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Increase of Serotonin Receptors in Rat Uterus Induced by Estradiol*

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[^{3}H]Spiroperidol was used to label uterine membrane-binding sites that have the characteristics expected of serotonergic receptors. The characteristics of specific [^{3}H]spiroperidol binding to the uterine membrane of 17β-estradiol-3-benzoate-treated ovariectomized rats were studied. The specific [^{3}H]spiroperidol binding was rapid and reversible, and the half-maximal saturation, taken as the apparent dissociation constant (K_D) for [^{3}H]spiroperidol, was 5.16 ± 0.24 (n = 12) nM [^{3}H]spiroperidol. Scatchard plots of saturation curves of the specific [^{3}H]spiroperidol binding were convex and the Hill coefficient was 2.06 ± 0.11 (n = 12). Cinanserin, mianserin, metergoline (which are serotonergic antagonists), and serotonin (5-HT) inhibited the [^{3}H]spiroperidol binding with apparent K_i values of 21.2, 14.1, 14.1, and 176.5 μM, respectively. Concentrations of 1 mM sulpiride (a dopaminergic antagonist) and dopamine reduced [^{3}H]spiroperidol binding only 26 and 23%, respectively. 1 mM GTP reduced the potency of 5-HT (10^{-6}–10^{-3} M) to displace bound [^{3}H]spiroperidol. The uterine membranes were treated with various enzymes and protein-modifying reagents, and binding studies on the treated uterine membranes showed that protein(s), phospholipids, and *N*-acetylneuraminic acid in uterine membranes were important as specific binding sites of [^{3}H]spiroperidol.

Measurement of the specific binding of [^{3}H]spiroperidol to uterine membranes from untreated and estradiol-treated ovariectomized rats showed that estradiol significantly increased the number of specific binding sites of [^{3}H]spiroperidol, but did not change the apparent affinity of specific [^{3}H]spiroperidol binding. Estradiol also did not change the dissociation constant or the number of binding sites for [^{3}H]3-quinuclidinyl benzilate, which binds to muscarinic acetylcholine receptors.

These findings suggest that [^{3}H]spiroperidol mainly binds to 5-HT receptors in the uterine membrane of estradiol-treated ovariectomized rats. The finding that administration of estradiol significantly increased the number of [^{3}H]spiroperidol-binding sites is consistent with the specific increase in the contractile response to 5-HT observed in isolated uterus from ovariectomized rats treated with estradiol.

In several species of mammals, the response of the uterus to contractile agents is dependent upon the sex steroid status of the animal (1–4). Erspamer (5) and Robson *et al.* (6) reported that the contractile response to 5-HT[1] of ovariectomized rat uterus can be greatly increased by administration of estradiol, but not testosterone, progesterone, or other steroid hormones. Therefore, uteri isolated from ovariectomized rats after injection of estradiol have been widely used for bioassay of 5-HT (7). However, little is known about the mechanism(s) by which the contractile response to 5-HT in rat uterus is increased by administration of estradiol.

We recently confirmed that the contractile response of the uterus of ovariectomized rats to 5-HT was increased by administration of estradiol, and found that the effect of estradiol was specific for 5-HT, the contractile responses to ACh and oxytocin not being influenced by administration of estradiol.[2] This specific increase in the contractile response to 5-HT seemed to be due to change in the number of uterine 5-HT receptors, not to change in uptake or metabolic degradation of 5-HT.[2]

It has been reported that in the cerebral cortex and hippocampus of rat brain the radioligand [^{3}H]spiroperidol mainly binds to 5-HT receptors, although in the corpus striatum of rat brain only a small portion of the [^{3}H]spiroperidol binds to 5-HT receptors, most of the radioligand binding to DA receptors (8–12). However, little is known about the characteristics of [^{3}H]spiroperidol binding to uterine membrane.

In this work, we therefore use the radioligand [^{3}H]spiroperidol to label binding sites with the characteristics expected for 5-HT receptors in uterine membranes, and investigate the characteristics of specific binding of [^{3}H]spiroperidol to the uterine membrane from estradiol-treated ovariectomized rat. Our major findings were that (*a*) the radioligand [^{3}H]spiroperidol mainly binds to 5-HT receptors, (*b*) the number of specific binding sites of [^{3}H]spiroperidol is significantly increased by administration of estradiol, without any change in the apparent affinity of specific binding of [^{3}H]spiroperidol, and (*c*) the administration of estradiol does not change the apparent dissociation constant or the number of binding sites for the radioligand [^{3}H]QNB, which binds to muscarinic ACh receptors.

EXPERIMENTAL PROCEDURES

Virgin female Wistar rats weighing about 200 g were used. They were ovariectomized through a dorsal incision under ether anesthesia without regard to the stage of the estrous cycle. On day 15 after ovariectomy, they were given 10 μg of 17β-estradiol-3-benzoate once every 12 h for 48 h by intrasubcutaneous injection and then were killed. Untreated ovariectomized rats were used as controls.

Preparation of Uterine Membranes—Untreated and estradiol-treated ovariectomized rats were stunned by a blow on the head and the uterine horns were removed and placed in 50 mM Tris-HCl buffer (pH 7.4 at 30 °C). The uterine horns in buffer solution were freed

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[1] The abbreviations used are: 5-HT, serotonin; ACh, acetylcholine; GTP, guanosine 5′-triphosphate; QNB, 3-quinuclidinyl benzilate.

[2] S. Ichida, Y. Oda, H. Tokunaga, T. Hayashi, T. Murakami, and T. Kita, unpublished manuscript.

from fat and loosely bound connective tissue and homogenized in 70 volumes of ice-cold 50 mM Tris-HCl buffer (pH 7.4 at 30 °C) using a Physcotron (Niti-on Co.; setting 60–70) for 20 s. The homogenate was filtered through nylon mesh and then centrifuged at 42,000 × *g* for 20 min. The pellet was resuspended in 50 mM Tris-HCl buffer (pH 7.4 at 30 °C) and recentrifuged at 42,000 × *g* for 20 min. The pellet was suspended in 10 mM Tris-HCl buffer (pH 7.4 at 30 °C) at 2–3 mg of protein/ml (uteri from estradiol-treated ovariectomized rats) or 0.5–2 mg of protein/ml (uteri from untreated ovariectomized rats) and placed in an ice bath until used.

Binding Assay of Radiolabeled Ligands—For the binding assay of [^{3}H]spiroperidol, a 100-μl aliquot (50–300 μg of protein) of a suspension of uterine membranes was added to assay medium consisting of 62.5 mM Tris-HCl (pH 7.4 at 30 °C) and 0.25 mg/ml L-ascorbic acid with various concentrations (2.5–17.5 nM) of [^{3}H]spiroperidol. The final volume of the assay system was 500 μl. After incubation for 5 min at 30 °C, unless otherwise indicated, the mixture was rapidly filtered through a Whatman GF/F glass filter under vacuum and the filter was washed four times with 4 ml of ice-cold 50 mM Tris-HCl buffer (pH 7.4 at 30 °C). The filter was transferred to a counting vial, 5 ml of Triton/toluene-based scintillation mixture was added, and the radioactivity was counted in a liquid scintillation counter (Packard Model 3380). All assays were performed in duplicate or triplicate. The specific binding of the ligand was defined as the difference between the radioactivities bound in the presence and absence of 1 μM unlabeled spiroperidol. The specific binding of [^{3}H]spiroperidol was linear between 0.1 and 5 mg of protein/ml of uterine membranes from untreated or estradiol-treated ovariectomized rats.

For assay of binding of [^{3}H]QNB, which binds to muscarinic ACh receptors (13), the procedure was essentially the same as for measuring binding of [^{3}H]spiroperidol, except that L-ascorbic acid was not added to the assay medium. Atropine at 10^{-5} M was used to determine nonspecific binding.

Pretreatments of Uterine Membranes with Various Enzymes and Protein-modifying Reagents—The uterine membranes (0.4–0.6 mg of protein/ml) from estradiol-treated ovariectomized rats were preincubated with the indicated concentrations of enzyme or protein-modifying reagents for 20 min at 37 °C in the following buffers: phospholipase A_1 and trypsin in 50 mM Tris-HCl (pH 7.4 at 30 °C) with 5 mM $CaCl_2$; α-chymotrypsin in 50 mM Tris-HCl (pH 7.4 at 30 °C) with 40 mM $CaCl_2$; phospholipase D and neuraminidase in 100 mM CH_3COOH-CH_3COONa buffer (pH 5.6 at 30 °C) with 40 mM $CaCl_2$; various modifying reagents in 50 mM Tris-HCl (pH 7.4 at 30 °C). As controls, uterine membranes were pretreated under the above conditions, but without enzymes or modifying reagents. The final volume of the reaction systems in pretreatments was 1500 μl. Then, the preincubated mixtures were centrifuged at 42,000 × *g* for 20 min. The pellets were washed twice with ice-cold 50 mM Tris-HCl buffer (pH 7.4 at 30 °C) and resuspended at a final concentration of about 2 mg of protein/ml in ice-cold 10 mM Tris-HCl buffer (pH 7.4 at 30 °C). Then, the specific binding of [^{3}H]spiroperidol (2 nM final concentration) to pretreated and control uterine membranes was determined as described above.

Other Methods—Protein was measured by the method of Lowry *et al.* (14) with bovine serum albumin as a standard.

The equilibrium dissociation constant (K_D value) and maximal number of binding sites (B_{max} value) were obtained from Scatchard plots (15).

Hill coefficients of specific [^{3}H]spiroperidol binding to untreated and estradiol-treated preparations were obtained from Hill plots (16).

K_i values were calculated from IC_{50} values (reagent concentration inhibiting 50% of specific binding) according to the equation of Cheng and Prusoff (17).

Statistical analyses were made by Student's *t* test and a level of $p < 0.05$ was regarded as significant.

Reagents—17β-Estradiol-3-benzoate was purchased from Teikokuzoki Co. Guanosine 5′-triphosphate was from Yamasa Shoyu Co. Acetylcholine, *N*-bromosuccinimide, 5,5′-dithiobis(2-nitrobenzoic acid), dopamine, 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide, *N*-ethylmaleimide, 2-hydroxy-5-nitrobenzyl bromide, and serotonin were from Wako Co. Atropine, α-chymotrypsin, oxytocin, phospholipase A_2, phospholipase D, neuraminidase, and trypsin were from Sigma. [^{3}H]3-quinuclidinyl benzilate (40.2 Ci/mmol) and [^{3}H]spiroperidol (16 Ci/mmol) were from Amersham Co. or New England Nuclear Co. Cinanserin was a gift from Squibb (S. J. Lucania), metergoline was from Dr. K. Saito (Osaka University, School of Dentistry), mianserin was from Organon Co. (Japan; H. Mita), spiroperidol was from Janssen Pharmaceutica (Belgium; Dr. P. Laduron) and Esai Co. (Japan) and sulpiride was from Fujisawa Yakuhin Co. (Japan; Dr. T. Nuki).

All drugs were prepared in distilled water or 0.01 N HCl solution on the day of use and were neutralized when necessary before use.

RESULTS

Properties of [^{3}H]Spiroperidol Binding to Uterine Membranes of Estradiol-treated Ovariectomized Rats—The specific binding of [^{3}H]spiroperidol was saturable and reached a plateau at about 10 nM (Fig. 4*A*). Half-maximal saturation, taken as the apparent dissociation constant (K_D) for the interaction of [^{3}H]spiroperidol with binding sites, occurred with 5.16 ±

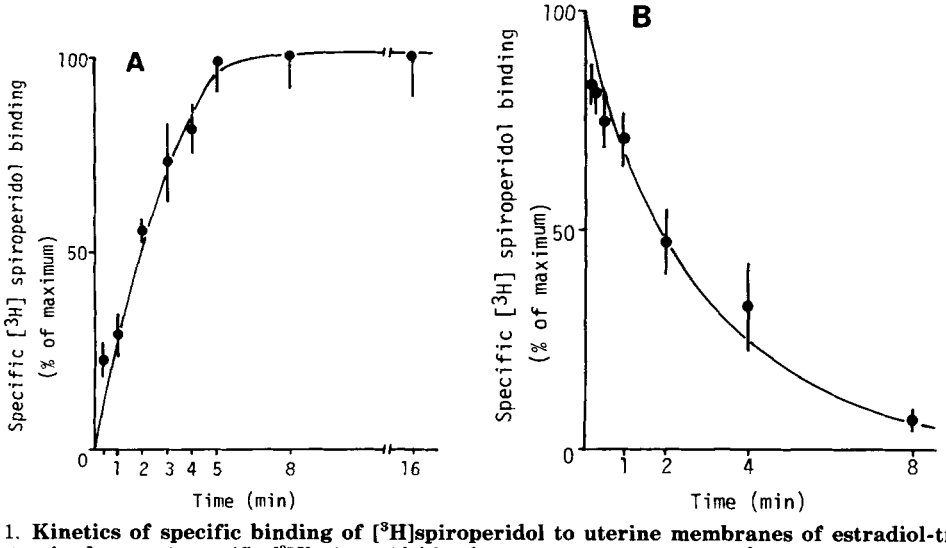

FIG. 1. **Kinetics of specific binding of [^{3}H]spiroperidol to uterine membranes of estradiol-treated ovariectomized rats.** *A*, specific [^{3}H]spiroperidol binding as a function of time. [^{3}H]spiroperidol (2 nM) was incubated with uterine membranes (200–300 μg of protein/tube) for the indicated times at 30 °C and specific binding was determined as described under "Experimental Procedures." *Points* are means for three or four separate animals. *Bars* indicate standard errors. *B*, reversibility of specific [^{3}H]spiroperidol binding. Membranes were incubated with [^{3}H]spiroperidol (2 nM) for 5 min at 30 °C and then a large excess of unlabeled spiroperidol (10 μM) was added. The time of spiroperidol addition is defined here as $t = 0$. At the indicated times subsequently, specific [^{3}H]spiroperidol binding was determined as described under "Experimental Procedures." *Points* are means for five to seven separate animals. *Bars* indicate standard errors.

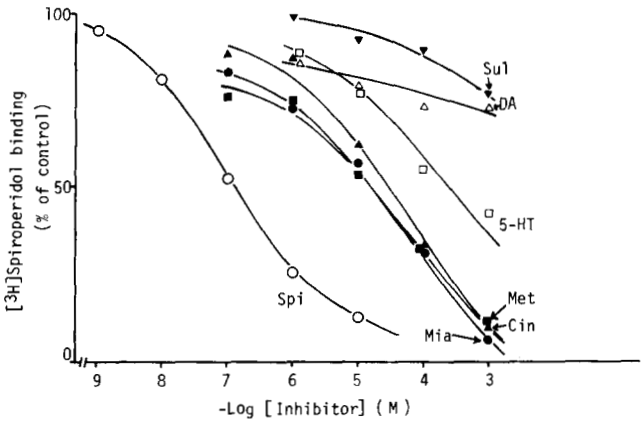

FIG. 2. **Inhibition of [^{3}H]spiroperidol binding by various agents.** Uterine membranes of estradiol-treated ovariectomized rats were incubated with [^{3}H]spiroperidol (2 nM) in the absence and presence of the indicated agents, and specific binding was determined as described under "Experimental Procedures." *Points* are means for four to six separate animals. *Sul*, sulpiride; *DA*, dopamine; *Met*, metergoline; *Cin*, cinanserin; *Spi*, spiroperidol;, *Mia*, mianserin.

0.24 (n = 12) nM [^{3}H]spiroperidol. The amount of [^{3}H]spiroperidol specifically bound at equilibrium was 1.23 ± 0.11 (n = 12) pmol/mg of protein. The specific binding accounted for about 70% of the total binding at a concentration of 2 nM, the amount of [^{3}H]spiroperidol used routinely in various other studies (data not shown). With an increase in the ligand concentration above 6 nM, the percentage of total binding attributable to specific binding decreased and with 14 nM [^{3}H] spiroperidol it was 51% of the total binding (data not shown). Nonspecific binding increased linearly with an increase in [^{3}H]spiroperidol concentration (data not shown).

The specific binding of [^{3}H]spiroperidol was time-dependent (Fig. 1*A*). At 30 °C, the specific binding reached a plateau after 5 to 16 min, and was half-maximal after about 2 min. The specific binding of [^{3}H]spiroperidol was also reversible (Fig. 1*B*).

The competitions of various reagents for [^{3}H]spiroperidol-binding sites were investigated (Fig. 2). The [^{3}H]spiroperidol binding was inhibited by cinanserin, mianserin, metergoline, and 5-HT, which caused half-maximal inhibitions at about 30, 20, 20, and 250 μM, respectively. Therefore, the apparent K_i values for cinanserin, mianserin, metergoline, and 5-HT are about 21.2, 14.1, 14.1, and 176.5 μM, respectively. Cinanserin, mianserin, and metergoline are known to be 5-HT antagonists. On the other hand, at concentrations of 1 mM, dopamine and sulpiride (which affect dopamine receptors) reduced [^{3}H]spiroperidol binding by only 26 and 23%, respectively. These findings suggest that the radioligand [^{3}H]spiroperidol mainly binds to 5-HT receptors in uterine membranes of estradiol-treated ovariectomized rats.

Fig. 3 shows the effect of 1 mM GTP on displacement of bound [^{3}H]spiroperidol by 5-HT in uterine membranes of estradiol-treated ovariectomized rats; 1 mM GTP reduced the potency of 5-HT (10^{-6}–10^{-3} M) to displace bound [^{3}H]spiroperidol. In the absence of 5-HT, GTP at concentrations of up to 5 mM did not affect specific [^{3}H]spiroperidol binding. These results also suggest that [^{3}H]spiroperidol labels sites at which 5-HT acts as an agonist in rat uterine membranes.

To determine which membrane components and functional groups are involved in [^{3}H]spiroperidol-binding sites, we treated uterine membranes with various enzymes and protein-modifying reagents and then measured their binding sites (Table I). [^{3}H]Spiroperidol binding was not reduced by treatment with low concentrations of trypsin (3.8 units/ml) or α-chymotrypsin (0.43 unit/ml), but was significantly reduced by higher concentrations of trypsin (1140 units/ml) and α-chymotrypsin (43 units/ml). These findings suggest that the binding sites of [^{3}H]spiroperidol involve protein structure. Pretreatments with phospholipase A_2, D, and neuraminidase significantly reduced the specific binding of [^{3}H]spiroperidol. The finding that pretreatment with neuraminidase reduced the specific binding of [^{3}H]spiroperidol is consistent with the observation that the contractile response of isolated smooth muscle to 5-HT was inhibited selectively by treatment of neuraminidase plus EDTA (18). These findings suggest that phospholipids and *N*-acetylneuraminic acid in uterine membranes are directly or indirectly important for specific binding sites of [^{3}H]spiroperidol. Specific [^{3}H]spiroperidol binding

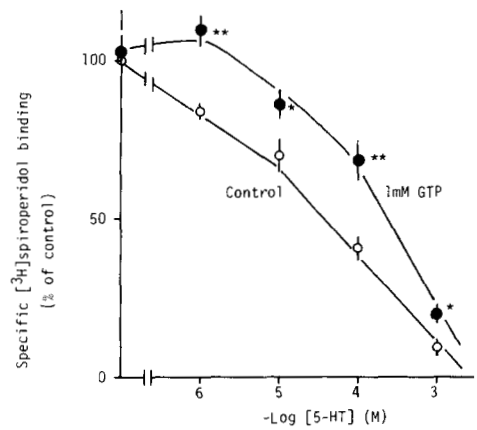

FIG. 3. **Effect of GTP on the displacement of bound [^{3}H] spiroperidol by 5-HT in uterine membranes of estradiol-treated ovariectomized rats.** Membranes were incubated with [^{3}H] spiroperidol (2 nM) in the absence and presence of 5-HT and/or GTP, and specific binding was determined as described under "Experimental Procedures." *Points* are means ± S.E. for three to five separate animals. *, $p < 0.05$; **, $p < 0.02$; significance of difference from percentage inhibition produced with 5-HT alone.

TABLE I

Effects of enzymatic treatments and protein-modifying reagents on specific [^{3}H]spiroperidol binding to uterine membranes of estradiol-treated ovariectomized rats

Uterine membranes were treated for 20 min at 37 °C, isolated by centrifugation, and washed twice with ice-cold 50 mM Tris-HCl buffer. The specific binding of 2 nM [^{3}H]spiroperidol to treated and control membranes was then determined as described under "Experimental Procedures."

Treatment	Specific [^{3}H]spiroperidol binding	p	n
	% control		
Enzymes			
α-Chymotrypsin, 0.43 unit/ml	96.8 ± 35.8	NS[a]	3
43 units/ml	47.7 ± 8.8	<0.02	6
Trypsin, 3.8 units/ml	93.0 ± 27.4	NS	3
1140 units/ml	45.9 ± 5.2	<0.05	3
Neuraminidase, 0.03 unit/ml	45.1 ± 10.3	<0.02	5
Phospholipase A_2, 1.3 units/ml	16.7 ± 10.5	<0.001	4
Phospholipase D, 2.0 units/ml	47.5 ± 7.4	<0.01	5
Protein-modifying reagents			
N-Bromosuccinimide, 10 μM	63.9 ± 7.7	<0.05	4
5,5′-Dithiobis(2-nitrobenzoic acid), 5 mM	75.0 ± 16.7	NS	3
N-Ethylmaleimide, 1 mM	53.2 ± 5.9	<0.05	4
1-Ethyl-3-(3-dimethylaminopropyl)carbodiimide, 1 mM	40.9 ± 5.4	<0.001	6
2-Hydroxy-5-nitrobenzyl bromide, 10 mM	126.5 ± 8.6	NS	3

[a] NS, not significant.

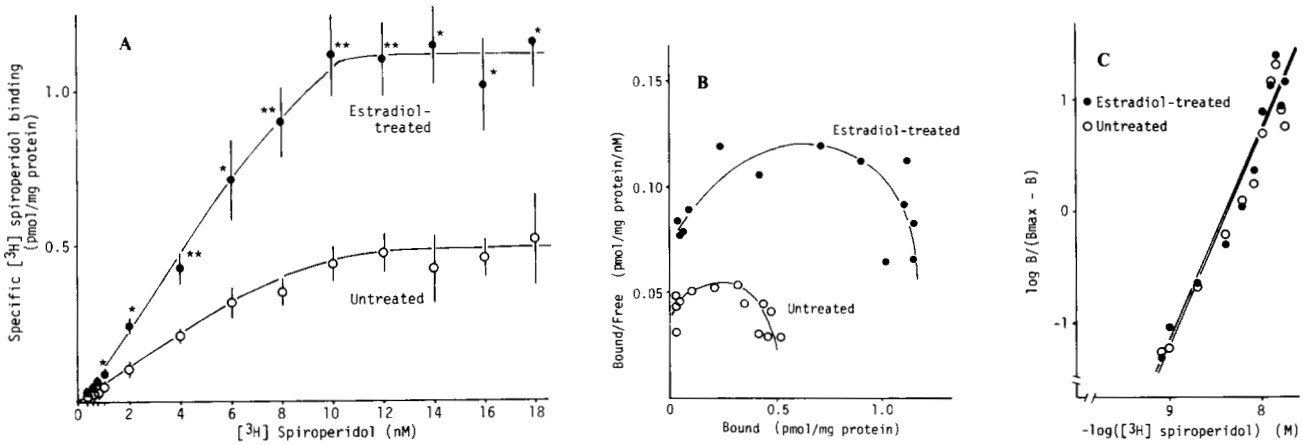

FIG. 4. **Equilibrium binding of [^{3}H]spiroperidol to uterine membranes of untreated and estradiol-treated ovariectomized rats.** *A*, specific [^{3}H]spiroperidol binding as a function of the concentration of [^{3}H] spiroperidol. [^{3}H]spiroperidol at the indicated concentrations was incubated with uterine membranes of untreated and estradiol-treated ovariectomized rats for 5 min at 30 °C and specific binding was determined as described under "Experimental Procedures." *B*, data shown as a Scatchard plot. *C*, data shown as a Hill plot. *Points* are means for 10 to 12 separate animals. *Bars* indicate standard errors. *, $p < 0.05$; **, $p < 0.02$; significance of difference from [^{3}H]spiroperidol bound to uterine membranes of untreated rats.

was sensitive to reagents which modify sulfhydryl groups. It was reduced markedly and similarly by pretreatments with 10 μM *N*-bromosuccinimide and 1 mM *N*-ethylmaleimide, and somewhat less by treatment with 5 mM 5,5′-dithiobis(2-nitrobenzoic acid). 1-Ethyl-3-(3-dimethylaminopropyl)carbodiimide (1 mM), a carboxyl group reagent, reduced specific [^{3}H] spiroperidol binding about 59%. Pretreatment with 10 mM 2-hydroxy-5-nitrobenzyl bromide, which alters tryptophan residues, did not reduce specific [^{3}H]spiroperidol binding. These findings suggest that sulfhydryl and carboxyl groups in uterine membranes are important to the specificity of the binding sites of [^{3}H]spiroperidol.

Effect of Estradiol Administration on Specific [^{3}H]Spiroperidol Binding to Uterine Membranes—Previously, we found that the contractile response of ovariectomized rat uterus to 5-HT was specifically increased by administration of estradiol; the contractile responses to ACh and oxytocin were not influenced by its administration.[2] Therefore, we measured 5-HT receptors quantitatively by determining [^{3}H]spiroperidol binding to the uterine membrane of untreated and estradiol-treated ovariectomized rats. In both preparations, specific binding of [^{3}H]spiroperidol was saturated at a concentration of about 10 nM, and the concentrations for half-maximal saturation were 5.22 ± 0.22 (n = 11) and 5.16 ± 0.24 (n = 12), nM, respectively (Fig. 4*A*). However, the amount of [^{3}H] spiroperidol specifically bound at equilibrium was significantly increased by administration of estradiol; that is, it was 0.56 ± 0.04 (n = 11) pmol/mg of protein in untreated preparations and 1.23 ± 0.11 (n = 12) pmol/mg of protein in estradiol-treated preparations. Scatchard analysis of saturation curves of specific [^{3}H]spiroperidol binding to untreated and estradiol-treated preparations gave convex curves (Fig. 4*B*) and the Hill coefficients were 2.10 ± 0.14 (n = 11) and 2.06 ± 0.11 (n = 12), respectively (Fig. 4*C*), indicating that specific [^{3}H]spiroperidol binding to untreated and estradiol-treated preparations shows positive cooperativity. ACh receptors were also measured quantitatively with [^{3}H]QNB under conditions similar to those for [^{3}H]spiroperidol binding (Fig. 5, *A* and *B*). The K_D value and the number of binding sites for [^{3}H]QNB were not significantly changed by administration of estradiol (K_D for [^{3}H]QNB, 0.43 nM for untreated and 0.33 nM for estradiol-treated preparations; number of binding sites for [^{3}H]QNB, 126 fmol/mg of protein for control and

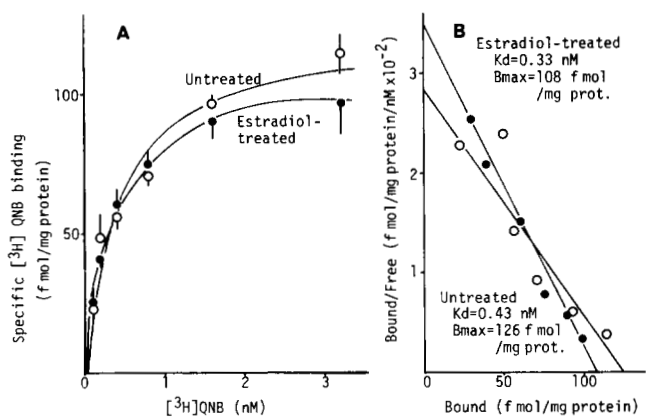

FIG. 5. **Equilibrium binding of [^{3}H]QNB to uterine membranes of untreated and estradiol-treated ovariectomized rats.** *A*, specific [^{3}H]QNB binding as a function of the concentration of [^{3}H]QNB. Experiments were performed as described in Fig. 4*B*; data are shown as a Scatchard plot. *Points* are means for four to six separate animals. *Bars* indicate standard errors.

108 fmol/mg of protein for estradiol-treated preparations). These findings suggest that the amount of 5-HT receptor was significantly increased by administration of estradiol, without any change in the apparent affinity of 5-HT receptor to 5-HT and that the amount and the apparent affinity of muscarinic ACh receptor did not change after administration of estradiol. Therefore, these findings are consistent with the observation that the contractile response to 5-HT, but not to ACh or oxytocin, was increased by administration of estradiol.[2]

DISCUSSION

The contractile response to 5-HT of isolated uterus was specifically increased by administration of estradiol (5, 6).[2] This specific increase in sensitivity to 5-HT could be due to (*a*) change in metabolic degradation of 5-HT, (*b*) change in 5-HT uptake, or (*c*) change in 5-HT receptors. Our results suggested that it was due to change in the 5-HT receptor, not to change in uptake or metabolic degradation of 5-HT.[2] In studies on the role of changes in 5-HT receptors in the increase of serotonergic sensitivity upon administration of

estradiol, we first used the radioligand [^{3}H]5-HT in binding studies. But we found that [^{3}H]5-HT binding to rat uterine membranes did not satisfy the so-called "basic criteria" (19) for a receptor, although it has been reported that [^{3}H]5-HT labels 5-HT receptors in the central nervous system (20, 21); that is, specific binding of [^{3}H]5-HT (at concentrations of 1, 10, and 100 nM) were not reversible and were not inhibited by 5-HT antagonists (metergoline, methysersid, and ergometrine) at high concentrations such as 10^{-4}–10^{-3} M, although the specific binding of [^{3}H]5-HT was saturable.[3] Therefore, we used the radioligand [^{3}H]spiroperidol to label binding sites with the characteristics expected for 5-HT receptors. Specific [^{3}H]spiroperidol binding appears to satisfy the criteria for identification of 5-HT receptors. Indeed, the specific binding of [^{3}H]spiroperidol was rapid, reversible, and saturable (Figs. 1*A*, 1*B*, and 4*A*). Moreover, cinanserin, metergoline, mianserin, and 5-HT inhibited the binding of [^{3}H]spiroperidol (Fig. 2). We also found that cinanserin, metergoline, mianserin, and spiroperidol specifically inhibited the contractile response to 5-HT in isolated uterus from estradiol-treated ovariectomized rats, because at concentrations that completely inhibited the contractile response to 5-HT they did not affect the contractile reponse to ACh or oxytocin.[4] Specific binding of [^{3}H]spiroperidol to uterine membranes could include binding to dopamine receptors (especially D_2 receptors). But, this possibility seems unlikely because the specific binding of [^{3}H]spiroperidol was scarcely affected by a concentration of sulpiride as high as 100 μM (Fig. 2) and Seeman (22) reported that sulpiride selectively inhibits dopamine receptors (especially D_2 receptors), while the binding of [^{3}H]spiroperidol was inhibited appreciably by 5-HT antagonists at 100 μM (Fig. 2). These findings, therefore, suggest that [^{3}H]spiroperidol mainly binds to 5-HT receptors in the uterine membrane of estradiol-treated ovariectomized rats, although there is a difference of about 3 orders of magnitude between the apparent K_i value (177 μM) of 5-HT deduced from the 5-HT displacement of [^{3}H]spiroperidol binding and the ED_{50} value (79.4 nM) of 5-HT deduced from the contractile response of uterus to 5-HT.[4]

Peroutka *et al.* (23) reported that GTP decreases the specific binding of [^{3}H]5-HT in membranes of mammalian brain, as observed previously in binding studies with agonists of other monoamines. If 5-HT acts as an agonist for the specific binding sites of [^{3}H]spiroperidol, GTP should reduce its affinity for this site and decrease its efficiency in displacing bound [^{3}H]spiroperidol. Indeed, we found that 1 mM GTP caused reductions in the potency of 5-HT (10^{-6}–10^{-3} M) to displace bound [^{3}H]spiroperidol, and in the absence of 5-HT GTP did not affect specific [^{3}H]spiroperidol binding at concentrations of up to 5 mM. Therefore, this finding also suggests that [^{3}H] spiroperidol labels sites at which 5-HT acts as an agonist in rat uterine membranes.

In this work, we found that administration of estradiol significantly increased the number of specific binding sites of [^{3}H]spiroperidol, but did not change the apparent affinity of specific binding of [^{3}H]spiroperidol (Fig. 4*A*). Administration of estrogens to ovariectomized rats or rabbit has been shown to increase the weight (24), dry weight (25), water accumulation (26), and quantity of contractile protein (27) of the uterus. Therefore, this change in number of specific binding sites of [^{3}H]spiroperidol by administration of estradiol could be due to change in some of the factors described above. However, this possibility seems unlikely because the number of specific binding sites of [^{3}H]QNB and their affinity in uterine membranes were not significantly changed by administration of estradiol.

L-Ascorbic acid has been shown to decrese [^{3}H]spiroperidol binding in a biphasic manner in the absence of EDTA (22, 28). Therefore, the increase in number of specific binding sites of [^{3}H]spiroperidol after administration of estradiol could be due to a difference between the inhibitory effects of L-ascorbic acid on the specific [^{3}H]spiroperidol binding to untreated and estradiol-treated preparations. However, this possibility seems unlikely because the amounts of specifically bound [^{3}H]spiroperidol (at concentrations of 0.4–18 nM [^{3}H] spiroperidol) in untreated and estradiol-treated preparations did not change irrespective of whether L-ascorbic acid (0.25 mg/ml) was present in the medium (data not shown).

We found that in isolated uterus the maximal contractile response to 5-HT, but not ACh or oxytocin, was significantly greater in estrus than in diestrus.[2] The estradiol level of rats is highest in proestrus and is still elevated during estrus (29, 30). Thus, the contractile response to 5-HT may be sensitive to changes in the level of sex steroid hormone during the estrous cycle, and the specific change in sensitivity to 5-HT may be important physiologically in some uterine function(s). However, the functional role of 5-HT receptors in the uterus and the physiological significance of their increase by estrogen are unknown. There are also reports that estrogen modulates the receptors for hormones and neurotransmitters in several systems of myometrium (31–35).

Although the mechanism(s) of the increase in number of 5-HT receptors on administration of estradiol is unknown, we consider that a change in 5-HT content of the uterus after administration of estradiol may be correlated with an increase in the number of 5-HT receptors upon administration of estradiol, because it has been reported that a decrease of the 5-HT content of the uterus of estradiol-treated ovariectomized rats occurred without apparent degradation of mast cells (36). Further investigations are needed on the mechanisms(s) of increase of 5-HT receptors after administration of estradiol.

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