Regulation of Rabbit Myometrial Alpha Adrenergic Receptors by Estrogen and Progesterone

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ABSTRACT The effects of estrogen and progesterone on uterine alpha-adrenergic receptors were investigated by direct receptor-binding studies. Immature female rabbits were primed with estrogen by intramuscular injections for 4 days. Other rabbits were primed with progesterone by injections of estrogen for 4 days followed by injections of progesterone for 4 days. The alpha adrenergic antagonist, [^{3}H]dihydroergocryptine, was used to directly assess the number and affinity of alpha adrenergic receptors in membranes derived from estrogen- and progesterone-primed uteri. Membranes from estrogen-primed uteri contained 257±52 fmol of [^{3}H]dihydroergocryptine-binding sites per mg protein whereas membranes from progesterone-primed uteri contained 83±11 fmol of binding sites per mg protein. This reduction of alpha adrenergic receptor-binding sites by progesterone was statistically significant ($P < 0.02$). In contrast, no significant difference in the binding site affinity was observed between the estrogen- and progesterone-primed groups. The progesterone-induced decrease in the number of uterine alpha adrenergic receptors provides a potential explanation for the reduced alpha adrenergic contractile response to epinephrine in the progesterone-primed myometrium.

INTRODUCTION

Regulation of the contractile state of the uterus is achieved by modification of endocrine, autonomic, and mechanical factors during adolescence, the menstrual cycle, normal pregnancy, premature labor, and parturition. One well-documented mechanism by which uterine contraction can be initiated is by catecholamine-induced stimulation of myometrial alpha adrenergic receptors (1–4). In contrast, inhibition of uterine activity can be induced by catecholamine stimulation of beta adrenergic receptors. Thus endogenous catecholamines can theoretically cause either excitation or inhibition of the uterus depending on whether alpha or beta adrenergic receptors predominate. The adrenergic responsiveness of the myometrium appears to be regulated by steroid hormones. Thus, when the uterus is predominantly under the influence of estrogen, an alpha adrenergic contractile response is elicitied by administered catecholamines or by sympathetic nerve stimulation. By contrast when estrogen-primed uteri are subsequently placed under the influence of progesterone, the alpha adrenergic contractile response is diminished significantly (5, 6). One possible mechanism by which progesterone might cause a decrease in alpha adrenergic responsiveness of the myometrium would be by a decrease in the number of functional alpha adrenergic receptors caused by a direct effect of progesterone on the myometrium. Thus, in the estrogen-primed uterus (in the absence of progesterone), alpha adrenergic receptors would be high in number and the myometrial response to catecholamines would be contraction, whereas under the influence of progesterone, the number of alpha adrenergic receptors would be reduced allowing the unmasking of the beta adrenergic inhibitor effect of the catecholamines on the myometrium.

Recently, techniques have been developed (7) for directly assessing the number and affinity of uterine alpha adrenergic receptors by radioligand-binding studies using the radioactively labeled alpha adrenergic antagonist [^{3}H]dihydroergocryptine. In the present investigation we utilized these binding technqiues to study the effects of estrogen and progesterone treatment on the number and affinity of uterine alpha adrenergic receptors. The results show that estrogen-primed uteri have a significantly higher number of alpha adrenergic receptors than progesterone-primed uteri.

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METHODS

Immature female New Zealand white rabbits (1–1.7 kg) were treated by one of two 8-day protocols.

Protocol I

Estrogen-primed rabbits. Rabbits received no injection for the first 4 days and received intramuscular estradiol valerate in peanut oil (0.25 mg/kg) every other day for the last 4 days.

Progesterone-primed rabbits. Rabbits received intramuscular estrogen (0.25 mg/kg) every other day for the first 4 days and then received intramuscular progesterone (10 mg/kg) daily thereafter for the last 4 days. This protocol was essentially identical to that previously reported by Miller and Marshall (5) to induce physiological estrogen or progesterone effects.

Protocol II

Estrogen-primed rabbits. Rabbits received intramuscular estradiol valerate in peanut oil (0.25 mg/kg) every other day for the first 4 days and no injection for the last 4 days.

Progesterone-primed rabbits. Rabbits were injected by a schedule indentical to progesterone-primed rabbits of protocol I.

On the 9th day all rabbits were sacrificed by air embolization and the uteri were immediately removed and used. Uteri were cleaned of fat, scraped of endometrium and homogenized in ice-cold buffer (0.25 M sucrose, 1 mM $MgCl_2$, 5 mM Tris-HCl, pH 7.5) as previously described (8). The homogenate was centrifuged at 300 *g* for 10 min to remove cellular debris. The supernate was then centrifuged at 28,000 *g* for 10 min. The final pellet was resuspended in buffer and used in the binding assay.

[^{3}H]Dihydroergocryptine ([^{3}H]DHE)[1] binding was assayed as previously described (8, 9) by incubating uterine membranes (4–9 mg/ml) with [^{3}H]DHE present at concentrations between 5 and 60 nM in 150 μl of buffer containing 15 mM $MgCl_2$ and 50 mM Tris-HCl (pH 7.5) at 25°C for 17 min. The incubation was terminated as previously described (8) by diluting a 125-μl incubation aliquot into 2 ml of incubation buffer (25°C) followed by rapid filtration through Whatman GFC glass fiber filters. The filters were immediately washed with 20 ml of incubation buffer (25°C) and then dried. The radioactivity on each filter was measured by liquid scintillation spectrophotometry at an efficiency of 40%. "Nonspecific" binding is defined as binding which is not displaced by a high concentration (10 μM) of phentolamine, a potent alpha-adrenergic antagonist which should occupy essentially all of the alpha adrenergic-binding sites. "Specific" or receptor binding is defined as total radioactivity bound minus nonspecific binding, and was generally 50–90% of the total counts bound to membrane protein, the percentage varying inversely with the concentration of [^{3}H]DHE utilized. "[^{3}H]DHE binding" in the figure refers to specific binding as defined above. Protein was determined by the method of Lowry et al. (10). Adenylate cyclase activity was measured as previously described (11).

[^{3}H]DHE was prepared by New England Nuclear (Boston, Mass.) as previously described (8). The purity and biological activity of this compound have been previously documented (8). Other compounds used in this study were (−) epinephrine bitartrate (Sigma Chemical Co., St. Louis, Mo.), phentolamine hydrochloride (Ciba-Geigy Corp., Ardsley, N. Y.), estradiol valerate (Delestrogen, Squibb Corp., New York) and progesterone (Prolutin, Schering-Plough Corp., Kenilworth, N. J.).

RESULTS

Previous publications from this laboratory have demonstrated that [^{3}H]DHE binds to uterine membranes rapidly and reversibly (8). The binding sites labeled by [^{3}H]DHE have the specificity and stereospecificity characteristics expected of alpha adrenergic receptors (8, 9). In the present study [^{3}H]DHE binding was used to measure the number of alpha adrenergic receptors in membranes prepared from estrogen- and progesterone-primed uteri. The data from each experiment were analyzed by a Scatchard plot (12) (Fig. 1) thus allowing the determination of receptor number (x-intercept) and the equilibrium dissociation constant (K_D) of [^{3}H]DHE for the binding sites (negative reciprocal of the slope of the line). The results (Table I) indicate that in the progesterone-primed uteri, the mean number of [^{3}H]DHE-binding sites (83±11 fmol/mg protein) is significantly ($P < 0.02$) lower than the mean number of [^{3}H]DHE-

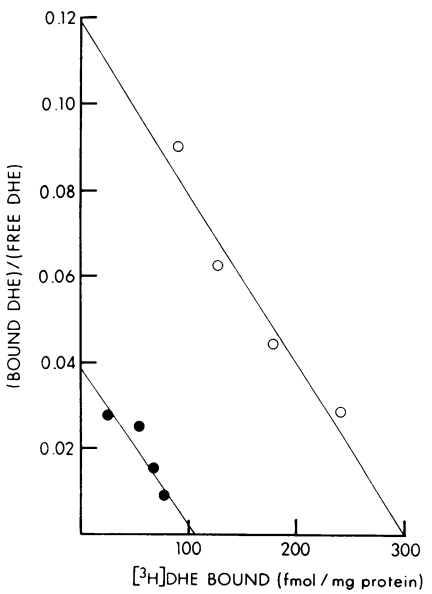

FIGURE 1 Scatchard analysis of [^{3}H]DHE binding in estrogen- and progesterone-primed uteri. Six rabbits were injected with estrogen alone (○) (estrogen-primed) by protocol II and six weight-matched rabbits were injected with estrogen followed by progesterone (●) (progesterone-primed). [^{3}H]DHE binding was determined as the mean of duplicate determinations over a range of [^{3}H]DHE concentrations from 5 to 60 nM and the data were analyzed by the method of Scatchard (12). Lines were determined by linear regression analysis ($r = 0.97$ for progesterone-primed and 0.92 for estrogen-primed).

[1] *Abbreviation used in this paper*: [^{3}H]DHE, [^{3}H]dihydroergocryptine.

TABLE I

Effects of Estrogen and Progesterone on the Number of Affinity of [^{3}H]DHE-Binding Sites in the Rabbit Uterus

	Experiment no.	[^{3}H]DHE-binding sites		K_D of [^{3}H]DHE	
		Progesterone-primed uteri	Estrogen-primed uteri	Progesterone-primed uteri	Estrogen-primed uteri
		fmol/mg Protein		*nM*	
A Protocol I	1	41	224	2	14
	2	93	437	6	11
	3	96	161	12	8
B Protocol II	4	80	161	17	14
	5	104	301	16	15
Mean±SEM		83±11	257±52 $P < 0.02$	11±3	12±2

Six rabbits were injected with estrogen alone (estrogen-primed) by either protocol I or II (Methods). Six weight-matched rabbits were injected with estrogen followed by progesterone (progesterone-primed). [^{3}H]DHE binding was determined as the mean of duplicate determinations over a range of [^{3}H]DHE concentrations from 5 to 60 nM and the data were plotted by the method of Scatchard (12) as in Fig. 1. Linear regression analysis was used to determine the x-intercept and slope from which the number of binding sites and the equilibrium dissociation constant (K_D) were determined. The SEM for the number of sites and K_D for all experiments were computed. Statistical significance was assessed by Student's t test.

binding sites (257±52 fmol/mg protein) in the estrogen-primed uteri.

The difference in number of [^{3}H]DHE-binding sites in the two groups of animals could not be attributed to a difference in membrane yield in the fractionation procedure for several reasons. First, the yields of final membrane protein were 7.9 and 7.8% of the initial homogenate protein from the estrogen- and progesterone-primed uteri, respectively. Second, the flouride-stimulated adenylate cyclase activity in the membranes from estrogen-primed uteri was 167 pmol cAMP/min per mg protein which was close to the value from progesterone-primed uteri (146 pmol cAMP/min per mg protein) thus demonstrating that this membrane marker was identical for the two groups of animals.

The mean K_D of [^{3}H]DHE for the binding sites in the estrogen-primed uterine membranes was not statistically different from that in the progesterone-primed membranes. Hence the affinity of the binding sites for the alpha adrenergic antagonist, [^{3}H]DHE, is apparently not altered when the number of binding sites is altered by these hormonal manipulations. The affinity of the binding sites for an adrenergic agonist was assessed by testing the ability of epinephrine to inhibit [^{3}H]DHE binding to uterine membranes from estrogen- and progesterone-primed uteri. The concentration of epinephrine required to half-maximally inhibit [^{3}H]DHE-binding in estrogen-primed uterine membranes (0.3 μM) was similar to that required to half-maximally inhibit binding in progesterone-primed membranes (0.2 μM). Thus, the reported decreased alpha adrenergic responsiveness to epinephrine in progesterone-primed uteri is not due to an alteration in the affinity of the sites for epinephrine.

In a single experiment, rabbits were injected by a modification of protocol II in which the dose of estrogen was much lower (0.01 mg/kg) than the dose used in other experiments. At this low dose of estrogen, the estrogen-primed uterine membranes had a threefold higher number of alpha adrenergic receptors than the progesterone-primed uterine membranes. Thus, even at a low dose of estrogen, the alteration in receptor number caused by these steroid hormones is observed. In a separate experiment, the binding of 8 nM [^{3}H]DHE was measured in uterine membranes from immature untreated control rabbits. The level of binding in these membranes was intermediate between the levels of binding in the estrogen- and progesterone-primed uteri, being 1.5-fold the value for the binding observed in progesterone-primed uterine membranes and 46% of the value observed in estrogen-primed uterine membranes.

DISCUSSION

The results of this investigation demonstrate that, as assessed by [^{3}H]DHE binding, the density of alpha adrenergic receptors in this particulate fraction from estrogen-primed rabbit uteri is threefold higher than the density of alpha adrenergic receptors in a similar fraction from progesterone-primed uteri. This result was statistically significant ($P < 0.02$) in five experiments involving over 60 animals. This alteration in number of alpha adrenergic receptors may represent a

mechanism by which estrogen and progesterone regulate the contractile sensitivity of the myometrium to alpha adrenergic stimulation. Thus, under the influence of progesterone, the number of functional alpha adrenergic receptors would be reduced and the ability of alpha adrenergic stimulation to elicit a contraction would be concomitantly reduced.

The results of this study are consistent with previous investigations of the effects of steroid hormones on adrenergic responsiveness of the rabbit uterus. Thus, Miller and Marshall (5) have demonstrated that if rabbits were treated with estrogen, their uteri contracted when the hypogastric nerve was stimulated or when norepinephrine was applied externally, whereas if rabbits were treated with estrogen followed by progesterone administration, their uteri would be inhibited by both nerve stimulation and by application of norepinephrine. Nesheim (6) confirmed and extended these findings by demonstrating that in estrogen-primed longitudinal uterine muscle there was a significantly greater response to alpha adrenergic stimulation than in the progesterone-primed muscle. The changes in the number of [^{3}H]DHE-binding sites observed in the present study parallel the reported changes in alpha-adrenergic contractile responsiveness (5, 6). Previous studies (6) have reported no difference in beta adrenergic responsiveness between estrogen- and progesterone-primed longitudinal uterine muscle. Thus far, attempts in our laboratory to measure uterine beta adrenergic receptors by binding studies using techniques described for beta adrenergic receptor identification with (−) [^{3}H]dihydroalprenolol (11, 13, 14) have not been successful because of high levels of nonspecific binding in the uterus.

The concept of one hormone (e.g. progesterone) regulating the receptors for another hormone (e.g. epinephrine) may apply to a number of biological systems. For example Wolfe et al. (15) have demonstrated that endogenous adrenal steroids regulate the number of hepatic beta adrenergic receptors. Similarly Williams et al. (16) have recently demonstrated that the number of cardiac beta adrenergic receptors can be regulated by thyroid hormone. Hence these regulatory mechanisms may be of general significance in understanding the physiology and pathophysiology of adrenergic responses.

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REFERENCES

1. Willems, J. L., and A. F. de Schaepdryver. 1966. Adrenergic receptors in the oestradiol and allyloestrenol dominated rabbit uterus, *Arch. Int. Pharmacodyn. Ther.* **161**: 269–274.
2. Cibils, L. A., and F. P. Zuspan. 1968. Pharmacology of the uterus. *Clin. Obstet. Gynecol.* **11**: 34–68.
3. Cibils, L. A., S. V. Pose, and F. P. Zuspan. 1962. Effect of l-norepinephrine infusion on uterine contractility and cardiovascular system. *Am. J. Obstet. Gynecol.* **84**: 307–317.
4. Pose, S. V., L. A. Cibils, and F. P. Zuspan. 1962. Effect of l-norepinephrine on uterine contractility and cardiovascular system. *Am. J. Obstet. Gynecol.* **84**: 297–306.
5. Miller, M. D., and J. M. Marshall. 1965. Uterine response to nerve stimulation: relation to hormonal status and catecholamines. *Am. J. Physiol.* **209**: 859–865.
6. Nesheim, B. 1974. Comparison of alpha and beta receptor stimulation in the circular and longitudinal muscle of the oestrogen and progesterone dominated rabbit uterus. *Acta Pharmacol. Toxicol.* **34**: 295–304.
7. Williams, L. T., and R. J. Lefkowitz. 1976. Alpha adrenergic receptor identification by [^{3}H]dihydroergocryptine binding. *Science (Wash. D. C.).* **192**: 791–793.
8. Williams, L. T., D. Mullikin, and R. J. Lefkowitz. 1976. Identification of alpha-adrenergic receptors in uterine smooth muscle membranes by [^{3}H]dihydroergocryptine binding. *J. Biol. Chem.* **251**: 6915–6923.
9. Williams, L. T., and R. J. Lefkowitz. 1977. Molecular pharmacology of alpha adrenergic receptors: utilization of [^{3}H]dihydroergocryptine binding in the study of pharmacological receptor alterations. *Mol. Pharmacol.* **13**: 304–314.
10. Lowry, O. H., N. J. Rosebrough, A. L. Farr, and R. J. Randall. 1951. Protein measurement with the folin phenol reagent. *J. Biol. Chem.* **193**: 265–275.
11. Williams, L. T., R. Snyderman, and R. J. Lefkowitz. 1976. Identification of beta adrenergic receptors in human lymphocytes by (−) [^{3}H]alprenolol binding. *J. Clin. Invest.* **57**: 149–155.
12. Scatchard, G. 1949. The attractions of proteins for small molecules and ions. *Ann. N. Y. Acad. Sci.* **51**: 660–672.
13. Williams, L. T., L. Jarett, and R. J. Lefkowitz. 1976. Adipocyte beta adrenergic receptors. *J. Biol. Chem.* **251**: 3096–3104.
14. Alexander, R. W., L. T. Williams, and R. J. Lefkowitz. 1975. Identification of cardiac beta adrenergic receptors by (−) [^{3}H]alprenolol binding. *Proc. Natl. Acad. Sci. U. S. A.* **72**: 1564–1568.
15. Wolfe, B. P., T. K. Harden, and P. B. Molinoff. 1976. Beta adrenergic receptors in rat liver: effects of adrenalectomy. *Proc. Natl. Acad. Sci. U. S. A.* **73**: 1343–1347.
16. Williams, L. T., R. J. Lefkowitz, A. M. Watanabe, D. R. Hathaway, and H. R. Besch. 1977. Thyroid hormone regulation of beta-adrenergic receptor number. *J. Biol. Chem.* **252**: 2787–2789.