170 ± 9	240 ± 9[a]	1.7 ± 0.2	1.3 ± 0.2
Amygdala	695 ± 62	895 ± 60[a]	2.0 ± 0.4	1.9 ± 0.4
Hypothalamus	395 ± 3	550 ± 28[a]	2.9 ± 0.6	3.0 ± 0.5
Cortex	373 ± 28	366 ± 25	2.4 ± 0.03	2.4 ± 0.07
Caudate	506 ± 69	516 ± 60	1.3 ± 0.1	1.5 ± 0.06

[a] $p < 0.05$; Student's t test.

TABLE II

Effect of manipulating pituitary hormones on serotonin binding and response to estradiol treatment in the amygdala

Parameters are as described in the legend to Table I. EB rats were killed 48 hr after the second of two 10-μg injections of estradiol benzoate in sesame oil. EB effects were found in the hypothalamus and preoptic area as well as in the amygdala of both hypophysectomized and ovariectomized rats. The animals with ectopic pituitaries had highly elevated prolactin levels.

Treatment	N[a]	B_{max}	K_d
		fmol/mg protein	*nM*
Ovariectomy + oil	5	695 ± 62	2.0 ± 0.4
Ovariectomy + EB		895 ± 60[b]	1.9 ± 0.4
Hypophysectomy + oil	4	534 ± 123	3.5 ± 1.0
Hypophysectomy + EB		738 ± 116[c]	4.2 ± 1.1
Ovariectomy	2	900, 900	2.5, 3.7
Ovariectomy + ectopic pituitary for 2 weeks		840, 850	2.7, 3.7

[a] N, number of experiments, each performed on tissue from six to eight animals.

[b] $p < 0.01$; two-tailed paired t test, compared to oil.

[c] $p < 0.0001$; two-tailed paired t test, compared to oil.

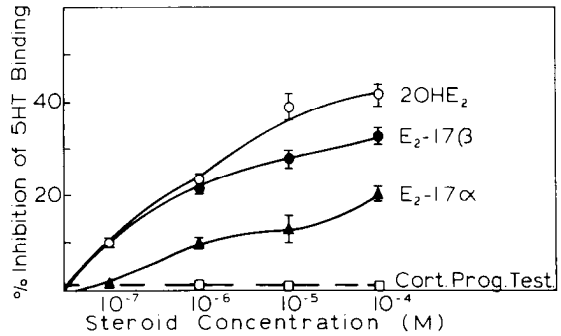

Figure 4. Effect of steroids *in vitro* on serotonin binding following simultaneous incubation for 10 min. The results are the means ± SEM of three separate experiments on cortical tissue from ovariectomized, otherwise untreated females. [^{3}H] 5-HT (2 nM) was incubated with: 2-hydroxyestradiol (*2OHE₂*), 17β-estradiol (*E₂-17β*), 17α-estradiol (*E₂-17α*), corticosterone (*CORT*), progesterone (*PROG*), and testosterone (*TEST*).

membrane. Their protocol includes 2 hr incubation of the steroid with the membranes in the presence of 3.5% polyvinylpyrrolidone (PVP). Following the same protocol, we found that estradiol in concentrations as low as 10^{-8} to 10^{-9} M significantly reduces the binding of serotonin (Fig. 5; Table III). This is due to a 20 to 25% decrease in B_{max}. The steroid also tends to increase the affinity of the binding relative to the PVP control, which is reminiscent of the size and direction of the effect of low doses of EB *in vivo* (Fig. 2). Estradiol in concentrations of 10^{-10} M or below had no effect.

Discussion

The present studies reveal a biphasic effect of estradiol on serotonin receptors: i.e., an acute reduction in the receptor concentration is followed by a delayed increase. The reduction in serotonin receptor levels 1 or 2 hr after an estradiol injection is evident throughout the brain of the ovariectomized female rat and is mimicked by a 2-hr preincubation of brain (e.g., cerebral cortex) membranes with low concentrations of estradiol in the presence of

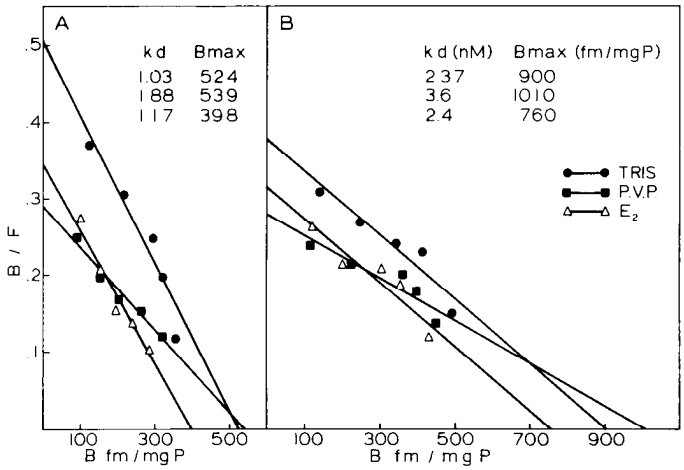

Figure 5. Effect of 2 hr incubation with estradiol *in vitro* on serotonin binding to brain membranes. *A*, Whole forebrain incubated with 10^{-9} M estradiol in PVP; *B*, cortex + hippocampus incubated with 10^{-8} M estradiol in PVP. Incubation with 10^{-10} or 10^{-11} M estradiol did not produce a significant effect. *B/F*, bound/free; *B fm/mg P*, bound in femtomoles per mg of protein.

TABLE III

Effect of estradiol in PVP, in vitro, on serotonin binding

A paired, two-tailed *t* test was performed on these data.

Treatment	*N*	K_d	B_{max}
		nM	*fmol/mg protein*
Tris buffer	4	2.6 ± 0.6	597 ± 109
PVP	6	4.5 ± 0.8[a]	768 ± 110
EB, 10 nM	6	3.0 ± 0.8[b]	514 ± 52[c]
EB, 50 nM	3	2.9 ± 0.7[b]	315 ± 30[c]

[a] $p < 0.03$, compared to Tris.
[b] $p < 0.05$, compared to PVP control.
[c] $p < 0.02$, compared to PVP control.

PVP as well as by concurrent incubation with high concentrations of steroids.

The increase in serotonin receptor levels 72 hr after estradiol benzoate injection is restricted to estrogen-concentrating brain regions (Pfaff and Keiner, 1973): i.e., mediobasal hypothalamus, amygdala, and preoptic area. In the cortex or the striatum, there is no effect. This effect can be elicited by EB in hypophysectomized animals and it is not mimicked by pituitary grafts under the kidney capsule.

Taken together, these findings may explain the observed cyclicity in serotonin receptors in basal forebrain during the estrous cycle. On proestrus, in the presence of peak levels of estrogen, we may expect to see the 20 to 25% decrease in serotonin receptor number, such as is evident after injecting a low dose of EB or after incubation of membranes with nanomolar concentrations of estradiol. Forty-eight hours later, when the circulating levels of estrogens are low, we might expect to see the delayed, receptor-mediated effect of estrogen on serotonin receptors, resulting in a 20 to 30% increase in receptor number. Thus, the biphasic effect of the hormone may result in a total 40 to 50% difference between proestrus and diestrus in brain areas which display both effects. We are conducting a further analysis of these effects in individual brain regions by microsampling and autoradiography. It shall be noted that a biphasic effect of estrogen is probably not unique to the serotonergic system, since the pituitary response to luteinizing hormone-releasing hormone has been shown to be under biphasic regulation by estrogen (Libertun et al., 1974; Vilchez-Martinez et al., 1974).

Although we observed decreased receptor levels in all brain regions following acute estrogen treatment, these acute effects might very well be restricted to estrogen-concentrating areas in the intact, cycling animal. Goodman (1977, 1978) has shown that estrogen levels in the hypothalamus of the intact, proestrus female rat are 5-fold higher than in the cortex, which, in turn, has twice the levels in the circulation. Since proestrus blood estradiol levels are of the order of 0.1 to 0.2 nM (Smith et al., 1975), one would predict that the hypothalamus in the intact rat might be exposed to estrogen concentrations 10-fold higher (i.e., 1 to 2 nM). From our data, this seems to be the lowest concentration able to produce a measurable reduction in receptor number. Estrogen levels in the cortex may never rise enough to produce such effects.

Estrogens have been shown to induce increases in receptor number in a number of tissues: e.g., adrenergic receptors in the uterus (Roberts et al., 1978) and testosterone receptors in the chick oviduct (Tokarz et al., 1978). In the brain, receptor induction by estrogen seems to follow the same time course and regional selectivity as in the present study. The levels of progestin receptors (MacLusky and McEwen, 1978), β-adrenergic receptors (Vacas and Cardinali, 1980), and cholinergic muscarinic receptors (Rainbow et al., 1980a) are increased by estrogen in the hypothalamus but not in the cortex or striatum. The genomic mediation of these actions of estrogen is supported further, in one case, by the fact that progestin receptor induction is blocked by a protein synthesis inhibitor (Rainbow et al., 1980b). Therefore, our working hypothesis is that the increase in serotonin binding is due to an estrogen receptor-mediated, genomic action of the hormone. Obviously, this assumption will have to be supported by further experiments involving estrogen antagonists and protein synthesis inhibitors. In any event, a pituitary hormone-mediated action is unlikely, since, unlike the estrogen-induced increase in striatal dopamine receptors (Hruska et al., 1980), the increase in serotonin receptors is not abolished by hypophysectomy or mimicked by pituitary grafts under the kidney capsule.

Much less is known about the possible mechanism underlying the acute reduction in serotonin receptors. A few examples relevant to this effect do exist. Estrogen has been shown to decrease luteinizing hormone-releasing hormone receptor binding in the pituitary following both *in vivo* and *in vitro* treatment (Heber and Odell, 1979) and to decrease responsiveness to serotonin in the guinea pig ileum preparation (Seaman et al., 1977). The fact that these interactions also occur *in vitro* in preparations that presumably do not contain intracellular estrogen receptors strongly suggests that a direct interaction with the membrane is involved. The fact that the change is in receptor number but not affinity argues against the idea of estradiol competing for the serotonin receptor. An attractive possibility is that estradiol is taken up into the membrane (as has actually been re-

ported for 2-hydroxyestradiol (Schaeffer et al., 1980)), causing an overall change in the microenvironment of receptors. Serotonin receptors have been found to be very sensitive to this type of manipulation when it was performed using cholesterol, which actually increases serotonin binding (Heron et al., 1980). The changes in membrane microviscosity and receptor binding reported by these investigators require a long preincubation in the presence of PVP. In our initial *in vitro* experiments reported in Figure 3, we utilized concurrent incubation of steroid and [^{3}H]5-HT and obtained reductions of binding only with micromolar concentrations of estrogens. Other steroids, like progesterone, were ineffective up to 100 μM. In subsequent *in vitro* experiments, we utilized a 2-hr preincubation of membranes with steroids in the presence of PVP and we obtained estrogen effects on [^{3}H]5-HT binding at nanomolar steroid concentrations. These actions had the same characteristic effect of enhancing the apparent affinity while reducing the B_{max} (Fig. 5) as we obtained using concurrent incubation with higher estrogen concentrations (McEwen et al., 1981). Furthermore, in recent experiments, we have determined that, using preincubation with PVP, progesterone does not decrease [^{3}H]5-HT binding even in the micromolar range (A. Biegon, B. S. McEwen, and L. Snyder, unpublished observation).

In contrast to the nanomolar sensitivity of [^{3}H]5-HT binding reported in this study, higher steroid concentrations in the micromolar range have been reported to reduce dopamine and noradrenaline binding to synaptic membranes from rat brain (Inaba and Kamata, 1979), to block histamine-dependent cyclic AMP production in rat hypothalamus (Portaleone et al., 1980), and to compete with α_1-adrenergic receptor antagonists in binding to rat cerebral cortex membranes (McEwen et al., 1981). By introducing longer incubation times and PVP to the α_1-adrenergic receptor assay, we were unable to change the nature or the dose range of the competitive estrogen effect nor did we obtain any estrogen effect upon 5-HT$_2$ receptors under similar conditions (B. S. McEwen, A. Biegon, and L. Snyder, unpublished observation). This suggests a considerable degree of selectivity of estrogen action toward neurotransmitter receptors, which again is supported by observations of Heron et al. (1980) that some membrane receptors are not affected at all by changes in membrane microviscosity, whereas others, like opiate receptors, are moderately affected.

Modulation of serotonin receptors by estrogen may be one of the components necessary for facilitation of sexual behavior in the female rat, a process which appears to be under serotonergic inhibitory control (Meyerson et al., 1974; Biegon, 1980). Similar mechanisms may be operative in humans. Serotonin has been implicated repeatedly in human affective disorders (Asberg et al., 1975; Van Praag and Korf, 1971). Estrogen-induced changes in serotonin receptors during the menstrual cycle or pregnancy may contribute to the known emotional liability associated with the premenstrual and postpartum periods (Dalton, 1964, 1971). Recent reports of estrogens themselves being efficient antidepressants in drug-resistant female patients (Klaiber et al., 1979) and essential for the expression of antidepressant action in rats (Fludder and Tonge, 1977; Kendall et al., 1981) further support the idea that estrogen modulation of the serotonergic system plays an important role in control of affect in susceptible women (e.g., Warren et al., 1979).

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