Opposing effects of estradiol and progesterone on oxytocin receptors in rabbit uterus

(steroid/induction/contractility/hormone binding)

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ABSTRACT Estradiol-17 β administration to young (10- to 12-week-old) rabbits to produce the “estrogen-dominated” uterus increased the uterine contractile response to both oxytocin and methacholine *in vitro*. In “progesterone-dominated” uteri, obtained from rabbits that received progesterone for 4 days after estrogen pretreatment, the contractile response to oxytocin *in vitro* was selectively abolished; the response to methacholine was unaffected. Parallel changes were observed in the concentration (but not affinity) of specific sites in uterine microsomal membranes that bind [3 H]oxytocin with selectivity features expected for oxytocin receptors. Thus, estrogen-dominated uteri have an increased number of specific [3 H]oxytocin binding sites per mg of membrane protein relative to untreated controls, whereas specific oxytocin binding sites are reduced to barely detectable levels in the progesterone-dominated uterus. Similar results are obtained when binding sites are measured in membranes from the myometrium of estrogen- or progesterone-dominated uteri. Short-term (24-hr) progesterone administration to estrogen-pretreated rabbits decreased, but did not abolish, specific [3 H]oxytocin binding; the concentration of specific [3 H]oxytocin binding sites was reduced without influence on the affinity of these sites. A sublethal dose of actinomycin D, administered over a 24-hr period to rabbits pretreated with estradiol for 4 days, likewise reduced specific oxytocin binding; additive effects were not observed when progesterone and actinomycin D were administered together. These results suggest that the regulatory effects of estrogens and progesterone upon the rabbit uterine contractile response to oxytocin are achieved, at least in part, by the opposing actions of these steroids in regulating the number of oxytocin receptors in smooth muscle cells. Estradiol increased the concentration of uterine oxytocin receptors; the maintenance of high receptor levels appears to depend upon the continuous *de novo* synthesis of oxytocin receptors. In contrast, progesterone, like actinomycin D, appears to act at the nuclear locus to repress synthesis of oxytocin receptors.

The response of the uterus to contractile agents in several species, but particularly in rabbits, is critically dependent upon the sex steroid status of the animal (1–5). Thus, the “estrogen-dominated” rabbit uterus is highly responsive to oxytocin whereas the “progesterone-dominated” uterus (whether due to exogenous or endogenous progesterone, as in pregnancy) is relatively nonresponsive. The decreased sensitivity of the progesterone-dominated uterus to oxytocin has likewise been observed in other species, including human beings (6), rats (7), and ewes (8).

The precise mechanisms by which sex steroids control myometrial contractile activity are not known. The effect of estrogens to augment the uterine response to contractile agents has been ascribed to a uterotrophic effect, which leads to an increase in the concentration of actin and myosin (9). Estrogens have also been reported to increase the affinity as well as the

number of oxytocin receptors in rat uterus (10). The effect of progesterone to induce uterine insensitivity has been attributed to hyperpolarization of smooth muscle cells so that localized action potentials, once generated, are poorly conducted to neighboring cells (11, 12); such a progesterone-induced conduction block would be expected to apply to all contractile agents. This “progesterone-block” theory has been challenged both on experimental and theoretical grounds (13).

Independent of the precise changes in membrane ion conductance produced by progesterone in uterine smooth muscle cells, we have found that the progesterone-dominated rabbit uterus exhibits a selective loss of responsiveness to oxytocin *in vitro* while retaining the capacity to respond to another contractile agent, methacholine, an acetylcholine analog. The selective loss of oxytocin sensitivity raised the possibility that progesterone, in addition to other effects at the cell membrane, might also influence that element of the uterine oxytocin response system responsible for hormone selectivity—the oxytocin receptor. This possibility was examined by measuring specific [3 H]oxytocin binding in uterine membrane fractions derived from rabbits treated with exogenous steroids, on the basis that such specific binding sites may represent oxytocin receptor units.

MATERIALS AND METHODS

Materials. Young (10- to 12-week-old, 2.5–3.0 kg) virgin female New Zealand white rabbits, obtained from Scientific Small Animal Farms (Arlington Heights, IL), were used for these studies. Progesterone, estradiol-17 β , and methacholine were obtained from Sigma Chemical Co. (St. Louis, MO). Bovine serum albumin was obtained from Armour Co. (Chicago, IL), and was purified as described (14). Actinomycin D was obtained from Merck, Sharp and Dohme (West Point, PA). All other chemicals used were of reagent grade quality. [3 H]oxytocin, (specific activity 31 Ci/mmol) was prepared and purified as described (15). The labeled hormone was radiochemically homogeneous in several thin-layer chromatography systems, and had identical activity to that of a synthetic standard unlabeled oxytocin sample (500 units/mg) in the Holton (16) rat uterotonic assay and in a bovine renal medullary adenylate cyclase assay system.[‡]

Steroid Administration. Steroid hormones dissolved in ethanol were added to peanut oil (final concentration, 1%

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‡ The adenylate cyclase assay involves partially purified renal medullary plasma membranes, prepared from perfused bovine kidneys by a polyethylene glycol–dextran double-phase technique to be reported elsewhere (Nakahara, T., Terada, S., Pincus, J., Flouret, G. & Hechter, O., *J. Biol. Chem.*, in press). Adenylate cyclase activity was assayed at 30° containing Bis/Tris/propane (pH 8.5), 2.0 mM MgCl₂, 1.4 mM EDTA, 100 μ M [32 P]ATP, 1 mM cyclic AMP, 20 mM creatine phosphate, and 0.2 mg of creatine phosphokinase per ml.

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ethanol) and administered by subcutaneous injection. Estrogen-dominated uteri were obtained by injecting rabbits daily with estradiol-17 β (200 μ g/kg) for 4 days. To obtain progesterone-dominated uteri, rabbits were first injected daily for 4 days with estradiol-17 β (200 μ g/kg) followed by four daily injections of progesterone (5.0 mg/kg). One group of estrogen-treated rabbits received the oil vehicle for 4 days instead of progesterone.

Uterotonic Assay. Uterine horns from rabbits (killed by decapitation) were quickly excised, dissected free of fat, and cut open longitudinally to obtain rectangular strips (about 0.5 $\times$ 1.5 cm). Strips were mounted vertically with one end fixed and the other end attached to a calibrated Grass FTO3C force-displacement transducer, and incubated at 30° in a bath containing 10 ml of oxygenated Munsick's (plus Mg²⁺) solution (17). Spontaneous contractions are eliminated under these conditions. Isometric tension was monitored on a dynograph recorder. The contractile response to maximal concentrations of methacholine (10 μ M) and oxytocin (1 μ M) was routinely measured at the beginning and end of each uterotonic study to verify that responsiveness of the strip was quantitatively maintained throughout the experiment. Uterine strips were "washed out" at least four times with 10 ml of Munsick's solution over a 10-min period between addition of different contractile agents and between different doses of the same agent, so that resting tension was restored prior to the next addition.

Oxytocin Receptor Assay. The approach used to study putative uterine oxytocin receptors was to measure "specific binding" of radioactive oxytocin to particulate fractions derived from homogenates of uterus, or myometrial strips. Specific oxytocin binding was differentiated from "nonspecific" binding by incubating homogenate fractions with various concentrations of [³H]oxytocin (0.1–10 nM) in the *absence* and *presence* of 10 μ M unlabeled oxytocin. The [³H]oxytocin binding that occurs in the presence of a large concentration of unlabeled oxytocin, designated as "nonspecific" [³H]oxytocin binding, represents ligand interaction with a large number of low-affinity sites, which are not saturable at abnormally high concentrations of oxytocin. The difference between total [³H]oxytocin binding and "nonspecific" binding is designated as specific oxytocin binding, and represents binding to a small number of saturable high-affinity sites. With intact uterine horns or myometrial strips from estrogen-dominated rabbits it was difficult to quantitatively estimate the number of specific [³H]oxytocin binding sites, since the values for "nonspecific" binding were very high relative to specific oxytocin binding. However, specific [³H]oxytocin binding could be demonstrated in estrogen-dominated uterine or myometrial strips, whereas specific oxytocin binding was not detected in the progesterone-dominated uterus. It was possible to quantitate the number and affinity of specific [³H]oxytocin binding sites when particulate fractions derived from homogenates were studied. Homogenate fractions derived from the pooled uteri obtained from at least three rabbits that had been treated identically (with exogenous steroids or the peanut oil vehicle) were prepared as follows: uterine horns from decapitated rabbits were dissected free of fat and thoroughly chopped into small fragments with a McIlwain chopper in the cold room (2°). The tissue fragments were first washed extensively with 0.9% NaCl/1.0 mM EDTA (to remove enzymes liberated during chopping) and then homogenized (20 strokes) in four volumes of 0.25 M sucrose/1.0 mM EDTA using a "loose" motor-driven Teflon pestle-glass homogenizer. The resulting homogenate was filtered through gauze and rehomogenized first with 20 strokes in a "loose" glass

Dounce homogenizer (followed by filtration through one layer of gauze), and then with 20 strokes of a "tight" glass Dounce homogenizer. The final homogenate obtained was differentially centrifuged successively at 2000 $\times$ *g* (10 min), 10,000 $\times$ *g* (20 min), and 95,000 $\times$ *g* (60 min). As previously described (18) in sow uterus, [³H]oxytocin binding activity was distributed in the various particulate fractions of the homogenate. The 10,000 $\times$ *g* and 95,000 $\times$ *g* pellets contained the bulk of the total [³H]oxytocin binding activity. The 95,000 $\times$ *g* pellet had 3–5 times greater specific [³H]oxytocin binding (expressed in terms of pmol of ligand bound per mg of protein) than the 10,000 $\times$ *g* pellet; the total number of binding sites was similar in the two fractions. Electron microscopy showed that the bulk of the particulate material sedimenting between 10,000 and 95,000 $\times$ *g* were typical "microsomal" membranes, with minimal mitochondrial contamination; the 10,000 $\times$ *g* pellet contained large amounts of mitochondria as well as other membranes. Changes produced by steroids in specific [³H]oxytocin in intact uterine or myometrial strips were accurately reflected by comparable changes in the binding activity of the microsomal membranes. Accordingly, microsomal membranes were used in the present study.

The influence of temperature, pH, electrolytes, and divalent cations upon specific [³H]oxytocin binding led us to adopt the following standard assay conditions. Uterine microsomal membranes (~60 μ g of protein) were incubated with various concentrations of [³H]oxytocin, in a final volume of 0.1 ml containing 25 mM Tris-HCl (pH 7.6), 2.0 mM MgCl₂, 0.1% bovine serum albumin, and, where appropriate, 10 μ M unlabeled oxytocin for estimation of nonspecific binding. Binding was terminated by addition of ice-cold buffer followed by rapid filtration (under reduced pressure) of the chilled suspension (within 4 sec) through 0.2- μ m EGWP cellulose acetate Millipore filters. The filters were then washed twice in the cold room (2–4°) by the addition and rapid filtration of 10 ml of ice-cold buffer. Specific binding with several concentrations of ligand (1–10 nM [³H]oxytocin) was at (or near) equilibrium after 15 min of incubation at 30°; accordingly, a standard 15-min incubation for oxytocin binding was adopted. In the absence of membranes, [³H]oxytocin binding to the filters was minimal, and was not reduced by the presence of 10 μ M unlabeled oxytocin. Using uterine membrane preparations from estrogen-dominated rabbits we showed that: (i) [³H]oxytocin incubated with the isolated membrane preparations was not significantly degraded, as evaluated by thin-layer chromatography in several systems; (ii) the *bound* radioactivity associated with membranes previously incubated with [³H]oxytocin, designated as specific [³H]oxytocin binding, was extractable with 0.1 M formic acid and migrated identically with authentic unlabeled oxytocin in several thin-layer chromatography systems; (iii) specific [³H]oxytocin binding sites were present in much higher concentration in microsomal membrane fractions of myometrium than of whole uterus; and (iv) for nine analogs of oxytocin there was a direct correlation between the calculated *K*_D values for analog binding obtained from competitive binding curves and the peptide concentrations required for half-maximal uterotonic activity. In some experiments, membranes prepared from myometrial strips of one horn were compared to membranes derived from the other horn (endometrium plus myometrium) in groups of rabbits treated with either estrogen alone or with estrogen plus progesterone.

RESULTS

Uterotonic Studies. Representative dynograph recordings of the oxytocin- and methacholine-induced contractile response

FIG. 1. Isometric tension developed *in vitro* by uterine strips from untreated (A), estrogen-dominated (B), and progesterone-dominated (C) rabbits. A fourth group (B') received the oil vehicle (instead of progesterone) for 4 days after estradiol. Representative tracings from the original dynograph recordings of a typical experiment are shown; uterine strips were incubated for at least 30 min prior to addition of contractile agents to establish resting tension (0.5 g). The results obtained in three additional experiments were similar. Time 0 represents addition to the bath of methacholine (MC) or oxytocin (OT) at the final concentrations shown. Contractile responses to other concentrations of these agents (not shown) demonstrated that maximal effects were obtained at 10^{-6} M oxytocin and 10^{-5} M methacholine. W indicates the beginning of at least four successive washouts of methacholine or oxytocin after 5 min of exposure; resting tension was restored by this procedure prior to each hormone addition.

of uteri from rabbits receiving exogenous steroid treatment are shown in Fig. 1. As expected, treatment of young rabbits with estradiol augmented the contractile response of the isolated uterus to both oxytocin and methacholine. The increased responsiveness of the estrogen-dominated uterus to oxytocin persisted for at least 4 days after cessation of estrogen injection. Administration of progesterone for 4 days (after estradiol treatment) to produce the progesterone-dominated state resulted in a complete loss of the uterine contractile response to oxytocin, even at concentrations as high as $1 \mu\text{M}$. Despite insensitivity to oxytocin, the progesterone-dominated rabbit uterus remained responsive to methacholine *in vitro* and there was no significant change in the dose-contractile response curve with methacholine (not shown).

[^3H]Oxytocin Binding Studies. Specific [^3H]oxytocin binding was measured in uterine microsomal membranes derived from rabbits treated with exogenous steroids (Fig. 2). A 4-day treatment with estradiol resulted in greatly increased specific [^3H]oxytocin binding, an effect that persisted for at least 4 days after cessation of estradiol injections. Administration of progesterone, after estradiol, produced a decrease in specific [^3H]oxytocin binding which was marked after 1 day and even greater after 4 days of progesterone. Fig. 3 shows the linearity of Scatchard plots of the specific oxytocin binding data of Fig. 2, indicating a single class of high-affinity binding sites. The 4-day estradiol treatment produced a 3-fold increase in the concentration of specific [^3H]oxytocin uterine binding sites, as compared to the untreated controls (from 0.10 to 0.32 pmol/mg of protein), without apparent influence upon affinity. Progesterone administration for 4 days after estradiol pretreatment markedly reduced the number of specific [^3H]oxytocin binding sites per mg of microsomal membrane protein to such low levels that Scatchard analysis of binding parameters was not possible. When the oil vehicle, instead of progesterone, was administered

FIG. 2. Specific [^3H]oxytocin binding to uterine microsomal membrane fractions from untreated (A), estrogen-dominated (B), and progesterone-dominated (C) rabbits. Other rabbits received estradiol for 4 days, followed by either the oil vehicle for 4 days (B') or progesterone (20 mg/kg) for 1 day (C'). The mean ($\pm$ SEM) of specific oxytocin binding, expressed as pmol of [^3H]oxytocin bound per mg of particulate protein, is plotted as a function of the log of molar concentration of added [^3H]oxytocin. Four or more membrane preparations (each derived from pooled membranes, obtained from at least three rabbits) were used for each group; the oxytocin binding curves for each preparation were done in duplicate. The points shown thus represent eight (or more) individual measurements for each hormone concentration ($n > 8$). B' and B are significantly different from A; C is likewise significantly different from A, but C' is not.

for 4 days to rabbits pretreated with estradiol, the concentration and affinity of specific oxytocin binding sites was virtually unchanged. Thus the loss of oxytocin-induced contractile activity in the progesterone-dominated uterus is associated with a marked reduction of specific [^3H]oxytocin binding sites.

High-affinity oxytocin binding sites have been reported in the endometrium as well as the myometrium of ewes (19); on the day of estrus, the binding capacity per mg of endometrial particulate protein was more than twice that of myometrial

FIG. 3. Scatchard plots of specific [^3H]oxytocin binding to uterine membrane fractions from rabbits treated with exogenous steroids (see legend of Fig. 2 for explanation of symbols). For each concentration the ratio of specifically bound [^3H]oxytocin to free [^3H]oxytocin is plotted against specific [^3H]oxytocin binding. It was not possible to obtain a Scatchard plot for the data on the progesterone-dominated uterus (Fig. 2C) due to the low specific binding values obtained. Dissociation constants (in nM), calculated from the slopes, were: A, 4.0; B, 4.1; B', 3.0; C, 2.6. Maximum specific binding values (in pmol/mg of protein), calculated from the x-intercepts, were: A, 0.10; B, 0.32; B', 0.26; C', 0.07. Data were linearized by the method of least squares.

protein. The changes in [3 H]oxytocin binding observed in the present study with "estrogen-dominated" and "progesterone-dominated" rabbit uteri, in contrast, appear to reflect changes in the number of specific oxytocin binding sites primarily in myometrium.[§]

The effect of progesterone to decrease specific [3 H]oxytocin binding in estradiol-pretreated rabbits can be demonstrated within 24 hr; administration of progesterone (5.0 mg/kg, four times) over a 24-hr period, after estradiol, significantly decreased the number of specific oxytocin binding sites from 0.32 to 0.07 pmol/mg of protein, without much change in the apparent affinity of these sites for oxytocin (Fig. 3).

There is considerable evidence that steroid hormones act via a common reaction pattern wherein a specific steroid-receptor complex acts at the gene locus to influence the synthesis of specific species of RNA and proteins (20). To examine in preliminary fashion the possibility that the observed steroid-induced changes in oxytocin receptor binding might be mediated via changes in uterine protein synthesis, we studied the effects of actinomycin D upon specific oxytocin binding in microsomal membrane fractions from estrogen-dominated uteri. Rabbits, pretreated for 4 days with estradiol, were injected with a sublethal dose of actinomycin D (80 μ g/kg, four times) over a 24-hr period after cessation of estradiol treatment. Fig. 4 shows that administration of actinomycin D reduced specific [3 H]oxytocin binding in estradiol-pretreated rabbits to about the same extent as a 24-hr treatment with progesterone. When progesterone and actinomycin D were administered together, additive inhibitory effects upon specific [3 H]oxytocin binding were not obtained.

DISCUSSION

The present results confirm and extend previous findings that have demonstrated the profound influence of estrogens and progesterone upon the contractile response of the rabbit uterus to oxytocin. Administration of estradiol to young rabbits resulted in an augmented uterine response (*in vitro*) to contractile agents such as oxytocin and the acetylcholine analog methacholine, in agreement with the previous findings of Csapo (21). The augmentation of contractility produced by estradiol has been ascribed to the increased concentration of actin and myosin in smooth muscle cells (22, 23) resulting from the uterotrophic action of estradiol. Our finding that estradiol increases the concentration of specific [3 H]oxytocin binding sites in membrane fractions from rabbit myometrium strips as well as whole uterus raises the possibility that estradiol promotes the synthesis of specific membrane receptors in uterine smooth muscle cells

[§] Thus, pooled membranes obtained from groups of five rabbits were prepared from myometrial strips obtained from one horn and compared to the pooled membranes derived from the contralateral uterine horns (containing endometrium plus myometrium). The myometrial and uterine membranes derived from "estrogen-dominated" rabbits showed no significant differences in the apparent affinity of [3 H]oxytocin for specific binding. However, the number of specific oxytocin binding sites per mg of protein was increased more than 2-fold in myometrial membranes as compared to membranes prepared from whole uterus. In similar experiments with progesterone-treated (4 days) rabbits, specific oxytocin binding sites were not detected either in myometrial or uterine membranes incubated with [3 H]oxytocin at concentrations ranging from 2–50 nM. These observations demonstrate that: (i) changes in specific oxytocin binding sites mainly reflect changes in the number of oxytocin receptors in myometrium and (ii) the specific [3 H]oxytocin binding capacity per mg of protein of the myometrial membranes is greater than that of the endometrium in estrogen-dominated rabbit uterus, in contrast to that reported for ewes.

FIG. 4. Specific [3 H]oxytocin binding to uterine microsomal membranes obtained from rabbits treated as shown. Rabbits received either the oil vehicle or estradiol (200 mg/kg per day) for 4 days. Some estradiol-pretreated animals were further injected with the oil vehicle or progesterone (5 mg/kg per day) for 4 days; other estradiol-pretreated rabbits received progesterone (20 mg/kg) and/or actinomycin D (320 mg/kg) over a 24-hr period after estradiol. The number of membrane preparations tested in each group is shown within the bar graph. Each membrane preparation was incubated with 40 nM [3 H]oxytocin, approximately equivalent to the peptide concentration required for half-maximal binding in membranes from estradiol-treated rabbits. The binding value for each membrane preparation was determined in duplicate; thus each binding value represents the mean ($\pm$ SEM) for six or more determinations ($n \geq 6$).

as well as the proteins of the contractile apparatus. Once induced by estradiol treatment, the increased concentration of specific [3 H]oxytocin uterine membrane binding sites are maintained for at least 4 days after termination of estrogen injections. Concurrently, the enhanced responsiveness of such uteri to oxytocin and methacholine (*in vitro*) is retained. The retention of uterine contractile responsiveness and of high concentrations of specific [3 H]oxytocin uterine binding units upon withdrawal of estrogen may be variously explained, depending upon whether or not tissue depots of previously injected estradiol serve to maintain the circulating levels of estradiol.[†] Independent of this specific issue, progesterone administration for 4 days after estradiol pretreatment markedly reduced the concentration of specific oxytocin binding sites to levels so low that quantitation was not possible. The fact that long-term progesterone treatment abolished the uterotonic response to oxytocin and concomitantly reduced specific oxytocin binding to undetectable levels is consistent with the idea that the specific oxytocin binding sites assayed indeed represent physiological receptors. The progesterone effect to reduce specific [3 H]oxytocin uterine binding activity in estradiol-pretreated rabbits can be demonstrated within 24 hr; in this case, progesterone treatment reduced the concentration (but not the affinity) of specific [3 H]oxytocin uterine binding sites.

The present results suggest that the regulatory effects of estrogen and progesterone on the *in vitro* responsiveness of the uterus to oxytocin are achieved, at least in part, by regulation of the number of oxytocin receptors in smooth muscle cells of the myometrium. Soloff (10) reported that a single dose of di-

[†] If the postulated "estrogen reservoir" does not in fact apply, then it would be necessary to postulate that either estrogen serves as a "trigger" to initiate oxytocin receptor synthesis or that oxytocin receptors, once synthesized, have a very long "half-life." The view that oxytocin receptors once synthesized are "stable" with a long "half-life" is not consistent with the actinomycin D results (Fig. 4).

ethylstilbestrol administered to rats castrated for 12 days increased both the affinity and total number of uterine [^3H]-oxytocin binding sites. The affinity change was apparent 6 hr after estrogen injection, whereas the increase in the number of uterine oxytocin binding sites, first detected at 12 hr, was marked only after 24 hr; the estrogen-induced increase in the number of oxytocin binding sites in rat uterus was apparent only if the number of sites was expressed in terms of total uterine weight rather than per mg of particulate protein, indicating that oxytocin receptors were increased to the same degree as other particulate protein components of the uterus. In contrast, our results with rabbits show that estrogen treatment produced a 3-fold increase in the number of specific oxytocin uterine binding sites expressed on a protein basis, without influence on the affinity of these sites for [^3H]oxytocin. Although there are obvious differences between our studies and those of Soloff, in terms of the species used as well as the time intervals of estrogen treatment, other factors may account for the reported differences in the effect of estrogens upon the affinity of oxytocin binding units. Thus in the present study, estrogen was administered to young rabbits in which endogenous estrogen is present (albeit at low levels), whereas Soloff used long-term castrated rats. Another difference, perhaps critical, is that we evaluated affinity by Scatchard analysis of the specific [^3H]oxytocin binding data, corrected for "nonspecific" [^3H]oxytocin binding, whereas Soloff applied the Scatchard plot to total [^3H]oxytocin binding, apparently on the assumption that "nonspecific" binding was negligible and could be ignored. If, as seems likely, the nonspecific fraction of total [^3H]oxytocin binding is relatively high in castrate uterus and decreases as the concentration of specific [^3H]oxytocin binding sites is increased by estrogen, this might be interpreted as an affinity increase that is more apparent than real. In subsequent studies on the ewe uterus (19) attention was given to the need for correcting for nonspecific oxytocin binding.

The present results strongly suggest that the concentration of oxytocin receptors in the rabbit uterus is controlled by sex steroid hormones, estrogens acting to increase and progesterone to decrease the concentration of membrane oxytocin receptors in smooth muscle cells. The detailed mechanisms involved in this action of the sex steroid remain to be definitively established, but our preliminary results with actinomycin D are consistent with the general view that these steroid hormones produce their effects by modification of gene activity and protein synthesis. Thus, administration of a sublethal concentration of actinomycin D to rabbits previously treated with estradiol markedly decreased uterine specific [^3H]oxytocin binding over a 24-hr period, to about the same extent as that produced by 24-hr treatment with progesterone. Although it cannot be unequivocally stated that the antibiotic directly inhibited nuclear RNA synthesis in uterine smooth muscle cells, the observed decrease in oxytocin binding is consistent with decreased oxytocin receptor synthesis. These results also suggest that oxytocin receptors, once synthesized, turn over within 24 hr so that maintenance of high levels of oxytocin receptors in the estrogen-dominated uterus requires continuous *de novo* synthesis of oxytocin receptor units. The finding that progesterone given in conjunction with actinomycin D over a 24-hr interval failed to produce an additive inhibitory effect may be taken to indicate that the progesterone effect to decrease the concentration of oxytocin receptors likewise involves the nuclear locus of smooth muscle cells in the uterus.

The physiological implications of these findings for human beings remain to be explored. In a number of species, including human beings, it has been reported that the progesterone-dominated uterus of early-mid pregnancy displays a relatively poor contractile response to oxytocin (1-8). This effect has been widely ascribed to a generalized decrease in uterine contractile activity, resulting from progesterone-induced alteration of membrane excitability in uterine smooth muscle cells (11, 12). Our finding that methacholine, but not oxytocin, retains its effectiveness as an *in vitro* contractile agent in the progesterone-dominated rabbit uterus, considered together with the [^3H]oxytocin binding data, indicates that progesterone-induced uterine sensitivity is due, in part, to an effect of progesterone to reduce the number of oxytocin receptors. If women resemble rabbits in this respect, the relative insensitivity of the gravid human uterus to oxytocin may also be due, in some part, to the lack of functional oxytocin receptors. As parturition approaches, the uterus becomes increasingly dominated by estrogens and the responsiveness of the gravid uterus to oxytocin increases greatly (6). The question of whether estrogen-induced synthesis of oxytocin receptors is a critical component in the development of this oxytocin sensitivity remains to be established.

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