

Nongenomic Inhibition of Oxytocin Binding by Progesterone in the Ovine Uterus¹

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ABSTRACT

Progesterone (P₄) has been reported to inhibit oxytocin (OT) binding to its receptor in isolated murine endometrial membranes. The purpose of the present research was to 1) examine the *in vivo* and *in vitro* effect of P₄ on the binding of OT to its receptor in the ovine endometrium and 2) determine whether the endometrial plasma membranes have high-affinity binding sites for P₄. Ovariectomized ewes were pretreated with a sequence of estradiol-17β (2 days) and P₄ (5 days) before being treated with estradiol-17β plus either vehicle (corn oil), P₄, or P₄ + mifepristone (RU 486) for 3 consecutive days. Treatment of ewes with 10 mg P₄/day for 3 days suppressed binding of OT ($P < 0.01$) compared with that of controls, whereas concomitant treatment with the progestin antagonist RU 486 (10 mg/day) blocked the effect of P₄. Similarly, incubation of endometrial plasma membranes with P₄ (5 ng/ml) inhibited binding of OT ($P < 0.05$), whereas this effect of P₄ was blocked by the presence of RU 486 (10 ng/ml). By radioreceptor assay, the endometrial plasma membranes were found to contain a high-affinity binding site for P₄ and the progestin agonist promegestone (K_d 1.2 × 10⁻⁹ and 1.74 × 10⁻¹⁰M, respectively). Incubation of endometrial plasma membranes with P₄ (5 ng/ml) significantly increased the concentration of progestin binding sites. Binding of labeled promegestone (R 5020) was competitively inhibited by excess unlabeled R 5020, P₄, RU 486, and OT but not by estradiol-17β, cortisol, testosterone, and arginine vasopressin. These data suggest a direct suppressive action of P₄ on the binding of OT to OT receptors in the ovine endometrial plasma membrane.

mechanisms of hormone action, oxytocin, progesterone, progestin receptor, uterus

INTRODUCTION

During the estrous cycle of the ewe, endometrial concentrations of oxytocin receptor (OTR) and, hence, oxytocin (OT) binding appear to be regulated by progesterone (P₄) and estrogen [1, 2]. Endometrial concentrations of OTR mRNA and protein are maximal beginning at proestrus, coincident with the presence of increased levels of estrogen receptor (ER) [3]. By contrast, concentrations of OTR are markedly reduced during the luteal phase of the cycle when there is an absence of nuclear P₄ receptor and low levels of ER in the luminal and superficial glandular epithelium. By use of ovariectomized (OVX) ewes treated with ovarian steroids to mimic cyclic changes in these hor-

mones, it has come to be generally accepted that P₄ and estradiol acting via nuclear receptors promote down- and up-regulation of endometrial OTR [4, 5].

In recent years, there has been increasing experimental evidence that steroids can act at the level of the plasma membrane in various species to evoke biological responses. As an example, P₄ has been found to bind to a membrane receptor in rat granulosa cells [6], *Xenopus laevis* oocytes [7], human sperm [8, 9], and seatrout ovaries [10]. More than a decade ago, it was reported that *in vitro* contractility of rat uterine segments was inhibited by P₄ and certain P₄ metabolites when added to the incubation media [11]. Because the inhibition occurred within 2 min of addition, it is doubtful the effect of the steroids was mediated via nuclear receptors. Particularly germane to the actions of ovarian steroids in regulating uterine OTR is the report by Grazzini et al. [12] indicating that P₄ in the rat uterus may be acting via a nongenomic pathway to inhibit binding of OT to its receptor. Similarly, in ewes subjected to chronic estradiol treatment during the estrous cycle to prolong luteal life span, the increased concentrations of P₄ may act, at least in part, by a nongenomic mechanism to limit OT binding in the endometrium [13].

The research presented herein was undertaken to determine whether P₄ can act at the level of the plasma membrane to alter OT binding in the ovine endometrium and, if so, whether there exists a high-affinity binding site for P₄ in the plasma membrane. In the event that membrane P₄ binding was found to exist, experiments were planned to determine whether specificity or concentration of P₄ binding sites were altered by progestins and other steroids.

MATERIALS AND METHODS

Experiment 1. OT Binding after *In Vivo* Exposure of Endometrium to P₄

To evaluate the *in vivo* effect of P₄ on the binding of OT in the endometrium as determined by OTR analysis, 11 OVX ewes were pretreated with subcutaneous (SC) injections of estradiol-17β (25 μg/day for 2 days) followed by P₄ (10 mg/day for 5 days). Ewes were then assigned randomly to immediately receive SC injections of 25 μg estradiol-17β plus the following steroids on each of 3 consecutive days: group 1 (n = 4), vehicle (corn oil); group 2 (n = 4), 10 mg P₄; group 3 (n = 3), 10 mg P₄ + 10 mg mifepristone (RU 486, a P₄ antagonist). On each treatment day, estradiol was administered in the morning to up-regulate and maintain OTR, and vehicle P₄ or P₄ + RU 486 was injected 12 h later. Endometrium removed via midventral laparotomy [14] 12 h after the final injection was quick-frozen in liquid N₂ and stored at -80°C until OTR assay (see assay details below).

All experimental procedures and protocols involving ewes were reviewed and performed in accordance with the Institutional Animal Care and Use Committee guidelines at Oregon State University.

Experiment 2. OT Binding after *In Vitro* Exposure of Endometrial Plasma Membranes to P₄

To evaluate the *in vitro* effect of P₄ on the binding of OT to its endometrial receptor, endometrium was removed via a midventral laparotomy from five OVX ewes subjected to the 10-day sequence of steroid

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treatments for control ewes as described above for experiment 1. Upon removal, the endometrial tissue was placed into cold saline (4°C) and transported to the laboratory. Tissue (1.5–3 gm) was added to 15 ml of cold buffer A (25 mM Tris-HCl, 0.25 M sucrose, pH 7.4) and homogenized by use of a Tekmar Tissumizer (Tekmar Co., Cincinnati, OH) (5-sec bursts five times with 30-sec pause between bursts). Subsequently, the crude homogenate was transferred to a Dounce tissue grinder (Wheaton Science Products, Millville, NJ) at 4°C and subjected to 10 strokes of the pestle to further homogenize remaining clumps of tissue. The homogenate was decanted into a centrifuge tube to which was added a 2-ml buffer rinse of the tissue grinder. The homogenate was then subjected to differential centrifugation to remove nuclei and mitochondria and finally centrifuged at $100\,000 \times g$ for 90 min (4°C) to acquire the membrane pellet. After discarding the supernatant, the membrane pellet was gently washed three times with 5 ml of buffer B (25 mM Tris-HCl, 0.01% NaN₃, pH 7.4) and the pellet then resuspended in 3 ml of the latter buffer. The resuspended membrane was centrifuged at $100 \times g$ to remove any membranous aggregates that failed to disperse upon resuspension. The supernatant was retained and analyzed for protein content by use of the BCA protein assay (Pierce Chemical Co., Rockford, IL). Aliquots of the membrane preparation (1 mg protein/ml) were adjusted to a total volume of 1.5 ml with 25 mM Tris-HCl, 0.01% NaN₃ and 15 mM EDTA (pH 7.4) for a total of three sets of duplicate tubes. Samples in duplicate received the following additions: group 1, vehicle (30 μ l absolute ethanol); group 2, P₄ to achieve a final concentration of 5 ng/ml + 15 μ l absolute ethanol; and group 3, 5 ng/ml P₄ + RU 486 at a final concentration of 10 ng/ml. P₄ and RU 486 were each dissolved in 15 μ l of absolute ethanol. The ethanol in each tube represented less than 2% of the final volume. All samples were incubated for 60 min at 25°C according to the methods of Grazzini et al. [15]. Subsequently, the samples were placed on ice to stop the reaction and centrifuged at $100\,000 \times g$ for 60 min (4°C) to recover the membrane pellet, which was resuspended in buffer B to a concentration of 1.5 mg protein/ml. Samples were immediately subjected to OTR assay.

Experiment 3. Specificity of P₄ and Promegestone Binding to Endometrial Plasma Membranes

This experiment consisted of two separate phases. In phase one, endometrium removed from OVX ewes (n = 5), subjected to the 10-day sequence of steroid treatments for control ewes as described in experiment 1, was homogenized and processed as in experiment 2 to isolate plasma membranes. The plasma membranes from each ewe were evaluated for presence of high-affinity progesterin binding sites by use of a radioreceptor exchange assay as described below. Results of this initial trial demonstrated the presence of high-affinity binding sites for P₄ and promegestone (R 5020, progesterin agonist) that were saturated with 8 nM of either ligand (see below). Therefore, a second trial was conducted to determine whether these high-affinity binding sites were specific for progestins. For this trial, endometrial plasma membranes were isolated from ewes (n = 5) as above. Duplicate aliquots of plasma membranes from each ewe were exposed to a saturating concentration of [³H]-R 5020 or the labeled ligand plus a 200-fold excess of one of the following unlabeled competitors: R 5020, P₄, RU 486, OT, estradiol-17 β , cortisol, testosterone, and arginine vasopressin. Data on binding of [³H]-R 5020 in the presence of each competitor are expressed as percentage of [³H]-R 5020 bound in the absence of the competitor.

Experiment 4. In Vitro Effect of P₄ on Membrane Progesterin Binding Sites

On the basis of the results of experiment 2, it appeared that P₄ could act at the level of the endometrial plasma membrane to alter binding of OT, whereas the results of experiment 3 were interpreted as evidence for the presence of high-affinity binding sites for this steroid in the membrane. Therefore, an experiment was conducted to quantify the concentration of P₄ binding sites after in vitro exposure of the membrane to P₄ and P₄ plus RU 486. An aliquot of endometrial plasma membranes isolated from OVX ewes (n = 4) that had been subjected to steroid treatment to up-regulate OTR as in experiment 1 was distributed to each of three tubes containing 1.5 ml of 25 mM Tris-HCl, 0.01% NaN₃ and 15 mM EDTA (pH 7.4) and to which was added vehicle, P₄ or P₄ + RU 486 as described above for experiment 2. Tubes were incubated for 1 h at room temperature (25°C). After incubation, the plasma membranes from each ewe were individually processed as for experiment 2, and the resulting pellet was resuspended in a membrane-diluting buffer (25 mM Tris-HCl, 0.01% NaN₃, pH 7.4) to a concentration of 1.5 mg protein/ml. Aliquots of resuspended membrane

were subjected to a radioreceptor exchange assay as described below by incubation with 8 nM of [³H]-R 5020 alone (total binding) or in the presence of a 200-fold excess of unlabeled R 5020 (to establish nonspecific binding). Concentration of specifically bound progesterin was determined by subtracting the nonspecifically bound progesterin from the total quantity that was bound.

OTR Assay

Endometrial tissue was evaluated for OTR binding by use of a modified procedure of Hazzard and Stormshak [14] adapted from original methods of Mirando et al. [16]. Specific binding of OT to its receptor was determined with [³H] OT (range of concentrations 0.625–10 nM; 44.5 Ci/mmol, New England Nuclear, Boston, MA) and labeled ligand plus a 200-fold excess of unlabeled OT to correct for nonspecific binding. Data from the equilibrium binding curve revealed that the population of receptors was saturated at an OT concentration of 5 nM. Thus, this saturating concentration was chosen for routine use in subsequent assays conducted to quantify tissue concentrations of OTRs. Realizing that this point was slightly greater than that determined by Hazzard and Stormshak [14], it still was within the range described by Vallet et al. [4] and thereby acceptable for use. Scatchard analysis of the saturation data revealed a K_d value of 1.01×10^{-10} M suggesting measurement of a single binding site with high affinity for OT.

Exchange Assay of Membrane Progesterin Binding Sites

Endometrial tissue collected from ewes was homogenized and subjected to differential centrifugation as described in experiment 2 to recover the plasma membranes. While gently vortexing, 100 μ l aliquots of membrane preparation resuspended in 25 mM Tris-HCl, 0.01% NaN₃ and pH 7.4 (1 mg/ml) were added to four tubes to permit individual analyses in duplicate. To two tubes was added [³H] P₄ (114.4 Ci/mmol, New England Nuclear) or [³H] R 5020 (84 Ci/mmol, New England Nuclear) in concentrations ranging from 0.5 to 16 nM. These tubes containing the sample and labeled ligand were utilized to estimate total binding of [³H] progesterin at each concentration included in the assay. To the remaining two tubes was added the labeled ligand (in 2.5 μ l absolute ethanol) plus a 200-fold excess of unlabeled ligand (in 2.5 μ l absolute ethanol) for the purpose of determining nonspecific binding. The volume of all tubes was adjusted to a total of 155 μ l by addition of 50 μ l of TBM buffer (25 mM Tris-HCl, 0.01% NaN₃, 0.2% BSA, 20mM MnCl₂, pH 7.4). All tubes were gently vortexed and then incubated for 18 h (4°C). Subsequently, all tubes were placed into an ice bath and to each was added 750 μ l TBM buffer (4°C) and 1 ml of pelleting buffer (25 mM Tris-HCl, 0.01% NaN₃, 40% [wt/vol] polyethylene glycol, pH 7.4). Tubes were vortexed and centrifuged at $2000 \times g$ for 15 min (4°C). The supernatant was decanted and the tubes inverted 10 min (4°C) to drain excess buffer. The pellet was resuspended in 1 ml each of TBM and pelleting buffer, vortexed, and centrifuged as above. The supernatant was decanted and the tubes again inverted to drain for 10 min (4°C). The pellet was resuspended in 500 μ l of 25 mM Tris-HCl, 0.01% NaN₃ (pH 7.4) and decanted into vials containing 5 ml of CytoScint (ICN Biomedicals, Inc., Irvine, CA) for counting. Vials were vortexed, shielded from light for 12 h, and counted. Specifically bound progesterin for each concentration used was determined by subtracting nonspecific binding from total binding. Data from the generated equilibrium binding isotherm revealed that the population of binding sites was saturated at a concentration of 8 nM P₄ and 8 nM R 5020. Scatchard analysis of the specifically bound progesterin revealed the presence of a high-affinity binding site for P₄ and R 5020 (K_d 1.2×10^{-9} and 1.74×10^{-10} M, respectively). Because of similarities in binding profiles and Scatchard analyses between P₄ and R 5020, the latter ligand was routinely used for measurement of binding sites as in experiment 4 above. It should be noted that an exchange assay, as the name implies, permits the measurement of both occupied as well as unoccupied sites.

Statistical Analysis

All data generated for experiments 1 through 4 were subjected to one-way ANOVA. Differences among means were tested for significance by use of the Least Significant Difference test.

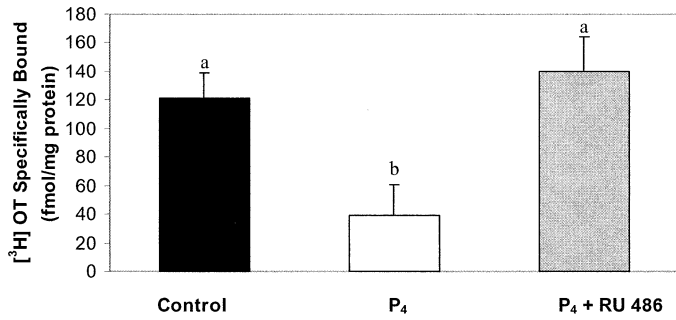


FIG. 1. Effect of treatment of OVX ewes (n = 3 or 4/group) with vehicle (control), P₄, and P₄ + RU 486 on endometrial concentrations of specifically bound OT. ^{a,b}Means ± SEM with different superscript letters differ (P < 0.01).

RESULTS

Experiments 1 and 2. OT Binding after Exposure of Endometrium or Its Plasma Membranes to P₄ In Vivo or In Vitro

Treatment of OVX ewes with 10 mg P₄/day for 3 days (experiment 1) significantly suppressed the quantity of available sites in the endometrium for OT binding (mean ± SEM; control, 121 ± 18 vs. P₄, 40 ± 21 fmol/mg protein; P < 0.01), whereas concomitant treatment with the antagonist RU 486 blocked the suppressive effect of P₄ (P₄ + RU 486, 140 ± 24 fmol/mg protein, compared with control, P > 0.05; Fig. 1).

Incubation of endometrial plasma membranes isolated from control ewes with P₄ reduced the binding of OT to its receptor as compared with that of controls (mean ± SEM; control, 273 ± 42 vs. P₄, 128 ± 33 fmol/mg protein; P < 0.05), whereas the presence of RU 486 in the incubation medium inhibited the suppressive effect of P₄ (P₄ + RU 486, 245 ± 36 fmol/mg protein, compared with controls, P > 0.05; Fig. 2). The in vitro effects of P₄ and P₄ + RU 486 on the binding of OT to its endometrial receptor were similar to those observed when P₄ and the antagonist were administered to ewes.

Experiment 3. Specificity of P₄ and R 5020 Binding to Endometrial Plasma Membranes

Analysis of endometrial plasma membranes for presence of progestin binding sites by using both [³H]-P₄ and [³H]-R 5020 as ligands revealed by Scatchard analysis the presence of a high-affinity binding site for these ligands. The

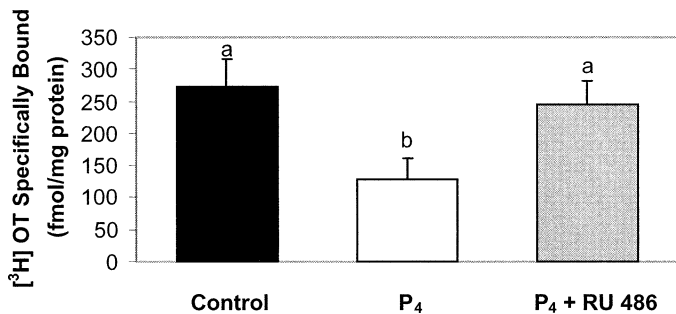


FIG. 2. [³H]-OT specifically bound to isolated endometrial plasma membranes after in vitro exposure of membranes to vehicle (control), P₄, or P₄ + RU 486. Each bar represents the mean of individual measurements of five ewes. ^{a,b}Means ± SEM with different superscript letters differ (P < 0.05).

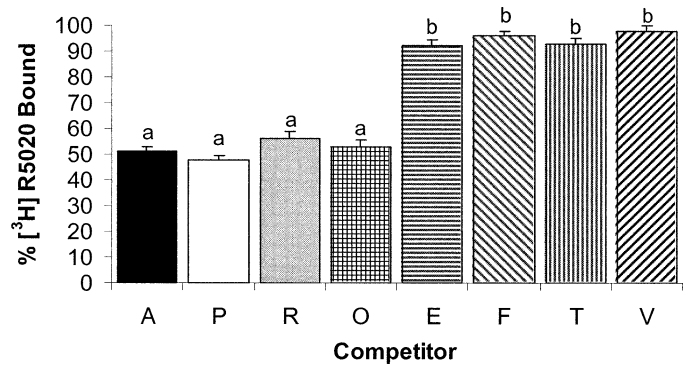


FIG. 3. Effect of various steroid and peptide competitors on the binding of [³H]-R 5020 to isolated endometrial plasma membranes from five ewes as determined by radioreceptor exchange assay. Each bar represents the mean percentage of the maximum bound [³H]-R 5020 determined for each ewe. The excess unlabeled competitors tested were A (R 5020), P (P₄), R (RU 486), O (oxytocin), E (estradiol-17β), F (cortisol), T (testosterone), and V (arginine vasopressin). ^{a,b}Means ± SEM with different superscript letters differ (P < 0.05).

calculated K_d values by use of labeled P₄ and R 5020 (see above section on progestin binding assay) are comparable with those reported for membrane P₄ binding sites by others [10, 12].

To test the specificity of progestin binding, endometrial plasma membranes containing an enriched population of OTR were isolated from OVX ewes (n = 5) and binding of labeled R 5020 determined in the presence of a 200-fold excess of unlabeled peptide or steroid competitor. The results of this competition experiment are depicted in Figure 3 in terms of mean ± SEM percentage of maximum [³H]-R 5020 specifically bound. A 200-fold excess of unlabeled R 5020, P₄, RU 486, and OT significantly suppressed binding of [³H]-R 5020 (mean ± SEM, 51.2 ± 1.8, 48.2 ± 1.7, 56.4 ± 2.9, 53.4 ± 2.6%, respectively) compared with that of estradiol-17β, cortisol, testosterone, and arginine vasopressin (mean ± SEM, 91.6 ± 2.4, 95.4 ± 1.7, 93.2 ± 2.0, 96.3 ± 1.8%, respectively). Thus, out of the eight competitors utilized, inhibition of binding of labeled R 5020 occurred in response to two progestins, a progestin antagonist and OT.

Experiment 4. In Vitro Effect of P₄ on Membrane Progestin Binding Sites

The effects of incubating plasma membranes with P₄ or P₄ plus RU 486 on mean concentrations of progestin binding sites are depicted in Figure 4. Although the binding of

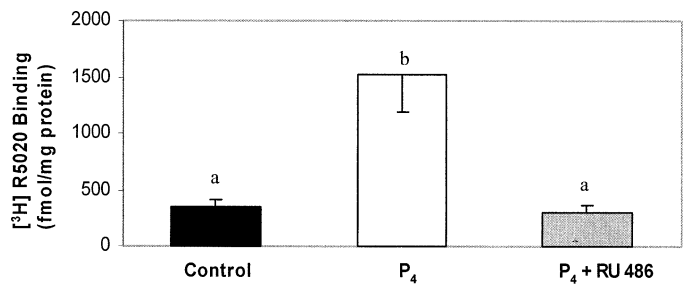


FIG. 4. [³H]-R 5020 specifically bound to isolated endometrial plasma membranes after incubation of membranes with vehicle (control), P₄, or P₄ + RU 486. Specifically bound ligand was determined for each of four ewes per group. ^{a,b}Means ± SEM with different superscript letters differ (P < 0.05).

R 5020 was similar between control membranes and those exposed to P_4 plus RU 486 (mean \pm SEM; control, 347 ± 64 vs. P_4 + RU 486, 297 ± 74 fmol/mg protein; $P > 0.05$), there was a marked increase in the concentration of progesterin binding sites in membranes that were incubated with P_4 (P_4 , 1529 ± 343 fmol/mg protein, compared with control, $P < 0.05$). Incubation with P_4 nearly quadrupled the number of measured binding sites over those present in the control membranes.

DISCUSSION

Administration of P_4 to estrogen-primed OVX ewes in the present study suppressed the binding of OT to its endometrial receptor, which is consistent with data reported by others on P_4 -induced changes in OTR concentration [4, 5, 13, 17]. One might logically conclude that the primary mode of action of P_4 is via regulation of transcription of the OTR gene. Indeed, exposure of the ovine uterus to P_4 has been shown to essentially prevent expression of the OTR gene [13]. Further, concurrent administration of the antagonist RU 486 with P_4 blocked the apparent suppressive effect of P_4 on endometrial OTR and, hence, binding of OT. Because RU 486 antagonizes binding of P_4 to the nuclear P_4 receptor [18], these data support the above premise regarding the genomic mode of action of P_4 .

Exposure of isolated endometrial plasma membranes from estrogen-primed OVX ewes to P_4 for 60 min during in vitro incubation (experiment 2) suppressed the binding of OT to its receptor. The suppressive in vitro effect of P_4 on OT binding to the membrane OTR was reversed by concurrent incubation with RU 486. The pattern of changes in bound OT among membranes exposed in vitro to P_4 and P_4 + RU 486 compared with that of controls was similar to that observed after in vivo treatment of ewes with these steroids. Although the patterns of responses to P_4 and antagonist are similar, the membrane concentration of OTR (experiment 2) appears to be greater than in the endometrial tissue analyzed from ewes in experiment 1. This may be attributed to differences among animals, a more homogeneous tissue preparation in experiment 2, or loss of receptors because of freezing and thawing of tissue in experiment 1. Nevertheless, our data support the hypothesis that P_4 , in addition to regulating available OTR via a genomic mechanism, might also act at the level of the endometrial plasma membrane to interfere with the binding of OT to its receptor in the ewe. The ability of P_4 to act at the level of endometrial plasma membrane of a ruminant to interfere with binding of OT is supported by the recent report of Bogacki et al. [19]. These investigators provide experimental evidence that P_4 can act in a nongenomic manner to attenuate binding of OT to its receptor in the bovine endometrium. The observed nongenomic action of P_4 is also similar to that reported by Grazzini et al. [12], who demonstrated a suppressive effect of physiologically relevant concentrations of P_4 on OT binding to OTR in rat uterine membranes and Chinese hamster ovary (CHO) cells stably transfected with the rat OTR expression vector.

In contrast to our in vitro data and those of Grazzini et al. [12] (i.e., data that were acquired by using a near physiological concentration of P_4), Burger et al. [20] also reported a similar effect of P_4 on binding of OT to human OTR transfected into CHO cells, but this occurred only when using a nonphysiological concentration of the steroid. This nongenomic and rapid action of P_4 to inhibit a uterine response was documented earlier [11]. In this latter study, in vitro contractility of rat uterine segments was inhibited

within 2 min of addition of the steroid to the medium, a response too rapid to be promoted via the classical genomic mechanism of action of the steroid.

The in vitro effects of P_4 and P_4 plus RU 486 on OT binding suggest the possibility that the ovine endometrial membranes contain a progesterin binding site. By use of a radioreceptor exchange assay with [3 H]- P_4 and [3 H]-R 5020, the existence of a high-affinity binding site for these progestins has been identified. Results of an experiment to determine the specificity of progesterin binding revealed that excess R 5020, P_4 , and RU 486 could compete with labeled R 5020 for the binding site. The fact that both P_4 and R 5020 apparently bind to the same site with almost identical affinities, as based on the K_d values, and that RU 486 can compete with R 5020 for binding, suggests that the site may be comparable with the ligand binding site of the nuclear P_4 receptor. There is some basis for this proposition. A 60-kDa membrane P_4 binding protein isolated from rat granulosa cells was found to contain a sequence of amino acids identical to the ligand-binding domain of the nuclear P_4 receptor [6]. On the other hand, Zhu et al. [10] have identified a putative 40-kDa serpentine progesterin binding protein in the plasma membrane of seatrout ovaries that possesses no homology with the nuclear steroid receptors. In our competition analysis, steroids such as estradiol-17 β , cortisol, and testosterone were not competitive with R 5020 for binding. Surprisingly, the nanopeptide OT was as effective as the progestins in competing for the binding site. However, arginine vasopressin with a molecular weight and secondary structure comparable with OT was not competitive. These data suggest that a protein closely associated with the OTR or the OTR itself contains the binding site for P_4 . The fact that OT can compete with R 5020 should not be interpreted to signify that both molecules bind to the same intramolecular site. It is conceivable that a progesterin bound to the OTR or to a closely associated protein may cause a conformational change in the OTR, thus preventing the binding of OT. Likewise, the binding of OT is apparently able to negatively affect the binding of progestins, perhaps by a similar mechanism. Regardless, data from the present experiments are the first to demonstrate that a high-affinity progesterin binding site exists in the endometrial plasma membrane of the sheep as has been detected in other species.

Results of experiment 4 were interpreted as suggesting that exposure of the endometrial plasma membrane to P_4 markedly increases the number of available sites for the steroid as determined by radioreceptor exchange assay. The precise reason for the observed increase in progesterin binding sites is not known with certainty. However, it may be speculated that the increase in number of progesterin binding sites reflects a scenario in which the binding site is closely associated with the monomeric units of the OTR. Evidence has recently been presented that the serpentine OTR can form homo- and heterodimers [21]. Dimerization of OTR is essential for binding to OT; therefore, binding of P_4 to the monomeric units of this receptor, or a closely associated protein, may prevent dimerization and binding of OT as discussed above and result in an apparent increase in measurable P_4 binding sites. If this phenomenon occurs, then competitive inhibition of progesterin binding by RU 486 would conceivably allow dimerization of OTR and binding of OT with an apparent reduction in measurable progesterin binding sites as occurred in experiment 4. It is doubtful that exposure of the plasma membranes to progestins simply results in supersaturation of the plasma membrane with ste-

roid to increase the number of apparent binding sites. Evidence against such an effect of progestins is provided by the results of experiment 3, which demonstrate progestin binding to be specific.

The results of studies reported herein are interpreted to suggest that P_4 in the ewe may act not only via a genomic pathway but also at the level of the plasma membrane to regulate the response of endometrium to OT. It may be questioned whether there is any biological basis for the nongenomic action of this steroid in the ewe. Because the ovine conceptus is a source of P_4 [22], it is conceivable that the steroid synthesized by the developing conceptus acts at a local level to regulate the functional activity of the endometrium. From a physiological standpoint, potential competition between OT and P_4 for the endometrial plasma membrane binding site for P_4 , as demonstrated in this study, might conceivably occur as luteolysis progresses during the estrous cycle. At this time, luteal secretion of P_4 in the ewe is waning, whereas systemic levels of OT are increasing [23].

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