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RAPID RECOVERY OF NUCLEAR ESTROGEN RECEPTOR AND OXYTOCIN RECEPTOR IN THE OVINE UTERUS FOLLOWING PROGESTERONE WITHDRAWAL

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Summary—We previously showed that progesterone rapidly down regulates nuclear estrogen receptor (Re) in the estrogen-primed rodent uterus. We have now extended these studies to test the response of the Re system in sheep uterus to progesterone withdrawal. Since the estrogen–Re complex is believed to regulate hormone-dependent gene expression, it was of interest to determine whether withdrawal of progesterone under constant estrogen stimulation would lead to the recovery of nuclear Re levels and estrogen action, i.e. oxytocin receptor (R_{OT}) synthesis. Ovariectomized ewes were primed with estradiol-17β and serum steroid levels were maintained by constant infusion of estradiol (0.5 μg/h) and progesterone (500 μg/h) for 5 days. The animals were anesthetized with fluothane/O_2, and uterine samples were excised 1 h before and 3, 6 and 12 h after progesterone withdrawal. Estradiol infusion was continued during the experiment in order to maintain estrogen levels at a steady state (14 pg/ml plasma). Re, R_{OT} and progesterone receptor (Rp) were measured in endometrium and myometrium using standard ^{3}H-hormone binding assays. Following progesterone withdrawal, the nuclear Re concentration increased in both uterine compartments, and the nuclear Re level was correlated significantly with the R_{OT} concentration in the membrane fraction of both uterine tissues (endometrium, $r = 0.79$; myometrium, $r = 0.86$). Although cytosol Re rose between 6 and 12 h in the endometrium, cytosol Re levels remained unchanged in myometrium. Cytosol Rp appeared to increase in endometrium but not in myometrium. Uterine tissue sampled from a control animal before stopping the progesterone infusion revealed that the observed changes in receptor concentration following progesterone withdrawal were not due to regional differences in receptor levels. These results demonstrate that the recovery of nuclear Re in the ovine endometrium and myometrium following progesterone withdrawal represents a selective effect on Re retention in the nucleus rather than on cytosol Re availability or Re activation which was controlled by constant estrogen infusion. Thus, these results are consistent with the hypothesis that progesterone induces an Re regulatory factor which acts to down regulate nuclear Re, and that the activity of this factor diminishes rapidly after progesterone withdrawal.

INTRODUCTION

One hormone may act to alter the expression of another hormone, and the modulation of estrogen action by progesterone in female target tissues is a classic example of this phenomenon [1]. However, the underlying mechanism responsible for progesterone-induced changes in estrogen action is unknown. It may be pertinent that progesterone down regulates the estrogen receptor (Re) system [2], and the site of progesterone action appears to reside in the target cell nucleus [3, 4]. Recently, we noted that progesterone mediates a selective loss of the occupied form of Re from the uterine cell nucleus [5], and our studies with the estrogen-primed rodent uterus suggested that progesterone promotes nuclear Re turnover by a process involving a receptor regulatory factor which may function to release Re from nuclear acceptor sites and thereby modify estrogen-dependent gene expression [6, 7].

We have now turned our attention to the recovery response of the Re system in the progesterone-dominated uterus upon hormone withdrawal. Thus, we have measured Re, oxytocin receptor (R_{OT}) and progesterone receptor (Rp) responses at 3, 6 and 12 h after progesterone withdrawal in the sheep uterus. With this large animal model, we were able to obtain multiple uterine samples from individual animals and to compare receptor responses of the endometrium and myometrium with time after progesterone deprivation. Since the nuclear Re–estrogen complex appears to mediate estrogen-dependent gene expression, it was our objective to determine whether progesterone withdrawal under steady state estrogen exposure would lead to the recovery of nuclear Re levels and estrogen-sensitive events such as R_{OT} and Rp synthesis.

EXPERIMENTAL

Chemicals and buffers

Labeled steroids (90 Ci/mmol) and [2,4,5,7-^{3}H]estradiol-17β [1,2,6,7-^{3}H]progesterone

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(97 Ci/mmol), were obtained from New England Nuclear (Boston, MA) and stored in ethanol (100 μ Ci/ml) at −10°C. [Tyrosyl-^{3}H]oxytocin (20 Ci/mmol) was generously supplied by Dr J. S. Roberts, Worcester Foundation for Experimental Biology (Shrewsbury, MA). Radioinert steroids were from Sigma Chemical Co. (St Louis, MO) and unlabeled oxytocin was from Parke-Davis (Detroit, MI). Other chemicals were obtained from commercial sources at reagent grade or better.

Scintillation counting solution was toluene–Triton X-100 (2:1, v/v) with 5 g diphenyloxazole (PPO) and 50 mg 1,4-*bis*[2-(5-phenyloxazolyl)]-benzene (POPOP) per l. Buffers used in Re and Rp assays: A_{30} (50 mM Tris–HCl, 1 mM EDTA, 12 mM monothioglycerol, 30% glycerol (v/v), pH 7.5); TG (10 mM Tris–HCl, 10% glycerol (v/v), pH 7.5); B (10 mM Tris–HCl, 1 mM EDTA, 12 mM monothioglycerol, pH 7.5); buffered saline (10 mM Tris–HCl, 150 mM NaCl); and Dextran-charcoal (0.5 g Norite A (Sigma), 50 mg Dextran-70 (Pharmacia) per 100 ml buffer B). TMG buffer used in the R_{OT} assay was 50 mM Tris–maleate, 5 mM $MnCl_2$, 1% (w/v) gelatin, pH 7.6.

Animal preparation

Sheep, ovariectomized at least 2 weeks previously, were primed by the intravenous infusion of estradiol-17β (0.5 μg/h for 24 h) using a constant infusion pump (Harvard Apparatus Co, Model No. 600-910/920). In the presence of the continued infusion of estradiol-17β, a constant infusion of progesterone (500 μg/h) for 5 days was begun. This infusion rate created plasma levels of progesterone approximating those found during the luteal phase of the ovine estrous cycle [8]. Plasma estradiol and progesterone levels were measured by specific RIA as described elsewhere [9]. On day 6 of infusion, each animal was anesthetized with fluothane/O_2, and uterine samples were taken 1 h before and 3, 6 and 12 h after termination of progesterone infusion. Tissue samples were removed starting at the tubal end of the uterine horn, and four serial samples were obtained per horn in each case taking care to avoid disturbing the blood supply to the remaining uterine tissue. Tissues were chilled on ice immediately after excision, and the endometrium was dissected away from myometrium in preparation for receptor analysis. A control animal was used to determine whether regional differences in the distribution of hormone receptors existed throughout the uterine horn, and similar uterine biopsies were removed immediately prior to stopping the 5-day progesterone infusion in the presence of steady-state estrogen.

Receptor assays

Myometrial and endometrial tissues were each minced and homogenized in buffer A_{30} (1:8, w/v) with a Polytron Pt-10 (Brinkman Instruments, Westbury, NY). Care was taken to maintain the samples at 0–4°C at all times. Cytoplasmic and nuclear fractions were separated by centrifugation of the homogenate at 800 *g* for 10 min. The low-speed cytoplasmic fraction was centrifuged at high speed (170,000 *g*) for 30 min to prepare cytosol and a high-speed pellet. The high-speed pellet containing the membrane fraction was homogenized in TMG buffer for R_{OT} assay as described by Soloff [10] and modified by Pearlmutter and Soloff [11]. Cytosol and nuclear KCl extract were prepared for Re and Rp assay as detailed elsewhere [9]. These assays measured unoccupied cytosol Re, total cytosol Rp and total nuclear Re. Protein and DNA were determined according to Lowry *et al.*[12] and Burton[13], respectively, using BSA and calf thymus DNA as standards. Statistical treatment of the results is described in the text.

RESULTS

Measurement of serum estradiol and progesterone by RIA established that estradiol remained at steady state levels (14 ± 0.9 pg/ml, mean ± SEM) and that progesterone declined from 3.7 ng/ml to 0.3 ng/ml during the first 3 h of progesterone withdrawal (Fig. 1).

To study the effect of progesterone on Re, R_{OT} and Rp response, we measured the content of these receptors in endometrium and myometrium before and during progesterone withdrawal. The receptor results from 3 ewes are presented in Fig. 2. Prior to progesterone withdrawal, some differences in receptor concentrations were observed comparing endometrium and myometrium in individual animals. After progesterone withdrawal, cytosol Re increased in the endometrium of 2 out of 3 cases, but no cytosol Re response was noted in the myometrium. Similarly, cytosol Rp appeared to increase somewhat in the endometrium (3/3 animals) but not in myometrium during the 12 h withdrawal period.

Nuclear Re and R_{OT} increased in the endometrium and myometrium of all 3 animals following progesterone withdrawal (Fig. 2). There was a highly

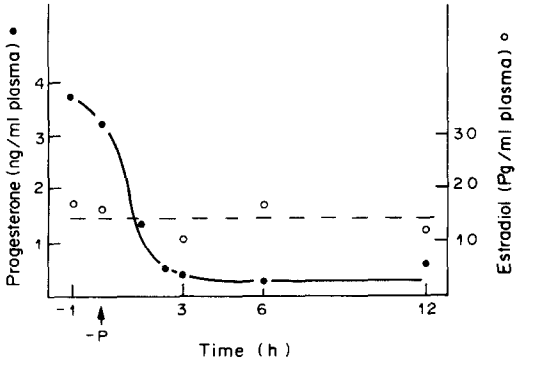

Fig. 1. Plasma estradiol and progesterone levels in peripheral blood samples taken during the experimental period. Progesterone withdrawal (-P) occurred at 0 h, and steroids were measured by specific RIA.

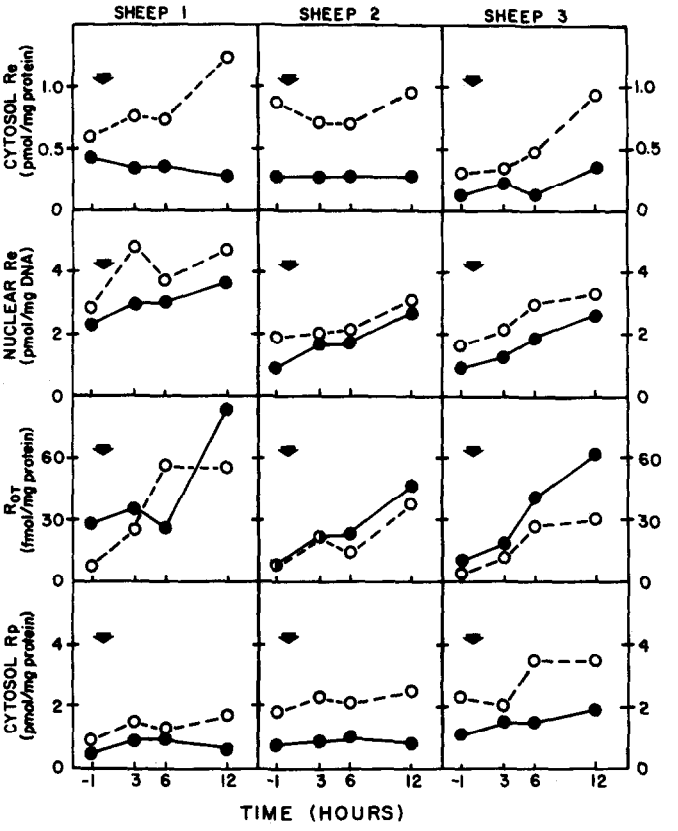

Fig. 2. Time course of uterine receptor responses to progesterone withdrawal in 3 sheep. Progesterone withdrawal (arrow) occurred at 0 h. Endometrium = open circles, dashed line. Myometrium = closed circles, solid line.

significant ($P < 0.001$) correlation between nuclear Re and R_{OT} concentrations in both endometrium ($r = 0.79, n = 12$) and myometrium ($r = 0.86, n = 12$) (Fig. 3). However, there was no such correlation in a control uterus which was sampled in the same manner during maintenance of progesterone infusion (endometrium, $r = 0.33$, $n = 8$; myometrium, $r = 0.06, n = 8$). Although cytosol Rp levels appeared to increase somewhat in the endometrial compartment after progesterone withdrawal, there was no significant correlation between nuclear Re and cytosol Rp responses.

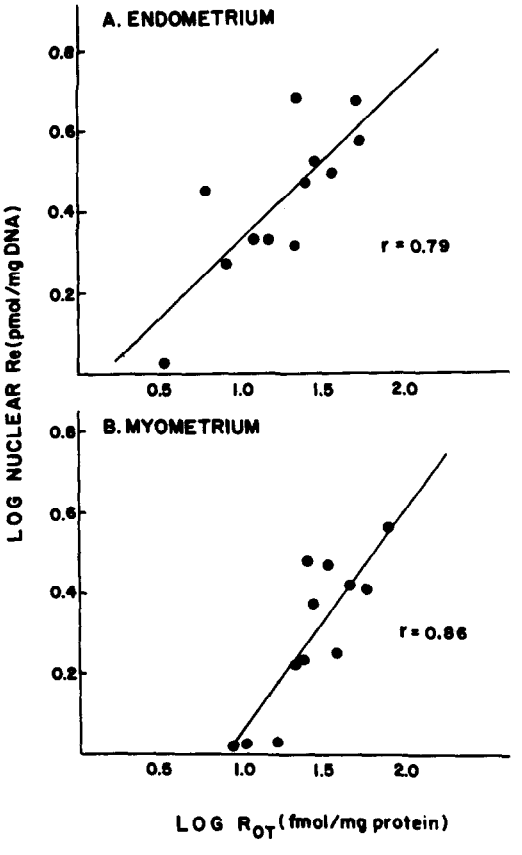

Fig. 3. Correlation of nuclear Re and R_{OT} levels in sheep endometrium (panel A) and myometrium (panel B). The correlation coefficient (r) was obtained by regression analysis. $P < 0.001$ for both endometrium and myometrium.

DISCUSSION

Estrogen and progesterone exert opposing effects on Re, R_{OT} and Rp availability in the uterus [2]. Estrogen stimulates macromolecular synthesis leading to the accumulation of Rp, R_{OT} and Re sites in the uterine target cell [14–16]. In contrast, progesterone down regulates these receptors by processes that are not fully understood [17–19]. Thus during

pregnancy, progesterone appears to down regulate Re, Rp and R_{OT} levels until the time of parturition [20, 21].

The present results demonstrate a rapid (6–12 h) recovery of nuclear Re in sheep endometrium and myometrium after progesterone withdrawal that is associated with an estrogen-induced response, i.e. R_{OT} production. A similar relationship was found between nuclear Re and R_{OT} synthesis in pregnant guinea-pig and rat myometrium prior to parturition when plasma estrogen rises relative to progesterone [20, 21]. However, in the latter studies both hormones changed at the same time so that it was not clear whether Re recovery resulted from a rise in estrogen or a fall in progesterone. By maintaining estrogen at steady state levels during progesterone withdrawal, we established previously that the removal of the inhibitory effect of progesterone results in the rapid accumulation of nuclear Re and induction of estrogen-dependent responses [2]. We have used that same experimental paradigm in the present study to show a rapid nuclear Re recovery in the ovine uterus. It is pertinent that the nuclear Re response to progesterone withdrawal observed in both the endometrium and myometrium is likely the result of a selective effect on Re retention in the target cell nucleus rather than a change in either cytosol Re availability (Fig. 2) or cytosol Re depletion which was controlled by constant estrogen infusion [22]. Additionally, the occupied form of Re is chronically down regulated in the progesterone-dominated hamster uterus, and it is the occupied Re that recovers rapidly in the nuclear fraction following progesterone withdrawal (W. W. Leavitt and W. C. Okulicz, unpublished observations).

Recent immunocytochemical evidence [23] and cell fractionation studies [24] suggest that the majority of Re may be localized in the nucleus of intact target cells. Thus, Re distribution between cytosol and nucleus may be an artifact of the cell fractionation procedure. If true, then we may be measuring loosely-bound (cytosol) and tightly-bound (nuclear) populations of Re. In this case, the present observations would indicate that progesterone acts only on the tightly-bound Re population. It sould be noted that our results provide further evidence for a selective effect of progesterone on the biologically active form of Re, i.e. nuclear-bound Re.

The present findings coupled with the previous demonstration that elevation of serum progesterone relative to estrogen suppresses nuclear Re in hamster[9], rat [25], and sheep uterus [26, 27] supports the hypothesis that progesterone may induce a factor which regulates nuclear Re retention [2]. It will be of interest to determine whether progesterone supports the production of an Re regulatory factor in the sheep uterus which functions to suppress nuclear Re retention. If so, the present results suggest that the activity of this factor decays rapidly upon progesterone withdrawal. It is conceivable that Re regulatory factor, or its activity, changes during pregnancy, and such an alteration might relate to the action of other hormones at term. For example, oxytocin and prostaglandins are two important myometrial stimulators which appear to be involved in triggering parturition in certain species such as the human. Fuchs *et al.*[28] proposed that the up regulation of R_{OT} in response to estrogen action in the human myometrium and prostaglandin production and release in response to fetal oxytocin action on the decidua could both contribute importantly to the initiation of labor. Since serum estrogen and progesterone levels don't change before parturition in the human, it is possible that a decline in Re regulatory factor activity could be involved in the up regulation of R_{OT} in the human uterus at term.

It has not escaped our attention that receptor regulation may play an important role in the control of corpus luteum function during the sheep estrous cycle. Oxytocin-induced secretion of prostaglandin $F_{2\alpha}$ from the ovine endometrium is believed to be a significant event leading to luteolysis in this species [29, 30], and a potential explanation for oxytocin action in this regard may be related to R_{OT} recovery at the end of the luteal phase. Such a mechanism would involve an increase in endometrial sensitivity to oxytocin action based on the recovery of nuclear Re and an attendant increase in endometrial R_{OT} levels. Recent findings with the human [31] and monkey [32] demonstrate that progesterone secretion becomes pulsatile during the luteal phase of the menstrual cycle with a decrease in the frequency of pulses as the luteal phase progresses. It is tempting to speculate that an increase in the interval between progesterone pulses might account for Re and R_{OT} recovery and the secretion of prostaglandins. Additional study is needed to test this novel hypothesis.

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