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Regulation of Myometrial Adrenoreceptors and Adrenergic Response by Sex Steroids

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SUMMARY

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Myometrial adrenergic response and receptors are modulated by the hormonal environment. Uteri from rabbits treated with estrogen demonstrate *alpha*-adrenergic predominance and have an increased number of *alpha*-adrenergic receptors, measured by [^{3}H]dihydroergocryptine binding, relative to animals treated with estrogen followed by progesterone. In rabbits treated with estrogen followed by progesterone, *beta*-adrenergic response predominates; however, the number of *beta*-adrenergic receptors measured by [^{125}I]iodohydroxybenzylpindolol binding is similar in these animals and in those treated with estrogen. Comparing *alpha*-adrenergic receptors and response in uteri from estrogen-treated and -untreated animals reveals that an estrogen-induced increase appears to be responsible for the increased *alpha*-adrenergic sensitivity. The increased sensitivity is manifest by a 4.5-fold decrease in the ED_{50} for norepinephrine in rabbits treated with estrogen. This increased potency of norepinephrine is not explained by treatment-induced change of agonist affinity for *alpha*-adrenergic receptors, catecholamine reuptake, or a nonspecific increase in contractile sensitivity. The enhanced potency of norepinephrine secondary to increased receptor concentration suggests the presence of spare receptors. This is supported by pharmacological experiments in which reducing the number of available *alpha*-adrenergic receptors results in an increase in the ED_{50} for norepinephrine. In contrast to the *alpha*-adrenergic predominant state, the *beta*-adrenergic predominance produced by progesterone treatment cannot be explained solely by changes in adrenergic receptors. *Beta*-adrenergic receptor number, the ratio of *beta*- to *alpha*-adrenergic receptors, and the affinity of agonists for *alpha*- or *beta*-adrenergic receptors seem unable to account for the development of *beta*-adrenergic predominance. Thus, although modification of adrenergic receptor concentration by sex steroids can be explained by the myometrial *alpha*-adrenergic predominance produced by estrogen treatment, alteration in receptors cannot account for the *beta*-adrenergic predominance present when progesterone treatment follows estrogen.

INTRODUCTION

Myometrial contractile response to adrenergic stimulation is altered by gonadal steroids. In rabbits, elevated endogenous estrogen levels or estrogen administration increases the uterine contractile response to sympathetic stimulation in whole organ preparations or to norepinephrine with isolated myometrial strips (1). This contractile response is mediated by *alpha*-adrenergic mechanisms, since it can be blocked by *alpha*-adrenergic antagonists. In contrast, if estrogen treatment is followed by progesterone, sympathetic stimulation or norepinephrine inhibits contractility and this inhibition can be blocked by *beta*-adrenergic antagonists (1). We and others have previously reported that the *alpha*-adrenergic increase in contractility produced by estrogen alone was associated with a greater concentration of *alpha*-adrenergic receptors than was present in the uterus of rabbits treated with estrogen followed by progesterone (2, 3). In the same animals, we found no difference in the concentration of *beta*-adrenergic receptors with either treatment (2). These findings indicated that changes in number of *alpha*-adrenergic, but not *beta*-adrenergic recep-

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tors, accompany and perhaps mediate the change in myometrial responses to catecholamines.

The following studies were done to determine if the changes in adrenergic receptors induced by gonadal steroids might cause the change in adrenergic response and whether estrogen and/or progesterone was responsible for the changes in receptor concentration. We find that estrogen increases myometrial *alpha*-adrenergic receptor concentration and this change appears to mediate an increased *alpha*-adrenergic contractile sensitivity. In contrast, the predominant *beta*-adrenergic response which is present when progesterone treatment follows estrogen cannot be explained by changes in concentration or affinity of adrenergic receptors.

METHODS

Treatment regimens. Immature (2–2.5 kg) New Zealand rabbits were either untreated or treated with 150 μg/day of estradiol benzoate i.m. for 4 days or with the same dose of estradiol benzoate followed by 10 mg/day of progesterone i.m for 4 days. This treatment regimen has been previously demonstrated to result in *alpha*-(estrogen treatment) or *beta*-adrenergic predominance (estrogen followed by progesterone) (1, 2). Some animals were ovariectomized and studied 2 weeks after surgery.

Isometric contraction studies. Rabbits were killed by CO_2 narcosis and uteri were removed. Longitudinal strips of myometrium approximately 0.25 by 2 cm were suspended at 1-g tension in a modified Tris-Ringer buffer, pH 7.5, at 37°. After spontaneous activity stabilized (about 1 hr), tension was readjusted to 0.25-g tension and cumulative dose-response curves to agonists or antagonists were obtained. Strips were washed with buffer two to three times between each drug administration. Relative dose-response curves in individual animals remained constant over 3–5 hr, although spontaneous activity and absolute response in a series of animals were quite variable. Since we were attempting to determine adrenergic predominance, we did not routinely include *alpha*- or *beta*- adrenergic antagonists.

Subcellular particulate preparation. Uteri were removed and scraped free of endometrium. The myometrium was minced in a 250 mM sucrose, 5 mM Tris (pH 7.4) buffer containing 4 mM $MgSO_4$ and 1 mM DTT[3] at 4°. The slurry was kept on ice and further disrupted in a Vertis homogenizer (one 30-sec homogenization at half-speed, followed by four 15-sec homogenizations at full-speed with 30 sec between each homogenization). The crude homogenate was filtered through layered cheesecloth and then centrifuged at 600 and 10,000 × *g* for 15 min each. The pellets were discarded after each centrifugation and the resulting supernatant was centrifuged at 30,000 × *g* for 15 min. The pellets were resuspended in 50 mM Hepes, pH 7.5, 4 mM $MgSO_4$, 1 mM DTT, and were used for radioligand-binding assays immediately or were rapidly frozen and stored at −70° until assayed. Binding of both radioligands was unchanged after storage of particulates for at least 4 weeks under these conditions. The protein concentration was determined as described by Bradford (4) with bovine serum albumin as standard.

Alpha-adrenergic receptor assay. Aliquots (0.1 ml) of particulate suspension containing 0.22–0.44 mg of protein were added to 0.1 ml of a solution containing (final concentrations) 50 mM Hepes, pH 7.5, 4 mM $MgSO_4$, 1 mM DTT, 2% ethanol, DHE, and 0.02 ml of 1 mM HCl or 10 μM phentolamine in 1 mM HCl. Ethanol was included to ensure the solubility of DHE and at final concentration of 2% had no effect on binding. DTT at 1 mM concentration did not effect the binding of DHE or change the affinity of adrenergic agonists. Binding was a linear function of final protein concentration up to 3.5 mg/ml. Samples were incubated at 30° for 15 min. The binding reaction was terminated by adding 5 ml of 5 mM Hepes, pH 7.5, 23°, and immediately filtering samples over Whatman GF/C filters at low vacuum (1 ml/sec). The filters were washed with 15 ml of the same solution at 23°, dried by high vacuum, and counted in a liquid scintillation counter at 50% efficiency. Specific binding was defined as the difference between binding in the absence and presence of 10 μM phentolamine (2, 3).

Beta-adrenergic receptor binding assay. Beta-adrenergic receptor binding was determined by using an assay modified from our previously published method (5). Aliquots (0.1 ml) of particulate suspension containing 0.06–0.1 mg of protein were added to 0.1 ml of a solution containing (final concentration) 50 mM Hepes, pH 7.5, 4 mM $MgSO_4$, 1 mM DTT, 2% ethanol [^{125}I]IHYP, and 0.02 ml of 1 mM HCl or 1 mM HCl with agonist or antagonist. Ethanol was included to maintain conditions similar to the DHE binding assay, and at a final concentration of 2% had no effect on IHYP binding. DTT at 1 mM concentration did not affect the binding of IHYP or change the affinity of adrenergic agonists. Binding was a linear function of final particulate protein concentration to 1 mg/ml. Samples were incubated at 30° for 60 min and binding was terminated by adding 1.5 ml of 0.1 mM (±)-propranolol in 1 mM $MgSO_4$, 20 mm KH_2PO_4, pH 7.5 (37°), and immediately filtering over Gelman A/E glass fiber filters at low vacuum (1 ml/sec). The filters were washed with 30 ml of the same solution at 37°, dried at high vacuum, and counted in a gamma counter at 75% efficiency. Specific binding was defined as the difference between binding in the absence and presence of 10 μM (−)-propranolol (5).

Enzyme assays. Na-F stimulated adenylate cyclase activity in myometrial particulates was determined at a protein concentration of 0.25 to 0.5 mg/ml in a 10-min assay as previously described (6). Cyclic AMP generated was measured by a competitive protein-binding method (7).

5′-Nucleotidase activity was assayed at pH 9.0 (8) at a protein concentration of 15–30 μg/ml for 20 min. Free inorganic phosphorus (P_I) was determined by the method of Chen *et al.* (9).

Production formation (cyclic AMP or P_I) was a linear function of protein concentration and time under the assay conditions described.

Data analysis. The concentration of binding sites was determined by Scatchard analysis of data generated by

[3] The abbreviations used are: DTT, dithiothreitol; Hepes, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid; DHE, [^{3}H]dihydroergocryptine; IHYP, iodohydroxybenzylpindolol.

varying radioligand concentration from 0.1–8 times K_D. Dissociation constants for agents competing for radioligand (K_I) were determined as described by Cheng and Prusoff (10) in the equation

$$K_I = \frac{I_{50}}{1 + L_F/K_D}$$

where I_{50} is the concentration of competitor which reduces radioligand binding by 50%; K_D is the equilibrium dissociation constant of the radioligand, and L_F is the concentration of free radioligand. In all experiments, the concentration of binding sites was less than 0.1 K_D assuring that $L_F \cong L_T$ to satisfy the assumption upon which this equation is based [(free radioligand) is similar in the presence and absence of competitor].

The response to a dose of agonist was determined by measuring the area under the contractile curve for 1 min after the addition of agonist and is expressed as percentage of maximal response. All response determinations were corrected for spontaneous activity and the ED_{50} determined as the dose which resulted in half-maximal response.

Statistical comparison of data was performed by using Student's unpaired *t*-test or multivariant analysis.

RESULTS

Relationship of adrenergic receptor changes to alpha-adrenergic response. To demonstrate myometrial *alpha*-adrenergic response, we compared isometric contractile activity of myometrial strips from immature estrogen-treated, untreated, and ovariectomized rabbits. Norepinephrine increased tonus, frequency, and amplitude of contraction and the response was prevented by 10 μM phentolamine. The dose of norepinephrine which elicited half-maximal response (ED_{50}) was 3.2 ± 0.9 μM in preparations from untreated animals, which was similar to contractile sensitivity in ovariectomized rabbits (Fig. 1). Myometrial strips from immature rabbits treated with estradiol benzoate 150 μg/day i.m. for 4 days were more sensitive to norepinephrine than were strips from untreated animals. The ED_{50} for norepinephrine was 0.69 ± 0.4 μM, a 4.5-fold increase in sensitivity relative to untreated animals ($p < 0.05$). Myometrial strips from estrogen-treated animals were also more sensitive to norepinephrine in contraction studies in which 300 μM cocaine was included as an inhibitor of catecholamine reuptake and 1 μM propranolol was added to block *beta*-adrenergic receptors. (ED_{50} 0.14 ± 0.08 μM versus 0.82 ± 0.23 μM, $\bar{x}$ ± S.E.M. $p < 0.05$). The sensitivity of myometrial strips from treated animals to methoxamine, an *alpha*-adrenergic agonist not subject to reuptake, was also increased severalfold (ED_{50} 23.8 ± 5.5 μM versus 2.6 ± 0.76 μM) ($p < 0.05$). However, acetylcholine sensitivity of myometrial strips was unchanged by estrogen (3.7 ± 2.4 μM or 2.3 ± 0.7 μM).

To determine the role of changes in *alpha*-adrenergic receptors in the increased *alpha*-adrenergic sensitivity, we quantitated *alpha*-adrenergic receptors as measured by DHE binding in myometrial particulates prepared from estrogen-treated, ovariectomized, and untreated animals (Fig. 2). The number of *alpha*-adrenergic receptors was similar in ovariectomized (160, 110 fmoles/mg of protein) and untreated animals (175 ± 20 fmoles/mg of protein). Receptor concentration was increased 250%

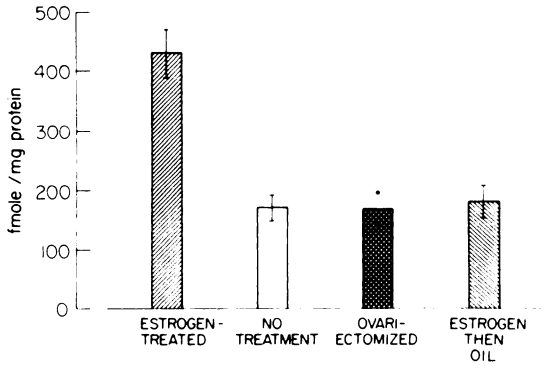

FIG. 2. *DHE binding in myometrial particulates from rabbits receiving different treatments*

Specific binding as defined under Methods was analyzed by Scatchard analysis to determine the concentration of DHE-binding sites. K_D was similar in all treatments. The data shown represent the mean and standard error of the mean of five experiments including two to three uteri each for treated and untreated rabbits, and the mean of two experiments with ovariectomized rabbits. The number of DHE sites in treated and untreated rabbits was statistically significantly different ($p < 0.01$).

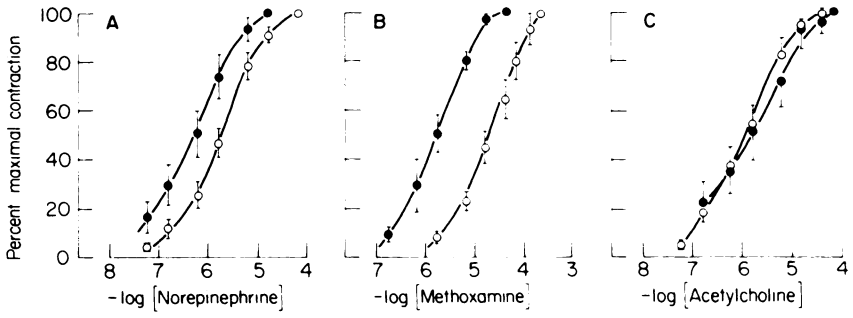

FIG. 1. *Isometric contraction studies of myometrial strips in response to increasing concentrations of norepinephrine, methoxamine, or acetylcholine from untreated* (○) *and estrogen-treated* (●) *rabbits*

Data are expressed as percentage of maximal response and are the mean and standard error of the mean of results from pairs of myometrial strips from five separate experiments. ED_{50} determined individually were: norepinephrine 3.2 ± 0.9 μM untreated, 0.69 ± 0.4 μM estrogen-treated ($p < 0.05$); methoxamine 23.8 ± 0.5 μM untreated, 2.6 ± 0.76 μM estrogen-treated ($p < 0.05$); acetylcholine 2.3 ± 0.7 M untreated, 3.7 ± 2.4 estrogen-treated ($p > 0.4$).

TABLE 1

Membrane marker enzyme recovery with different treatments

Results are the mean and standard error of the mean of duplicate determinations of the number of particulate preparations in parentheses. Multivariant analysis indicated no statistically significant difference of either enzyme determination with any treatment ($p > 0.5$).

Treatment	NaF-stimulated adenylate cyclase	5′ Nucleotidase activity
	(*pmoles cyclic AMP/mg protein^{-1} min^{-1}*)	(*mg P_i/mg protein^{-1} min^{-1}*)
Estrogen	175 ± 35 (5)	4.0 ± 0.7 (4)
Estrogen then progesterone	148 ± 25 (8)	4.2 ± 0.8 (4)
Untreated	153 ± 34 (8)	4.2 ± 0.5 (5)

after estrogen treatment (430 ± 40 fmoles/mg of protein). However, there was no change in the dissociation constant of the binding site for DHE (2, 3). To determine whether receptor concentration increased per unit membrane, we compared the concentration of the enzymatic membrane markers NaF-stimulated adenylate cyclase and 5′ nucleotidase in the particulates from myometrium of the differently treated rabbits. The activity of these enzymes was similar in myometrial particulates of estrogen-treated and untreated animals, and also particulates from rabbits in which estrogen treatment was followed by progesterone (Table 1).

Two-day treatment with doses of estradiol benzoate (10 μg/kg/day) did not change receptor number, but with 20 μg/kg/day the increase was nearly maximal (Fig. 3). The time course for the increase in receptor concentration indicated that 6 hr after the injection of 150 μg (70 μg/kg, a maximally effective dose) of estradiol benzoate, there was no increase in the number of *alpha*-adrenergic receptors, but by 12 hr, the response was maximal (Fig. 4). The increase in receptors was maintained for at least 14 days if estrogen therapy was continued. However, if

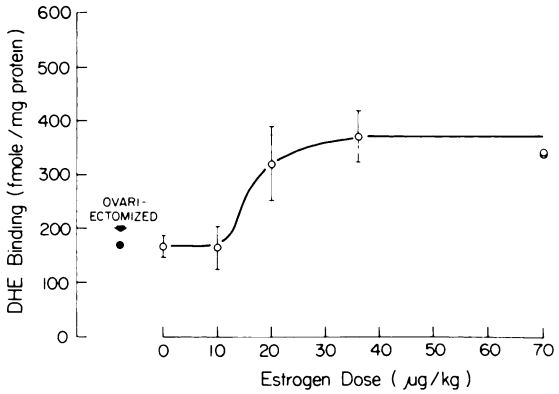

FIG. 3. *Dose dependency of estrogen-stimulated increase in alpha-adrenergic receptors*

Rabbits were treated with estradiol benzoate at different doses every 24 hr for 48 hr. At 48 hr uteri were removed and particulates were prepared. Binding data represent maximal number of sites determined by Scatchard analysis of several DHE concentrations or by incubating particulates at a DHE concentration that was 8–10 times K_D (70 nM). Data are mean and standard error of the mean for three to five experiments with doses to 45 μg/kg and the mean of two experiments in overiectomized animals and rabbits receiving 70 μg/kg of estradiol benzoate.

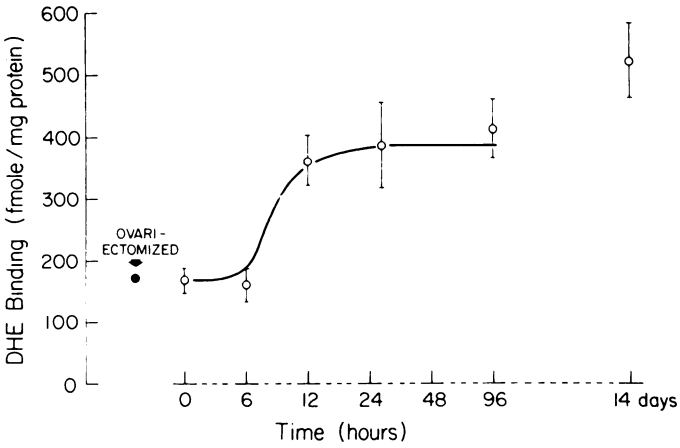

FIG. 4. *Time course of increase of estrogen-stimulated increase in alpha-adrenergic receptors*

Rabbits were treated with 150 μg of estradiol benzoate every 24 hr. At the times indicated, uteri were removed and particulates prepared. DHE binding was determined at a concentration of DHE that was 10 times K_D (70 nM). Data points are the mean and standard error of the mean of specific binding determined in four experiments at each time and the mean of two experiments with ovariectomized rabbits.

estrogen treatment was stopped, the number of DHE-binding sites returned to levels found prior to treatment by the 4th day after cessation of therapy (Fig. 2).

To determine whether the increased sensitivity of myometrial strips to norepinephrine might be due to changes in affinity of agonists for the *alpha*-adrenergic receptor, we determined the dissociation constant of adrenergic agonists for DHE-binding sites. The ability of

TABLE 2

Agonist dissociation constant for alpha- and beta-adrenergic receptors

	Alpha-adrenergic receptors		
	Epinephrine	Norepinephrine	Isoproterenol
	μM	μM	μM
Untreated	0.66 ± 0.3	1.1 ± 0.5	>50
Estrogen	0.80 ± 0.04	0.7 ± 0.15	>50
Estrogen, then progesterone	0.57 ± 0.15	0.69 ± 0.2	>50

	Beta-adrenergic receptors			
	Ritodrine	Isoproterenol	Epinephrine	Norepinephrine
	μM	μM	μM	μM
Estrogen	6.6 ± 0.4	54 ± 12	>100	
Estrogen, then progesterone	3 ± 1 32 ± 14 26 ± 10	48 ± 18	>100	

[1] The affinities of these agonists are considerably less than *beta*-adrenergic agonist affinities in several other systems and our previously reported values for rabbit myometrium. We could not explain these differences initially, but to ensure that results were comparable with different treatment, we performed all assays under identical conditions, including protein concentration. Burgisster *et al.* (11) have pointed out that with racemic radioligands such as IHYP, K_I values, and K_D values will vary with protein concentration. Since the modification of our original assay included reducing assay volume to increase protein concentration, we feel this may explain the decreased *beta*-adrenergic agonist affinities in our recent studies.

epinephrine, norepinephrine, and isoproterenol to compete for DHE binding was similar in estrogen-treated and untreated rabbits (Table 2).

We studied the relationship between the number of *alpha*-adrenergic receptors and *alpha*-adrenergic response by pharmacologically reducing the increased number of receptors in estrogen-treated animals toward the number observed in untreated animals. In order to do this, we initially tried to block the receptors by using the irreversible *alpha*-adrenergic blocker phenoxybenzamine, but we found that concentrations of phenoxybenzamine which shifted the dose-response curve for norepinephrine also affected response to acetycholine (data not shown). Thus, we used the competitive *alpha*-adrenergic antagonist phentolamine which did not alter myometrial response to acetylcholine (Fig. 5). By using the relative affinities of norepinephrine and phentolamine determined from DHE-binding experiments, we calculated the concentrations of phentolamine that would occupy fixed percentages of total receptor from the relationship

$$\frac{B}{R_T} = \frac{P}{P + K_P(1 + N/K_N)}$$

where B is receptor occupied by phentolamine, R_T is total receptor concentration, P is the concentration of phentolamine, K_P is the K_D of phentolamine (0.015 μM), N is the concentration of norepinephrine, and K_N is the K_D of norepinephrine (0.7 μM) determined in DHE-binding experiments.

We found that phentolamine concentrations calculated to occupy 80% of receptors under these conditions (that is, reducing receptor number toward a level observed under basal conditions) increased the dose of norepinephrine necessary to elicit half-maximal response at least 10-fold (Fig. 5).

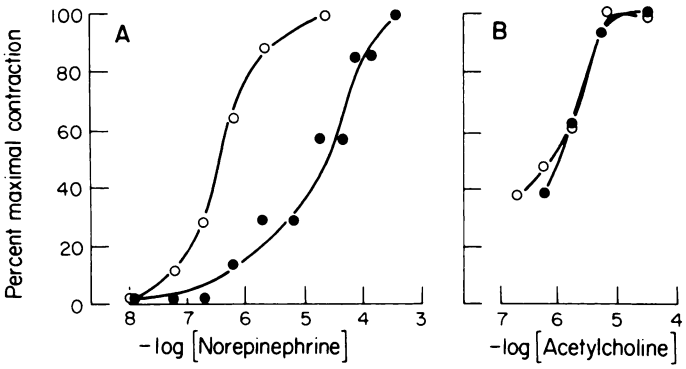

FIG. 5. *Myometrial contractile response in the absence and presence of phentolamine*

Isometric contraction of myometrial strips with increasing concentrations of norepinephrine (A) and acetylcholine (B) were examined before (○) and after (●) the addition of phentolamine. In the experiment with norepinephrine, phentolamine was included at different concentrations that were calculated to occupy 80% of the *alpha*-adrenergic receptors as discussed in the text. The concentrations (micromolar) of phentolamine versus norepinephrine in the muscle bath were: 0.06 versus 0.01; 0.065 versus 0.06; 0.074 versus 0.16; 0.12 versus 0.66; 0.21 versus 1.7; 0.63 versus 6.7; 1.5 versus 17; 5.2 versus 37; 5.8 versus 67; 9.5 versus 110; 14 versus 160. Phentolamine concentration was 10 μM in the experiment with acetylcholine. In three experiments, the ED_{50} of acetylcholaine was 5.5 ± 3.4 μM without phentolamine and 5.6 ± 2.3 μM with phentolamine.

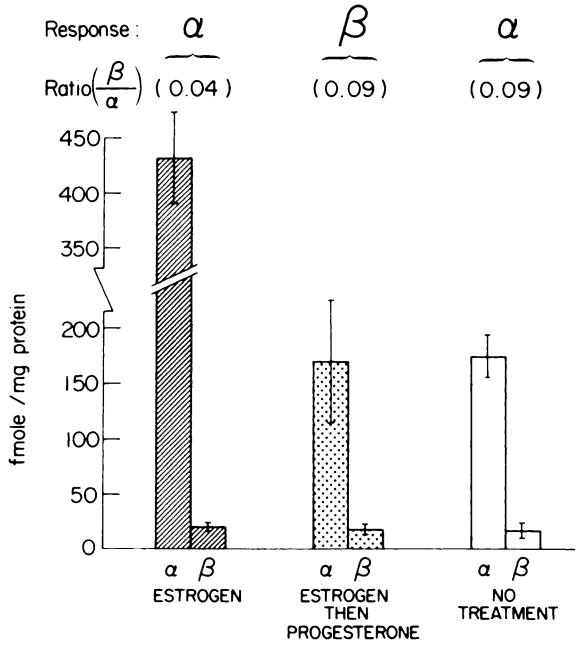

FIG. 6. *Relationship of the number of alpha- and beta-adrenergic receptors in estrogen, estrogen followed by progesterone, and untreated rabbits*

Particulates (1 mg/ml of protein) prepared from uteri of the treated or untreated animals were incubated with increasing concentrations of DHE or IHYP and specific binding was determined by Scatchard analysis. Data are the mean and standard error of the mean of five experiments with each treatment. There was no difference in the K_D of DHE or IHYP with the different treatments.

Relationship of adrenergic receptor changes to beta-adrenergic response. In order to examine *beta*-adrenergic receptors and response, myometrial strips from rabbits in which estrogen treatment was followed by 4 days of progesterone were studied. Such strips responded to norepinephrine with decreased spontaneous activity and this effect could be blocked by the *beta*-adrenergic antagonist propranolol (5). The binding of the radioligands DHE and IHYP to particulates from these myometrial preparations were examined to assess *alpha*- and *beta*-adrenergic receptors, respectively. We reported previously that *beta*-adrenergic predominance cannot simply be due to an increase in *beta*-adrenergic receptor concentration, because IHYP binding-site concentration is similar in uteri from estrogen-treated rabbits or animals in which estrogen treatment was followed by progesterone (2). In order to determine if the *beta*-predominant response results from altered affinity of the receptors for agonists, we examined the dissociation constants of adrenergic agonists for the DHE- or IHYP-binding sites after the different treatments. Treatment with either estrogen alone or estrogen followed by pregesterone yielded little difference in apparent affinities of several adrenergic agonists (Table 2).

As would be predicted from the selective decrease in *alpha*-adrenergic receptors observed when estrogen was followed by progesterone, the ratio of the number of

beta- to *alpha*-adrenergic receptors was greater in rabbits treated with estrogen followed by progesterone (0.096) than in animals treated with estrogen alone (0.045). That this increased *beta*/*alpha*-adrenergic receptor ratio alone cannot account for the *beta*-predominant state observed when estrogen treatment is followed by progesterone is indicated by the similarity of this ratio in untreated animals in whom *alpha*-adrenergic response predominates (Fig. 6).

DISCUSSION

The data presented here indicate that estrogen increases the number, but not the affinity, of myometrial *alpha*-adrenergic receptors, and that this increase in receptor number may account for the enhanced *alpha*-adrenergic sensitivity in myometrial strips prepared from estrogen-treated animals. The estrogen-stimulated increase in myometrial *alpha*-adrenergic-binding sites occurs in a time-dependent and dose-dependent manner. In our preparations, *alpha*-adrenergic receptor concentration did not increase until more than 6 hr after the injection of estrogen. This time course suggests that macromolecular synthesis may be required for increase in *alpha*-adrenergic receptor concentration in myometrium. This suggestion is consistent with other known effects of estrogen on induction of proteins by enhanced gene transcription (12) and the delayed response of estrogen on inhibiting the number of *alpha*-adrenergic receptors on rabbit platelets (13).

In contrast to the increase in *alpha*-adrenergic receptors produced by estrogen treatment, withdrawal of estrogen decreases *alpha*-adrenergic receptor number. Because the concentration of receptors in rabbits treated with estrogen followed by progesterone for 4 days is similar to concentrations of receptors in untreated rabbits or animals treated with vehicle rather than progesterone, cessation of estrogen treatment is sufficient to explain the decrease in receptor concentration after estrogen-primed animals are given progesterone.

The increase in *alpha*-adrenergic receptors produced by estrogen appears to be causally related to the increased *alpha*-adrenergic sensitivity observed in myometrial strips from estrogen-treated animals. In the absence of spare receptors, an increase in receptor concentration without a change in agonist affinity (as we find for *alpha*-adrenergic receptors in myometrium) would increase maximal contractile response without shifting the relative dose-response curves for agonists. If spare receptors exist, increases in receptor number would tend to shift relative dose-response curves to the left [i.e., decrease the ED_{50} of agonists stimulating contraction (14)]. We are unable to make firm quantitative conclusions regarding changes in maximal response of myometrial strips, because of the decrease in maximal force and the variability of spontaneous contractions which occur with time in individual strips. However, our finding that relative dose-response curves for *alpha*-adrenergic agonist-stimulated contraction are shifted to the left by estrogen treatment suggests the existence of spare receptors for *alpha*-adrenergic agonists in rabbit myometrium. The fact that reducing receptor concentration by a fixed amount with phentolamine reduces norepinephrine sensitivity supports this concept.

Our studies have emphasized the role of *alpha*-adrenergic receptors in mediating altered adrenergic response in myometrium. Although estrogen might modify adrenergic responses in myometrium by mechanisms independent of changes in receptors, these alternative mechanisms do not appear likely in our preparations. Such effects include the ability of estrogen to block effector cell uptake of catecholamine (Uptake 2) (15), to compete for catechol-*O*-methyl transferase as catechol estrogen (2 OH-estradiol) (16), and to increase the quantity of contractile proteins in the uterus (17). Inhibition of effector cell reuptake seems unlikely because our treatment regimen increased serum concentrations of estradiol (determined by radioimmunoassay) to a concentration of 0.7–1 nM, whereas 2 μM is the concentration necessary to inhibit Uptake 2 by 50% (15). It is also unlikely that concentrations of catechol estrogens produced by administered estradiol would be sufficient to compete substantially for catechol-*O*-methyltransferase, because the K_I of such inhibition by catechol estrogens is 19 μM (16). Moreover, the myometrial sensitivity to methoxamine, an adrenergic agonist neither subject to reuptake nor a substrate for catechol-*O*-methyltransferase, was increased with estrogen treatment and myometrial strips from estrogen-treated rabbits were more sensitive to norepinephrine than strips from untreated animals even in the presence of cocaine as an inhibitor of catecholamine reuptake. These results offer additional evidence that inhibition of uptake by estrogen and competitive inhibition of catechol-*O*-methyltransferase are of minor importance in producing enhanced adrenergic response in these animals. Because we were unable to demonstrate increased myometrial sensitivity to acetylcholine despite a 4- to 10-fold increase in *alpha*-adrenergic sensitivity, it appears that a nonspecific increase in contractile sensitivity, as might be produced by increased contractile proteins (17), cannot account for the enhanced adrenergic sensitivity produced by estrogen treatment.

Two additional aspects of our findings for *alpha*-adrenergic receptors in myometrium deserve mention. The assessment of changes in receptor concentration requires a point of reference which relates to receptor-bearing cell surface. We find an increased concentration of receptors after estrogen treatment, whether we express the results as receptors per microgram of DNA, or per wet weight as well as per milligram of membrane protein. In addition, we find that estrogen does not change the yield of membrane protein in our preparation (2). Since the activity of enzymatic marker enzymes is similar to both preparations, the increase in receptor concentration would also be evident if data were expressed per 5′ nucleotidase or NaF-stimulated adenylate cyclase activity.

A second point relates to our finding that estrogen increases *alpha*-adrenergic receptors approximately 250% in our myometrial particulate preparations. Hoffman *et al.* (18) have recently shown that *alpha*-adrenergic receptors defined by DHE binding in myometrial membranes are comprised of at least two subtypes of roughly equal concentration, $alpha_1$ and $alpha_2$. It is conceivable that these subtypes of receptors exist on

different cell types (for example, myometrial cells and vascular smooth muscle), and that the extent of increase in *alpha*-adrenergic receptors may be selectively occurring on one of those cell types. Preliminary studies in our laboratory indicate that estrogen increases primarily $alpha_2$ receptors. Further studies using $alpha_1$ and $alpha_2$ specific agents to compete for DHE-binding sites in control and estrogen-treated animals should be useful in testing this possibility.

Whereas increases in number of *alpha*-adrenergic receptors appears to explain the increased myometrial *alpha*-adrenergic sensitivity produced by estrogen treatment, changes in adrenergic receptors effecting occupancy cannot explain the *beta*-adrenergic predominance present when estrogen treatment is followed by progesterone. Neither absolute changes in *beta*-adrenergic receptor number, ratio of *alpha*- to *beta*-adrenergic receptor number, nor receptor affinities appear to account for the progesterone-stimulated *beta*-predominate state. Although it is possible that more detailed comparisons of changes of subtypes of *alpha*- and *beta*-adrenergic receptors (18) might indicate a selective defect in myometrial adrenergic receptors following treatment with progesterone, perhaps more likely is the possibility that progesterone alters adrenergic responsiveness through actions beyond or unrelated to the adrenergic receptors. For example, progesterone depresses myometrial contractility to several agonists, perhaps by hyperpolarizing the plasma membrane and preventing the initiaton of contraction or by decreasing the availability of calcium for the contractile machinery (19, 20). Such alternative mechanisms might account for the *beta*-adrenergic predominance observed in rabbits treated with estrogen followed by progesterone.

Heterologous regulation of hormone and neurotransmitter receptors is potentially an important mechanism of sex steroids for regulating physiological response. In addition to our finding in myometrium, estrogen modulates receptors for hormones and neurotransmitters in several systems. We previously reported that estrogen treatment, which decreases ADP-induced aggregation of rabbit platelets, decreases platelet *alpha*-adrenergic receptors (13). *Beta*-adrenergic receptor concentration is increased in homogenates of hypothalamus from ovariectomized rats treated with estrogen for 48 hr (21). In contrast, homogenates of cerebral hemispheres have decreased *beta*-adrenergic binding after 2 weeks of therapy (22). In rabbits, myometrial oxytocin receptors (23) and angiotensin II receptors (24) are also increased by estrogen, and in rats, myometrial adrenergic receptors can be modulated by estrogen (25). Studies of other estrogen responsive tissues and other receptors should prove useful in defining both the detailed molecular mechanism and overall importance of heterologous regulation of membrane receptors by estrogen and other steroids.

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