Rabbit Myometrial Adrenergic Sensitivity is Increased by Estrogen But is Independent of Changes in Alpha Adrenergic Receptor Concentration

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ABSTRACT
The ability of estrogen to increase the alpha-1 adrenergic sensitivity of rabbit uterine smooth muscle was compared with its effects on the concentration of alpha adrenergic receptor subtypes in myometrium. Compared to ovariectomized controls, estrogen treatment was found to increase the adrenergic contractile sensitivity of rabbit uterus determined in vitro. Estrogen treatment increased uterine alpha-2 adrenoceptor concentration nearly 5-fold. Mature rabbits (endogenous estrogen) had the same uterine sensitivity and alpha-2 adrenoceptor concentration as estrogen-treated rabbits. Alpha-1 receptor concentration was increased only in the estrogen-treated group, and was accompanied by increased maximal contractile response. Thus, the increase in adrenergic sensitivity after estrogen was correlated closely with an increased concentration of alpha-2 adrenoceptors. However, enhanced adrenergic sensitivity persisted after alpha-1 and alpha-2 receptor concentration returned to pre-estrogen levels in rabbits pretreated with estrogen before ovariectomy. Studies utilizing subtype-selective competitive antagonists verified that contractile response is mediated primarily by alpha-1 adrenoceptors, with no apparent influence of alpha-2 adrenoceptors. Finally, we found that adrenergic sensitivity is altered by temperature and divalent cation concentration, but these effects do not prevent the expression of the regulatory action of estradiol. We conclude that estrogen increases the alpha-1 adrenergic sensitivity of rabbit uterus without changes in alpha-1 receptors, and thus may act on postreceptor response events. The role of alpha-2 adrenoceptors in rabbit uterus is still unclear, although estrogen significantly increases their concentration.

Rabbit uterine smooth muscle contracts in response to NE or adrenergic nerve stimulation and this response is influenced by the gonadal steroids, estrogen and progesterone. Estrogen promotes a contractile response whereas progesterone promotes relaxation of myometrium (Miller and Marshall, 1965). In humans and rabbits, uterine contractile response to sympathetic nerve stimulation is mediated through the action of NE on alpha adrenoceptors whereas uterine relaxation is mediated by beta adrenoceptor activation (Digges, 1982).

In previous studies we have shown that estrogen treatment increases the concentration of alpha adrenoceptors nearly 5-fold but does not change the concentration of beta adrenoceptors in rabbit myometrium (Roberts et al., 1977), and that estrogen treatment increases myometrial contractile sensitivity to NE but not acetylcholine (Roberts et al., 1981).

Both alpha-1 and alpha-2 adrenoceptor subtypes are present in rabbit myometrium (Hoffman et al., 1979). The effect of estrogen on the individual subtypes was studied by Hoffman et al. (1981). They found that estrogen increases the alpha-2 subtype primarily, but the contractile response appeared to be mediated by alpha-1 receptors. These investigators also reported that they found no change in sensitivity to NE after estrogen treatment when contractility studies were conducted at 25°C in De Jalon's solution. However, as noted above, our studies carried out at 37°C in Kreb's solution demonstrated increased contractile sensitivity to NE after estrogen treatment of ovariectomized rabbits (Roberts et al., 1981). The use of De Jalon's solution for studies of uterine contractility is sometimes preferred (University of Edinburgh, 1970), as the reduced concentration of divalent cations (Ca++ and Mg++) and lower temperature suppress spontaneous contractions. In addition to reducing spontaneous activity, we postulated that the expression of alpha-2 adrenergic sensitivity may have been impaired under these conditions, such that the increase in sensitivity was masked.

The present studies were designed to compare parallel deter-
minations of contractile response under both in vitro conditions, using the alpha adrenergic subtype-specific antagonists yohimbine and prazosin to evaluate the relative contribution of alpha-1 and alpha-2 adrenoceptors, respectively. We also compared the effect of estrogen on uterine adrenergic sensitivity and the concentration of alpha adrenoceptor subtypes to test the role of receptor changes in altered sensitivity.

We demonstrate that the uterine response to NE is mediated by alpha-1 receptors and that the in vitro sensitivity of this response is regulated by temperature and by calcium and magnesium ions, independent of the regulatory action of estrogen. However, we also found that estrogen does indeed increase myometrial contractile sensitivity to NE, and that enhanced sensitivity is expressed under widely different calcium and magnesium concentrations and at different temperatures. We did not find evidence for the direct contribution of alpha-2 adrenoceptors to uterine contractility in vitro and therefore failed to support the hypothesis that alpha-2 adrenoceptors contribute to the increase in sensitivity NE. The increase in adrenergic sensitivity after estrogen is not dependent on an increased concentration of alpha-1 or alpha-2 adrenoceptors, but appears to be due to the enhancement of a postreceptor mechanism by estrogen.

Methods

Animals and estrogen treatment. Female New Zealand White rabbits (1.5–3.5 kg b.w.) were used for all studies. Bilateral ovariectomy was performed under ketamine-xylazine anesthesia (White and Holmes, 1976) and a minimum period of 7 days was allowed for recovery from surgery before the animals were used for assay or steroid treatment. We used four groups of animals: ovex, mature, estrogen-treated, and E2-ovex rabbits to compare physiologic (mature, ca 30 pg of E2 per ml of serum) and pharmacologic (treated, ca 300 pg/ml) doses of E2. In some experiments we used “immature” animals which weighed 1.5 to 2 kg and had total uterine weights of 800 mg or less. The mature rabbits weighed approximately 3 kg, had mature ovaries and uterine weights of approximately 2 to 3 g and had a serum E2 concentration of 20 to 50 pg/ml. For estrogen-treated rabbits, E2 benzoate in sesame oil vehicle was administered for 4 successive days (50 mg/kg i.m. per day) and the animals were sacrificed 24 h after the last dose (200–300 pg/ml of serum E2 at sacrifice). The E2-ovex rabbits were first treated with E2 as described, then ovex, and then used for studies 7 days after surgery (serum E2, 11–24 pg/ml after ovariectomy).

Materials and drugs. [3H]Rauwolscine (79–88 Ci/mmol) and [3H] prazosin (81 Ci/mmol) were obtained from New England Nuclear (Boston, MA). [3H]HEAT (BE 2254, 2200 Ci/mmol) was obtained from New England Nuclear or HEAT HCl was obtained from Beiersdorf (Hamburg, F.R.G.) and iodinated and purified as described by Roberts and Kuhn (1985). Prazosin HCl was obtained from Pfizer Inc. (New York, NY). Phenolamine HCl was obtained from Ciba Pharmaceutical Co. (Summit, NJ). All other drugs were obtained from Sigma Chemical Co. (St. Louis, MO). All chemicals used were of reagent grade.

Evaluation of contractile activity. The two media used for these studies were Kreb’s and De Jalon’s solutions. Kreb’s (University of Edinburgh, 1970) solution contained the following salts (all in millimolar concentration): NaCl, 118; KCl, 4.7; MgSO4, 1.18; KH2PO4, 1.17; glucose, 11.1; NaHCO3, 25; and CaCl2, 2.5. Studies using this medium were conducted at a constant temperature of 37°C. De Jalon’s solution (Garcia de Jalon et al., 1945) had the following composition (all in millimolar): NaCl, 135; KCl, 5.6; glucose, 2.7; NaHCO3, 25; and CaCl2, 0.4. Studies in De Jalon’s solution were conducted at a constant temperature of 25°C. The bicarbonate concentration used for De Jalon’s solution in our studies was increased over that of the published values (exchange of NaCl) as the suggested concentration of 6 mM produced a pH of 6.8 (predicted by the Henderson Hasselbalch relationship) when the medium was “equilibrated” and aerated continuously with 95% O2–5% CO2 (e.g., 10 min at a gas flow of 1 liter/min).

Uteri were removed rapidly from rabbits sacrificed by anesthetic overdose (pentobarbital, 50 mg/kg i.v.) and maintained under continuous aeration. Longitudinal strips (0.25 × 2 cm) were suspended with one end fixed to a glass hook and the other end attached to a strain gauge transducer (Grass Instruments, Quincy, MA) under 2 g of tension in the appropriate buffer which was aerated continuously with 95% O2–5% CO2 to maintain pH at 7.4. The strips were washed a minimum of 3 times by overflow during the equilibration period of approximately 1 h. When resting tension was no longer declining, and spontaneous contractile activity had stabilized, resting tension was reduced to 1 g and the cumulative concentration-response relation for NE was determined. Propranolol, 2 μM, and cocaine HCl, 20 μM, were added to the tissue bath to block beta adrenergic response and catecholamine reuptake, respectively.

In experiments using competitive antagonists, control response was determined in strips to which no antagonist was added. These were run in parallel with strips to which antagonist was added. Initial experiments indicated that this procedure results in more reproducible data than that generated by use of each strip as its own control and correcting for desensitization and time-dependent changes in agonist sensitivity (Furchgott, 1972). A minimum of 30 min was allowed for the equilibration of antagonists with tissue preparations before concentration-response studies were begun. For the calculation of dose-ratios for Schild analysis (Arunlakshana and Schild, 1959) the EC50 in the presence of antagonist was compared with that of its paired control strips for each animal, and the Schild parameters for a given dose of antagonist from a number of rabbits were pooled for graphical analysis (Schid Plota).

Analysis of contractile response. The isometric contractile force was digitized at a frequency of 1 Hz using an Apple II microcomputer and stored on a magnetic disk. The integrated area under the tension vs. time curve for 90 sec after agonist addition was used to calculate the EC50 graphically. Maximal response (Emax) was the highest integrated area developed during the concentration-response. The KClmax was measured at the end of the cumulative concentration-response curves to NE with the agonist still present. Expression of the maximal response data as the ratio Emax/KClmax was used to normalize for variations in strip weight and contractility allowing each strip to serve as its own control. Statistical significance of differences in mean log EC50 value (i.e., geometric mean) was tested by analysis of variance. The contractile sensitivity (EC50) and maximal response (Emax and KClmax) are the responses of individual uterine strips determined from a graphical array of the data. Replicates from each animal were averaged to obtain the mean response from a number of rabbits. Graphical data displayed in the figures are the pooled responses for the number of strips indicated in the legend. The concentration-response curves generated in this manner are probably a more accurate estimate of the EC50 but are not as readily amenable to statistical evaluation.

Preparation of myometrial tissue for radioligand binding studies. The procedure used is modified slightly from that described by Roberts et al. (1981). Briefly, rabbits were sacrificed by pentobarbital injection (45 mg/kg) and the uterus scraped free of endometrium, minced and a 20% (w/v) suspension prepared by homogenization at 4°C in 50 mM Tris and 4 mM MgCl2, pH 7.4, with a Tekmar tissuemizer for 90 sec after agonist addition was used to calculate the EC50 graphically. Maximal response (Emax) was the highest integrated area developed during the concentration-response. The KClmax was measured at the end of the cumulative concentration-response curves to NE with the agonist still present. Expression of the maximal response data as the ratio Emax/KClmax was used to normalize for variations in strip weight and contractility allowing each strip to serve as its own control. Statistical significance of differences in mean log EC50 value (i.e., geometric mean) was tested by analysis of variance. The contractile sensitivity (EC50) and maximal response (Emax and KClmax) are the responses of individual uterine strips determined from a graphical array of the data. Replicates from each animal were averaged to obtain the mean response from a number of rabbits. Graphical data displayed in the figures are the pooled responses for the number of strips indicated in the legend. The concentration-response curves generated in this manner are probably a more accurate estimate of the EC50 but are not as readily amenable to statistical evaluation.
standard. Membrane preparations were solubilized with NaOH before assay and appropriate reagent blanks were used.

**Adrenergic receptor binding assays.** Alpha-2 receptor concentration in myometrial particulates was measured using \[^{[3]H}]prazosin essentially as described by Lavin *et al.* (1981) for the isomer \[^{[3]H}]yohimbine. The reaction was carried out in a total volume of 250 \(\mu\)l containing \[^{[3]H}]prazosin (0.25–16 nM) in 50 mM Tris and 4 mM MgCl\(_2\) (pH 7.4). Nonspecific binding was estimated by the inclusion of 10 \(\mu\)M phentolamine HCl, and was 10 to 15% of total radioligand binding at 6 nM \[^{[3]H}]prazosin. Membrane particulate (0.2–0.25 mg of protein) was added to start the reaction, and the incubation was continued for 30 min at 30°C then terminated by the addition of 5 ml of ice-cold Tris-Mg\(^{2+}\) (pH 7.4) and filtration on Whatman GF/C filters under low vacuum (1 ml/sec). The filters were washed with three additional 5-ml volumes of cold buffer, dried under high vacuum, and radioactivity was quantitated by liquid scintillation spectrometry. Binding affinity \(K_d\) of uterine alpha-2 receptors for \[^{[3]H}]prazosin was reduced slightly by estrogen, e.g., ovex 2.8 ± 1.1 nM \((n = 6)\) and estrogen-treated, 9.4 ± 1.3 nM \((n = 6)\), \(P < .05\).

Alpha-1 adrenergic receptor concentration was measured with \[^{[3]H}]HEAT (BE 2254), an alpha-1 adrenergic antagonist radioligand which has been shown to interact with alpha-1 adrenoceptors in brain, smooth muscle and other tissues (Glossman *et al.*, 1981; Minneman, 1983). The reaction was carried out in a total volume of 250 \(\mu\)l containing \[^{[3]H}]HEAT (25–1600 pM final concentration) in 50 mM Tris and 4 mM MgCl\(_2\) (pH 7.4). Nonspecific binding was estimated by the inclusion of 0.1 \(\mu\)M prazosin HCl (Pfizer), and was approximately 20% of total binding at 200 pM \[^{[3]H}]HEAT. Membrane particulate (40–50 \(\mu\)g of protein) was added to start the reaction, and the incubation was continued for 30 min at 30°C then terminated by the addition of 5 ml of ice-cold 50 mM Tris and 4 mM MgCl\(_2\), pH 7.4, containing 10% v/v polyethylene glycol (average MW, 400) followed by filtration under low vacuum on Whatman GF/C filters. Washing of filters was completed by the addition of three more 5-ml volumes of the same cold buffer solution. Radioactivity on the dried filters was quantitated by gamma emission. In all competition studies a receptor concentration which bound less than 10% of the total radioligand was used. Binding affinity \(K_d\) of uterine alpha-1 adrenoceptors was not changed by treatment, and was approximately 300 pM \[^{[3]H}]HEAT. We have also measured alpha-1 adrenoceptor concentration using \[^{[3]H}]prazosin and verified that \[^{[3]H}]HEAT and \[^{[3]H}]prazosin label the same concentration of alpha-1 receptors in uterine membranes (data not shown). Data from binding assays were analyzed by an iterative nonlinear curve fitting program in which bound ligand is calculated as a function of free ligand (Murlas *et al.*, 1982).

The data are represented as mean values ± S.E.M. Statistical significance of difference was tested by analysis of variance. A CL of 95% was the criterion for significance.

**Results**

**Effect of estrogen on adrenergic sensitivity and maximal response.** Intramuscular administration of 50 \(\mu\)g/kg of E2 for 4 days increased the sensitivity of rabbit uterus to NE 4-fold in Kreb’s solution (fig. 1) and 3-fold in De Jalón’s solution (table 1) compared to ovex animals. Both immature and ovex uteri displayed low sensitivity to NE, and the high sensitivity of estrogen-treated animals was similar to the sensitivity of strips obtained from mature animals (table 1). Thus, estrogen, whether endogenous or exogenous, increased adrenergic sensitivity of the uterus at least 3-fold (table 1) compared to ovex rabbits. However, estrogen treatment did not increase sensitivity beyond the level seen with endogenous estrogen (i.e., mature animals).

Table 2 shows the effect of estrogen treatment on maximal contractile response determined in Kreb’s solution. The maximal contractile response of a muscle is dependent on the mass as well as less easily controlled factors such as connective tissue content and degree of stretch during preparation. For this reason we also obtained the response of each strip to a depolarizing concentration of KCL, which causes a maximal activation, to evaluate the net response of each muscle strip. The ratio \(E_{\text{max}}/K\text{Cl}_{\text{max}}\) was then evaluated for the different treatment groups. Neither the \(E_{\text{max}}\) nor the \(K\text{Cl}_{\text{max}}\) was changed significantly with treatment \((P > .05)\), and there was substantial variation in these responses within most groups. However, a large reduction in variation was achieved by computing the ratio of these two responses. As judged by the ratio \(E_{\text{max}}/K\text{Cl}_{\text{max}}\), estrogen treatment increased maximal response, but estrogen treatment before ovariotomy (E2-ovex) resulted in a uterus with reduced maximal response compared to estrogen-treated rabbits. Uteri from mature rabbits also had a lower maximal response to NE than uteri from estrogen-treated rabbits.

**Effect of temperature and divalent cations on adrenergic sensitivity.** Whereas the ability of estrogen to increase adrenergic sensitivity is apparent using both physiologic salt solutions, the increase was greater in Kreb’s solution at 37°C than in De Jalón’s solution at 25°C (cf. table 1). We conducted a series of experiments designed to examine the relative influence of different concentrations of calcium and magnesium ions, and temperature on uterine sensitivity to NE. Figure 2 shows the \(EC_{50}\) values for NE-induced contraction, determined in parallel strips from estrogen-treated rabbits in each solution at 37 and 25°C. The data were analyzed by two-way analysis of variance to test for interaction between the factors of temperature and buffer composition. In Kreb’s solution, sensitivity was reduced significantly \((i.e., EC_{50} \text{ was greater})\) at 25°C compared with 37°C \((P < .025)\). In De Jalón’s solution, sensitivity was not different at the two temperatures. The combined effects of ionic composition and temperature interact to alter signifi-
TABLE 1
Sensitivity of rabbit uterus to contraction by NE

<table>
<thead>
<tr>
<th>Buffer</th>
<th>Ovex</th>
<th>Immature</th>
<th>Mature</th>
<th>E2</th>
<th>E2-ovex</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kreb's</td>
<td>746 ± 139</td>
<td>447 ± 140*</td>
<td>153 ± 1</td>
<td>164 ± 16*</td>
<td>1311 ± 12*</td>
</tr>
<tr>
<td>De Jalons</td>
<td>1210 ± 327</td>
<td>ND</td>
<td>208,722*</td>
<td>461 ± 98*</td>
<td>ND</td>
</tr>
</tbody>
</table>

The values for the two mature rabbits in De Jalons solution were not included in the statistical analysis and are provided for reference only.

* P < .05 by one-way analysis of variance.

TABLE 2
Maximal contractile response of rabbit uterus to NE (E_max) and KCl (KCl_max)

<table>
<thead>
<tr>
<th>Treatment</th>
<th>E_max</th>
<th>KCl_max</th>
<th>E_max/KCl_max</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ovex (5)</td>
<td>136 ± 19</td>
<td>92 ± 13</td>
<td>1.49 ± 0.07***</td>
</tr>
<tr>
<td>E2 (5)</td>
<td>433 ± 112</td>
<td>178 ± 43</td>
<td>2.48 ± 0.10</td>
</tr>
<tr>
<td>Mature (4)</td>
<td>212 ± 60</td>
<td>132 ± 27</td>
<td>1.52 ± 0.13***</td>
</tr>
<tr>
<td>E2-ovex (5)</td>
<td>366 ± 115</td>
<td>232 ± 57</td>
<td>1.48 ± 0.12***</td>
</tr>
</tbody>
</table>

*** P < .001 for significant difference from estrogen-treated rabbits by one-way analysis of variance.

Fig. 2. Comparison of the contractile sensitivity (EC_{50}) to NE (NOREPI) under different ionic and temperature conditions. The data are the mean EC_{50} values from two uterine strips obtained from five estrogen-treated rabbits. The effect of temperature was significant in Kreb's solution (P < .025) but not significant in De Jalon's solution. The interaction between temperature and ionic conditions was significant, P < .05 by two-way analysis of variance.

Significantly (P < .05) the adrenergic sensitivity of rabbit uterus, and this can account for the difference in sensitivity observed between Kreb's and De Jalon's solutions.

Subtype specificity of the alpha-adrenergic response to NE. The ability of the subtype-specific antagonists prazosin and yohimbine to inhibit competitively the response to NE was compared in Kreb's solution at 37°C and De Jalon's solution at 25°C to determine whether the expression of an alpha-2 receptor-mediated response was different under the two conditions. The effect of the alpha-2 antagonist yohimbine on the contractile response of estrogen-treated rabbits is shown in figure 3. Yohimbine was a poor antagonist of NE-stimulated contraction, producing only a modest shift at doses more compatible with antagonism of alpha-1 than alpha-2 adrenoceptors. Low concentrations of yohimbine failed to affect the EC_{50} and higher concentrations yielded K_i values too high for alpha-2 specificity (e.g., 200 nM yohimbine: K_i = 261 nM in Kreb's solution). Thus, we found no evidence to suggest that an alpha-2 response was obscured by lower temperature and calcium ion concentration.

In contrast to yohimbine, prazosin caused a rightward shift of the concentration-response curves (fig. 4A), as would be expected for a competitive antagonist. The effect of prazosin on NE contraction was analyzed by the method of Arunlakshana and Schild (1959) (fig. 4B), revealing a pA_2 for prazosin of 8.2 (K_i = 5.7 nM, 95% confidence interval of the regression: 0.3 to 14 nM) in Kreb's solution and a pA_2 of 8.5 (K_i = 2.9 nM, confidence interval, 0.2 to 10 nM) in De Jalon's solution, both of which are compatible with alpha-1 antagonism. In both buffers, the Schild plot was linear with a slope not significantly
different from unity (P > .05). Thus, the contractile response of rabbit uterus to NE appears to be mediated predominantly by alpha-1 adrenoceptors.

Uterine alpha adrenoceptor concentration changes with estrogen. Competition curves for $[^{125}\text{I}]$HEAT binding to uterine alpha-1 adrenoceptors are shown in figure 5. This radioligand displays the appropriate selectivity for the interaction with alpha-1 receptors.

The concentration of alpha adrenoceptors in myometria from the four treatment groups is shown in table 3. Alpha-1 adrenoceptor concentration measured by $[^{125}\text{I}]$HEAT binding was

\[ \text{Dose ratio} = \frac{\text{EC}_{50} \text{ with antagonist}}{\text{EC}_{50} \text{ without antagonist}} \]

unchanged except by treatment with estrogen, which produced a doubling in concentration (P < .025). In contrast, alpha-2 adrenoceptor concentration was increased nearly 5-fold in both mature and treated groups. In animals pretreated with estrogen before ovariectomy (E2-ovex), both alpha-1 and alpha-2 adrenoceptor concentrations had declined to levels comparable to ovex rabbits.

Discussion

The results of this study demonstrate that estrogen increases uterine adrenergic sensitivity determined in either Kreb's solution at 37°C or De Jalon's solution at 25°C, although the increase observed in De Jalon's solution was smaller. Temperature, and calcium and magnesium ion concentration, influence the in vitro sensitivity of the uterine adrenergic response. However, these changes are independent of and do not alter the regulatory influence of estrogen. Estrogen treatment did not increase sensitivity beyond the level seen with endogenous estrogen (i.e., mature animals), despite a 10-fold higher serum
E2 concentration and a doubling of alpha-1 adrenoceptor concentration. Also, treatment with pharmacologic doses of estrogen did not increase alpha-2 receptor concentration beyond the level achieved during normal maturation but it does increase alpha-1 receptor concentration.

We found that the adrenergic contractile response of rabbit uterus is mediated primarily by alpha-1 adrenoceptors, as reported previously by Hoffman et al. (1981) and that the predominant subtype mediating response is not influenced by temperature or ionic composition of the physiologic salt solution. We were unable to demonstrate a contractile response to alpha-2 adrenoceptor activation in uteri from estrogen-treated rabbits, despite the nearly 5-fold increase in receptor concentration.

The lack of a difference in NE sensitivity with estrogen treatment reported by Hoffman et al. (1981) may have resulted from the use of uterine strips obtained from rabbits which had begun to mature and had already increased their estrogen production sufficiently to increase NE sensitivity. We found that, even in the same strain, weight was an unreliable indicator of maturity and that ovariectomy was a consistently reliable way to obtain animals whose uteri exhibited a low sensitivity of NE.

Estrogen also increases maximal contractile response, and this was associated with the increase in alpha-1 receptor concentration. Evaluation of maximal response as the ratio between specific and nonspecific muscle activation achieved a large decrease in the intragroup variation in response. This suggests that the two responses were affected by the same factors (e.g., muscle mass and damage during isolation), and that the comparison is useful for correcting for variation in response. Simple correction for wet weight did not achieve as great a reduction in variation, although it did reveal a significant increase in maximal response to NE with estrogen treatment (data not shown).

Inasmuch as estrogen is known to inhibit extraneuronal catecholamine reuptake via "uptake II," this mechanism could conceivably increase the apparent sensitivity of the uterus to NE. In a previous study (Roberts et al., 1981) we examined this possibility by comparing the uterine responses to NE and methoxamine, an agonist which is not subject to reuptake. We found that estrogen treatment caused an increase in uterine sensitivity to both agonists, suggesting that the increase in sensitivity was not an artifact of changes in catecholamine reuptake. The uteri from ovex rabbits are usually one-half to one-third of the size (wet weight) of uteri from estrogen-treated rabbits and it is possible that this could contribute to the observed increase in sensitivity to NE. However, we feel that uterine size is unlikely to have contributed to the observed sensitivity change. In the previous study (cited above), we examined the contractile responses to both NE and acetylcholine, and we found that although adrenergic sensitivity was increased with estrogen treatment cholinergic sensitivity was not. Such agonist selectivity clearly argues against a nonspecific effect such as the influence of tissue size. Also, in the present study some of the ovex rabbits were quite large and the uteri were also large, yet they were as insensitive to NE as smaller uteri from smaller rabbits.

Several observations indicate that changes in alpha adrenoceptor concentration do not correlate with the observed changes in uterine contractile sensitivity. Alpha-2 receptor concentration increases with estrogen but these receptors do not appear to mediate contraction. In mature rabbit uteri, alpha-1 adrenoceptor concentration is the same as uteri from ovex rabbits, yet sensitivity is increased (i.e., EC50 is reduced). Finally, estrogen treatment before ovariectomy (E2-ovex) produces a uterus having increased alpha-1 adrenergic sensitivity but alpha-1 and alpha-2 adrenoceptor concentration is the same as uteri from ovex rabbits.

The choice of the E2-ovex experimental paradigm was quite fortuitous. Uteri from ovariectomized immature rabbits were very small, and we attempted to increase uterine size to allow the measurement of both alpha-1 and alpha-2 adrenoceptors in a single ovex rabbit uterus by treating animals with estrogen before ovariectomy. Because alpha adrenoceptor concentration returns to basal values within 4 days of estrogen withdrawal (Roberts et al., 1981), we felt that estrogen pretreatment might serve to increase uterine size without altering the response, and thus provide more tissue for study. However, in addition to inducing growth of the uterus, pretreatment with estrogen resulted in a persistent state of enhanced adrenergic sensitivity (fig. 1), despite the return of alpha adrenoceptor concentrations to levels present in uteri from ovex rabbits. The increased adrenergic sensitivity in these animals demonstrates clearly that increases in alpha adrenoceptor concentrations do not contribute to the sensitivity change. These results and the finding that treatment with pharmacologic doses of estrogen increases alpha-1 receptor concentration, but does not increase sensitivity to NE beyond the normal maturation process, suggest an important role for postreceptor mechanisms as mediators of estrogen-induced increases in adrenergic sensitivity. However, the locus of estrogenic action must be at a point before the final common pathway to myosin activation, as cholinergic sensitivity is not increased by estrogen (Roberts et al., 1981).

In a system containing substantial receptor reserve (spare receptors), an increase in receptor concentration correlates with increased sensitivity (reduced EC50). The results of this study indicate that there is no receptor reserve for the alpha-1 adrenergic contractile response of rabbit uterus. Contractile sensitivity was increased in two treatment groups (mature and E2-ovex) whose uteri did not have an increased concentration of alpha-1 adrenoceptors. Thus, no increase in receptor concentration was required for the sensitivity change. Moreover, in a system without receptor reserve, increased receptor concentration correlates with increased maximal response. This is precisely what was observed in uteri from estrogen-treated rabbits. The reduced maximal response while retaining enhanced sensitivity in the E2-ovex treatment group suggests that response and sensitivity can be attributed to separate entities, with the determinant of sensitivity having a longer half-life than that of maximal response. These data are consistent with a three component model: agonist-receptor-transducer, where receptor concentration affects maximal contractile response but changes in sensitivity require changes in the transducer moiety.

The role of the alpha-2 adrenoceptors in myometrium remains obscure. Inasmuch as most are not presynaptic (see Hoffman et al., 1981), they could act indirectly to prevent relaxation by inhibition of adenylate cyclase, and enhance alpha adrenergic response by preventing beta adrenergic relaxation. The inhibition of forskolin-stimulated CAMP levels by ephrine acting through alpha-2 adrenoceptors (beta adrenergic antagonist present) has been demonstrated in estrogen-treated rabbit uterus (unpublished results from our laboratory), sug-
gesting that uterine α2 receptors do inhibit adenylate cyclase activity. Because β antagonists and NE were used routinely in the contractility studies, this indirect effect of α2 receptors would not have been detected in these experiments.

In a previous study in which a β antagonist was not used, we concluded that there were spare α receptors in rabbit uterus. This conclusion was based on a decrease in sensitivity to NE after partial receptor blockade in an experimental paradigm using the reversible antagonist phentolamine (which recognizes both α1 and α2 adrenoceptors) in a manner designed to mimic the effect of an irreversible antagonist. If indeed there is an important effect of α2 adrenoceptors to oppose the effects of α1 adrenoceptor activation through the inhibition of adenylate cyclase, this could well have contributed to the observed reduction in sensitivity consequent to phentolamine treatment. Inasmuch as NE has efficacy at both α and β adrenoceptors, the effect of β adrenoceptor stimulation would be expected to be more profound with many of the α2 adrenoceptors blocked by phentolamine, and the increase in cAMP would be expected to reduce the apparent sensitivity of the α1 adrenergic contractile response. Thus, the reduction in α1 sensitivity (EC50 increase) with α1- and α2 receptor blockade observed in the previous study may not be due to the presence of spare receptors, but rather to the opposing influence of β receptor stimulation on α1 response. In the present studies, the presence of β receptor blockade precluded the detection of any postreceptor interactions between α1 and β receptors. The direct contribution of α2 adrenoceptors to the contractile response to NE is probably small, as judged by the lack of effect of yohimbine (fig. 3). However, because physiologic activation by NE or epinephrine would involve both α1 and α2, and also β receptors, the interaction between the postreceptor responses to the activation of these receptors (i.e., between cAMP and CA++ mobilization) deserves further study.

References


