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Progesterone Down-Regulation of Nuclear Estrogen Receptor: A Fundamental Mechanism in Birds and Mammals

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Progesterone is known to selectively down-regulate nuclear estrogen receptor (Re) in the mammalian uterus, and this process is functionally related to embryo retention. It is unclear if this mechanism is operative in the chick oviduct, where egg retention does not occur. We investigated the regulation of Re by progesterone in a mammalian model (proestrous hamster uterus) and an avian model (DES-primed chick oviduct), under the same assay conditions, in an effort to compare progesterone action in viviparous and oviparous species. Nuclear and cytosol estrogen receptor were measured with an assay employing pyridoxal 5′-phosphate (PLP). The PLP assay has the advantage of allowing exchange at low temperature, which results in improved receptor recovery, especially from the nuclear fraction. Parallel studies were done under two different hormonal settings, estrogen primed and estrogen + progesterone primed. Experiments were: (1) response of Re to acute progesterone treatment (5 mg progesterone, 4 hr) in estrogen-primed preparation, (2) time course of the Re down-regulation response (4, 8, and 12 hr after progesterone treatment), and (3) recovery of Re after progesterone withdrawal in estrogen + progesterone-primed preparation. Chick oviduct contained little cytosol Re (0.96 ± 0.32 pmol/g tissue) compared to hamster uterus (4.27 ± 0.15 pmol/g tissue), and progesterone treatment had no effect on cytosol Re levels in either species. Nuclear Re levels were similar for chick oviduct (2.68 ± 0.14 pmol/g tissue) and hamster uterus (2.64 ± 0.14 pmol/g tissue). Progesterone treatment reduced nuclear Re levels in both the hamster uterus and chick oviduct to about 50% of control levels. In the chick oviduct, down-regulation was transient, as evidenced by complete recovery of nuclear Re to control levels by 12 hr after progesterone administration. In the estrogen + progesterone-primed chick oviduct, nuclear Re increased within 6 hr after progesterone withdrawal and approached maximal levels by 12 hr. These data indicate that progesterone rapidly and selectively down-regulates the nuclear form of Re in the chick oviduct as in the hamster uterus. Thus, the regulation of Re by progesterone appears to be similar in the mammalian uterus and the chick oviduct, despite the basic differences in reproductive strategy between birds and mammals. This suggests that progesterone down-regulation of nuclear Re is a fundamental mechanism of progesterone action which is conserved in birds and mammals and is not restricted to organisms which exhibit egg or embryo retention.

Progesterone is generally considered a modulator of estrogen action in vertebrates (Leavitt *et al.*, 1983; Selcer and Leavitt, 1988). However, whether progesterone action is the same in all types of vertebrates remains to be determined. For mammals, progesterone is essential to the preparation of the uterus for implantation and for the maintenance of pregnancy. During pregnancy, progesterone increases the compliance and decreases the contractility of the myometrium, facilitating a prolonged period of embryo retention (Heap, 1972; Finn and Porter, 1975).

Progesterone action in mammals is generally antagonistic to that of estrogen (Brenner and West, 1975; Clark *et al.*, 1977; Nissenson *et al.*, 1978). The mechanism of this antagonism to estrogen is known to in-

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volve the rapid down-regulation of nuclear estrogen receptor (Re) by a process that is not fully understood (Leavitt *et al.*, 1983). We were interested in whether this mechanism of progesterone action is widely distributed among vertebrates, or if it is limited to species which exhibit prolonged egg or embryo retention.

Birds provide a marked contrast to mammals in terms of the length of egg retention after ovulation. The egg passes through the avian reproductive tract rapidly, and eggs are retained only for sufficient time to allow the deposition of the egg white proteins and the shell (Follett, 1984). In birds, progesterone action can be either antagonistic or synergistic with estrogen action (Oka and Schimke, 1969a, b; Mester and Baulieu, 1984), depending on the time that the hormones are administered relative to one another and on the amounts of estrogen and progesterone given.

The apparent dual nature of progesterone action in birds may extend to its effect on Re as well. Progesterone treatment has been demonstrated to either decrease or increase Re levels in secondary-stimulated chick oviducts, depending on the time the tissue is taken after progesterone administration (Seaver *et al.*, 1980; Sutherland *et al.*, 1980). A synergistic effect of estrogen and progesterone on Re levels has not been observed in mammals.

Understanding of the similarities and differences in progesterone action between birds and mammals requires that comparisons be made using comparable hormonal regimens for animals, and employing identical assay methods. We sought information of the regulation of Re by progesterone in a mammalian model (proestrous hamster uterus) and an avian model (DES-primed chick oviduct) using a pyridoxal 5′-phosphate (PLP) assay (Okulicz *et al.*, 1983). This assay has the advantage of allowing exchange of ligand at low temperature, and is the method of choice for studies of progesterone effects on Re in the hamster uterus (Leavitt and Okulicz, 1985). It was of interest to determine if the PLP method was applicable to the chick oviduct system. This is the first report of progesterone effects on Re in mammalian and avian reproductive tracts using identical assay conditions.

Our objective was to examine the regulation of Re by progesterone in the chick oviduct, using the hamster uterus as a reference. Two different hormonal settings were used, estrogen primed and estrogen + progesterone primed. Experiments were: (1) response of Re to acute progesterone treatment in estrogen-primed preparation (chick and hamster), (2) time course of the Re down-regulation response (chick), and (3) recovery of Re after progesterone withdrawal in the estrogen + progesterone primed-preparation (chick).

MATERIALS AND METHODS

Reagents. [^{3}H]Estradiol (17 β-estradiol, 1,2,6,7-^{3}H(N)-; 99 Ci/mmol) was obtained from DuPont/New England Nuclear (Boston, MA) and radioinert steroids were obtained from Sigma Chemical Co. (St. Louis, MO). Barbital buffer contained 20 m*M* sodium barbital (Mallincrodt Chemical Co., St. Louis, MO) and 5 m*M* dithiothreitol (Chemical Dynamics, South Plainfield, NJ), pH 8.0. Tris/glycerol (TG) buffer contained 50 m*M* Tris–HCl, 1 m*M* EDTA, 12 m*M* monothioglycerol, and 10% glycerol (vol:vol), pH 7.5. Phosphate-buffered saline (PBS) was 0.14 *M* NaCl, 1.5 m*M* KH_2PO_4, 8 m*M* NaH_2PO_4, and 2.7 m*M* KCl, pH 7.4. Dextran-coated charcoal (DCC) contained 0.5 g Norit A (Sigma) and 50 mg Dextran-70 (Pharmacia, Piscataway, NJ) in 100 ml of Tris–HCl and 1 m*M* EDTA, pH 7.5. Liquid scintillation cocktail was toluene–Triton X-100 (2:1 vol:vol) containing 5 g of diphenyloxazole (POP) and 50 mg of 1,4 bis[2-(5-phenyloxazole)]-benzene (POPOP) per liter.

Animals. Adult female Syrian golden hamsters were obtained from Harlan Sprague–Dawley (Indianapolis, IN) at 90–100 g (about 6 weeks of age) and housed under a 14:10 light:dark cycle at 22°. Regular 4-day estrous cycles were verified by checking for the presence of a postovulatory vaginal discharge (Day 1). All hamsters used in this study were taken during proestrus (Day 4). Progesterone (5 mg in 0.3 cc corn oil) or vehicle was administered at 0800 hr on the morning of Day 4. Animals were killed by cervical dislocation and the uteri were rapidly excised, cleaned of fat and mes-

entery, and placed into ice-cold PBS. Uteri were blotted dry, weighed, minced with scissors, and diluted with appropriate buffer (1:6 wt:vol).

Immature female chickens (Rhode Island Red) were obtained from a local hatchery at 3 weeks of age and housed under a 14:10 light:dark cycle at 22°. For Experiments 1 and 2, chicks were primed with daily sc injections of diethylstilbestrol (DES, 2.5 mg in 0.3 cc corn oil) for at least 10 consecutive days. Progesterone was administered in the morning, and DES was not given on the day of progesterone treatment. Chicks were killed by CO_2 asphyxiation, and the magnum portion of the oviduct was rapidly removed and placed into ice-cold PBS. Tissue was blotted dry, weighed, and minced in an appropriate volume of buffer (1:5 wt:vol).

For Experiment 3, chicks were implanted with 17β-estradiol pellets (25 mg; Innovative Research Inc, Toledo, OH) for 14 days, at which time they were treated with progesterone implants [Silastic tubing (Dow Corning, Midland, MI) 3 cm in length, packed with crystalline progesterone]. The progesterone implants were withdrawn after 3 days, and chicks were sacrificed at 0, 6, and 12 hr after implant removal. Tissues were removed and processed as above. The Re content of tissues was analyzed by the PLP method of Okulicz *et al.* (1983) as outlined below.

Preparation of cytosol and nuclear extract. Tissues were homogenized (1:5 wt:vol for chick, 1:6 wt:vol for hamster) in either barbital buffer (PLP method) or TG buffer (for ammonium sulfate preparations) with a Brinkman Instruments (Westbury, NY) polytron homogenizer at a rheostat setting of 6 using three bursts of 5 sec each. The homogenate was centrifuged at 1000 *g* for 10 min at 4°. The crude nuclear pellet was washed twice in barbital buffer and resuspended in 10 m*M* PLP (in barbital buffer, pH 8.0) for 1 hr. The PLP suspension was transferred to polyallomer tubes and centrifuged for 30 min at 170,000 *g* at 4°. The high-speed supernatant from the nuclear extract was diluted 1:1 (vol:vol) with 10 m*M* PLP and used as the nuclear extract for assay. The low-speed supernatant from the nuclear centrifugation was transferred to polyallomer tubes and centrifuged at 170,000 *g* for 1 hr at 4°. The resulting high-speed supernatant was diluted 1:1 (vol:vol) with 20 m*M* PLP in barbital buffer, and this constituted the cytosol preparation. DNA content was determined by diphenylamine reaction (Burton, 1956).

Measurement of nuclear and cytosol Re. Aliquots (300 μl) of cytosol and nuclear extract were incubated in a total volume of 500 μl with varying concentrations of [^{3}H]estradiol (0.10 to 3 n*M*) for determination of total binding. A parallel set of samples was incubated with radioinert estradiol (4 μ*M*) for determination of nonspecific binding. Samples were incubated for 16–20 hr at 4°. After incubation of cytosol or nuclear samples, free steroid was removed by addition of 500 μl DCC for 3 min, followed by centrifugation at 1500 *g* for 3 min. Sodium borohydride (50 μl of 120 m*M*) was added to 500-μl aliquots of the sample to blanch the yellow coloration of the PLP and prevent color quenching during scintillation counting. Specific binding was calculated as total binding minus nonspecific binding and plotted according to the method of Scatchard (1949).

Data analysis. Analysis of variance and Student's *t* tests were used, where appropriate, for detecting differences among treatment means. Student–Newman–Keul's test was used for a posteriori comparisons of means. Statistics were performed using the Statistical Analysis System (SAS Institute Inc., 1985). Probabilities of less than 0.05 were considered significant. Throughout the text, means are given ±1 SEM.

RESULTS

Measurement of cytosol Re with PLP (Experiment 1). Figure 1 shows Scatchard plots of [^{3}H]estradiol binding in cytosol from control and progesterone-treated hamster uterus and chick oviduct. There was no difference in the slope or position of

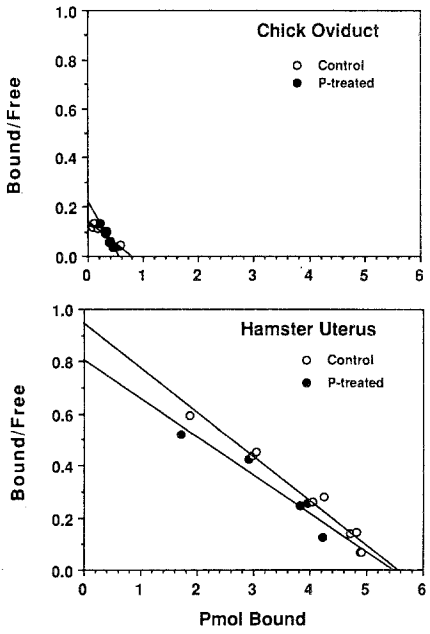

FIG. 1. Scatchard plots of specific [^{3}H]estradiol binding to cytosol PLP extracts of chick oviduct and hamster uterus comparing control and progesterone (P)-treated animals (Experiment 1). Animals were injected with 5 mg P and sacrificed 4 hr later.

the Scatchard plots comparing control and progesterone-treated cytosols from either hamster uterus or chick oviduct.

Figure 2 shows the mean values of multiple determinations of Re detected in control and progesterone-treated (4 hr) hamster uterine and chick oviduct cytosols. There was substantially less Re found in chick oviduct cytosol (0.96 ± 0.32 pmol/g tissue) as compared to hamster uterus cytosol (4.27 ± 0.15 pmol/g tissue). These data are in agreement with previous measurements of cytosol Re in hamster uterus (Leavitt and Okulicz, 1985) and estrogen-primed chick oviduct (Seaver *et al.*, 1980). No significant differences in levels of cytosol Re were detected between control and progesterone-treated hamster uterus (t = 1.27, 4 *df*, $P > 0.05$) or chick oviduct (t = 0.03, 4 *df*, $P > 0.05$).

Since cytosol Re levels in the chick appeared to be low, we tried using TG buffer, both alone and combined with ammonium sulfate fractionation (40%), in an effort to improve recovery of cytosol Re. As Fig. 3 shows, there is a substantial amount of [^{3}H]estradiol-binding material in crude cytosol prepared using TG buffer. However, there was a considerable amount of variation in the amount of binding detected using the TG buffer and, in several assays, the data could not be analyzed by Scatchard plot because a significant regression equation could not be resolved from the data. It does not appear that this [^{3}H]estradiol-binding material is estrogen receptor because it does not precipitate with 40% ammonium sulfate fractionation. Furthermore, the low Re values obtained after the ammonium sulfate fractionation are in agreement with those determined by the PLP method. Because of the low cytosol Re values, Experiments 2 and 3 did not include determinations of cytosol Re.

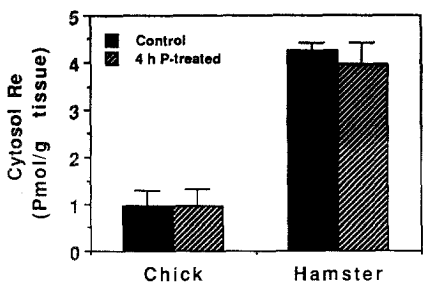

FIG. 2. Cytosol Re levels for control and progesterone-treated chick oviduct and hamster uterus (Experiment 1). Bars indicate the mean of three separate determinations, brackets indicate 1 SEM. There were no significant differences in Re between control and progesterone-treated animals for either hamster uterus or chick oviduct.

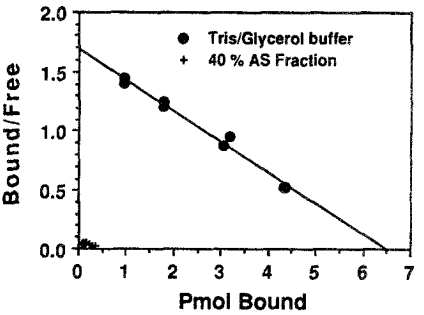

FIG. 3. Scatchard plots of specific [^{3}H]estradiol binding to chick oviduct cytosol extracts prepared in Tris–glycerol buffer with and without 40% ammonium sulfate (AS) fractionation.

Measurement of nuclear Re with PLP (Experiment 1). Figure 4 illustrates Scatchard plots of specific [^{3}H]estradiol binding to nuclear PLP extracts derived from hamster uterus and chick oviduct, with and without 4 hr of progesterone treatment. Progesterone had no effect on the slope of the Scatchard plot for either species, but lowered the x-intercept for both hamster uterus and chick oviduct to a level about 50% of control. This indicates that the effect of progesterone in both the hamster uterus and the chick oviduct was a reduction in the total number of nuclear Re-binding sites, rather than a change in the binding affinity of the Re.

Figure 5 shows the mean values of multiple determinations of nuclear Re for control and progesterone-treated hamster uterus and chick oviduct. Uterine tissue of

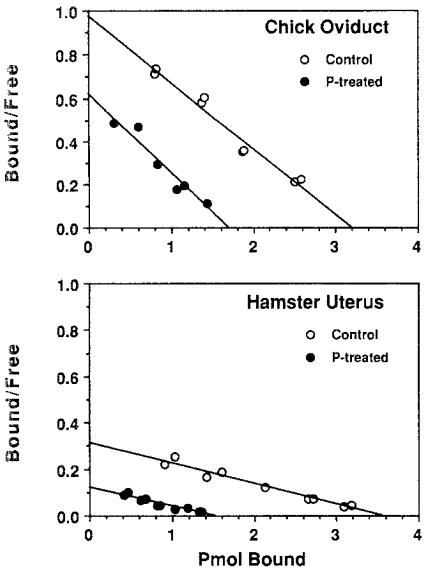

FIG. 4. Scatchard plots of specific [^{3}H]estradiol binding to nuclear PLP extracts of chick oviduct and hamster uterus comparing control and progesterone (P)-treated animals (Experiment 1). Animals were injected with 5 mg P and sacrificed 4 hr later.

control hamsters averaged 2.64 ± 0.14 pmol nuclear Re per gram of tissue. This value corresponds well with data obtained by Leavitt and Okulicz (1985) for proestrus hamster uterine tissue using the same assay method. Progesterone treatment decreased the mean nuclear Re value to 60% of the control value. Student's *t* test indicated that the difference in nuclear Re between control and progesterone-treated hamsters was statistically significant (t = 9.68, 6 *df*, $P < 0.05$).

Nuclear extract from the DES-primed chick oviduct averaged 2.68 ± 0.14 pmol nuclear Re per gram of tissue. In order to calculate the number of sites per cell, the data were adjusted for DNA content and it was assumed that there were 2.3 pg DNA/cell (Best-Belpomme *et al.*, 1975). Control animals averaged 3032 nuclear Re sites per cell. As with hamster uterus, chick oviduct nuclear Re levels were markedly reduced at 4 hr after progesterone administration (1668 sites per cell; statistically significant; t = 16.00, 4 *df*, $P < 0.05$).

Time course (Experiment 2). The time course of Re down-regulation was studied after 4, 8, and 12 hr of progesterone treatment. Figure 6 shows the relative response of Re at the time periods studied. Nuclear RE was reduced to 55% of control levels at 4 hr after progesterone treatment, it increased to 83% of control values at 8 hr, and exceeded (130%) control values by 12 hr. Thus, progesterone-induced down-regulation of nuclear Re was temporary.

Recovery of nuclear Re after progesterone withdrawal (Experiment 3). Figure 7 il-

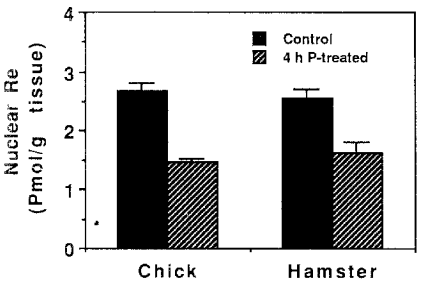

FIG. 5. Nuclear Re levels for control and progesterone-treated chick oviduct and hamster uterus (Experiment 1). Bars indicate the mean of three separate determinations, brackets indicate 1 SEM. The difference in Re between control and progesterone-treated animals was significant for both hamster uterus and chick oviduct (see text for statistics).

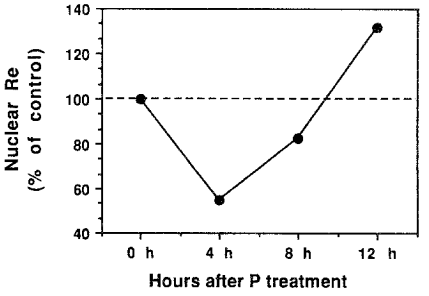

FIG. 6. Time course of the nuclear Re down-regulation response after a single progesterone treatment (Experiment 2). Data are expressed as a percentage of control animals. Values represent the mean of three separate determinations at all time periods except 12 hr, for which there were two determinations. Means ± SEM (pmol/g tissue) are as follows: 4 hr, 1.46 ± 0.14; 8 hr, 2.21 ± 0.16; and 12 hr, 3.526 ± 0.62.

lustrates the recovery of nuclear Re after progesterone withdrawal from estrogen + progesterone-primed animals. Nuclear Re relative to the progesterone-intact control animals increased 39% at 6 hr and threefold by 12 hr after progesterone removal. The amount of nuclear Re at 12 hr (2.8 pmol/g tissue) approximated the nuclear Re levels observed in DES-stimulated animals (Fig. 5), indicating that recovery was complete by 12 hr after progesterone withdrawal.

DISCUSSION

The present results demonstrate that progesterone down-regulates nuclear Re levels in DES-primed chick oviduct and proestrus hamster uterus, with no apparent effect on cytosol Re in either species. For both animal models, the reduction in nuclear Re attributable to progesterone action is about 50% and the response is quite rapid (<4 hr). Furthermore, we show that the effect of progesterone in the chick oviduct is reversible, with the nuclear Re levels recovering as early as 6 hr after withdrawal of progesterone.

It is known that the occupied form of the steroid hormone receptor mediates hormone action in the target cell nucleus. The mechanism by which estrogen elicits specific responses involves steroid binding to a specific receptor protein and for both mammalian (Anderson *et al.*, 1974) and avian (Mulvihill and Palmiter, 1976) systems, estrogen action has been shown to be dependent on the nuclear retention of receptor. Furthermore, recent studies indicate that the majority of estrogen receptor is localized in the nucleus and that cytosol receptor may be an artifact of tissue preparation (Walters, 1985). Receptor-binding sites in the nuclear chromatin, termed acceptor sites, appear to be responsible for the nuclear retention of receptor, and the receptor–acceptor site interaction is believed to be the transducer of the hormonal signal (Leavitt *et al.*, 1983). Our data on hamster uterus and chick oviduct indicate that progesterone antagonizes estrogen action by selectively down-regulating nuclear Re and thereby interrupting the transduction of the hormonal signal.

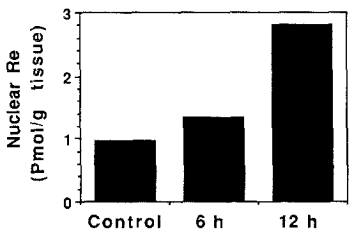

FIG. 7. Response of nuclear Re to progesterone withdrawal from estrogen + progesterone-primed chicks (Experiment 3). Bars indicate the mean of two separate determinations. Means ± SEM (pmol/g tissue) are as follows: control (0 hr), 0.97 ± 0.05; 6 hr, 1.34 ± 0.17; and 12 hr, 2.80 ± 0.07.

Progesterone effects on hamster uterine Re are well characterized (Leavitt and Okulicz, 1985; Leavitt, 1985), and our results agree with previously published reports on down-regulation of nuclear Re in proestrus hamster uterus (Leavitt and Okulicz, 1985). Progesterone rapidly and selectively reduces nuclear Re in the hamster uterus but has no short-term effect on cytosol Re (Evans *et al.*, 1980; this study). The decrease in nuclear Re in hamster uterus is noticable after only 2–4 hr of progesterone treatment, and it has been demonstrated that it is the occupied form of the nuclear Re which is selectively down-regulated (Okulicz *et al.*, 1981). Additionally, it is the occupied form of the nuclear Re which shows decreases during the progesterone-dominated stages of the estrous cycle and pregnancy (Leavitt and Okulicz, 1985). Thus, the mechanism of progesterone action in the hamster uterus appears to involve a selective effect on the retention or processing of the occupied form of the nuclear receptor (Leavitt *et al.*, 1987). In hamster decidual cell cultures, progesterone stimulates turnover of preexisting nuclear

Re within 3 hr and inhibits synthesis (replenishment) of new Re subsequent to the enhancement of Re degradation (Leavitt and Takeda, 1986). Progesterone appears to induce or activate a nuclear factor (termed ReRF) that rapidly blocks acceptor sites, and this way accounts for the decrease in receptor retention (Leavitt *et al.*, 1987).

Our results confirm the utility of the PLP assay procedure for studies of Re regulation in the chick oviduct. Previous studies of progesterone effects on Re in chick oviduct employed nuclear exchange assays done at elevated temperature (Seaver *et al.*, 1980; Sutherland *et al.*, 1980). Under these conditions, we found that the occupied form of the Re is unstable (Okulicz *et al.*, 1983), and thus the PLP assay was developed to maximize recovery of the occupied form of the receptor. The PLP assay has several advantages in studies of receptor regulation. First, PLP efficiently extracts nuclear receptor, thus eliminating the need for high salt extraction. Second, PLP stabilizes receptor and reduces degradation during preparation and assay. Third, PLP promotes dissociation of bound ligand from receptor, which allows assays to be performed at low temperature. To our knowledge, this is the first use of the PLP assay in a nonmammalian Re system.

Previous studies concerning progesterone regulation of Re in the chick oviduct used animals which had been estrogen primed, withdrawn, and then stimulated again with estrogen (secondary stimulation). Under this secondary stimulation regimen, the second administration of estrogen results in a fourfold increase in nuclear Re during the first 18 hr after estrogen administration (Sutherland *et al.*, 1980). Progesterone treatment at this time results in reduced nuclear Re levels within 2–6 hr as compared with controls (Seaver *et al.*, 1980; Sutherland *et al.*, 1980). However, under these conditions, the effect of progesterone is most likely attributed to the blockade of Re up-regulation by estrogen and not the down-regulation of preexisting Re.

Our paradigm involved long-term DES-primed chicks (primary stimulation) in which nuclear Re levels were fully up-regulated throughout the experiment. This is evidenced by the close concordance in nuclear Re levels between control chicks at 12, 18, and 24 hr after DES administration (corresponding to 4, 8, and 12 hr after progesterone administration in experimental chicks). Furthermore, the levels of nuclear Re we detected in control animals (≈3000 sites per cell) are consistent with the maximum levels reported by Seaver *et al.* (1980) after secondary estrogen stimulation. Under primary estrogen stimulation, we observed a reduction in nuclear Re attributable to progesterone of 45% at 4 hr after progesterone treatment. Under the primary stimulation paradigm, the blockade of Re up-regulation is not the cause of this reduction in Re. Rather, our data show that progesterone acts to down-regulate preexisting Re in the chick oviduct.

In mammals, the down-regulation of nuclear Re is reversible, and nuclear Re rapidly recovers when progesterone is withdrawn in the presence of estrogen (Leavitt, 1985; Leavitt *et al.*, 1985). The present study shows that the progesterone effect on nuclear Re in the chick oviduct is also reversible. Nuclear Re in the chick oviduct recovered from both acute and chronic progesterone down-regulation under the influence of DES and estradiol, respectively. Following acute progesterone treatment, there was an indication that nuclear Re levels overshoot (by 30%) those of control animals at 12 hr after progesterone administration. Similar findings were reported by Seaver *et al.* (1980) and Sutherland *et al.* (1980) under secondary stimulation.

We found no significant effect of progesterone on cytosol Re using the PLP assay. Furthermore, we detected very low levels of

cytosol Re under our DES injection regimen. Seaver *et al.* (1980) reported very low levels of Re in the cytosol of chicks under secondary estrogen stimulation, and they found no effect of progesterone on cytosol Re. However, Sutherland *et al.* (1980) reported that progesterone decreased cytosol Re in chick oviduct to the same extent that it decreased nuclear Re. Three different binders of [^{3}H]estradiol have been reported in chick oviduct cytosol (Smith and Taylor, 1971; Ruh and Toft, 1984), and at least one of these is not Re (Ruh and Toft, 1984). Thus, it is possible that we measured a different form of estradiol binder than the one studied by Sutherland *et al.* (1980), and that the discrepancy in the two data sets is a result of differing effects of progesterone on the two binders. Since we detected very little cytosol Re using the PLP method, we tried a TG buffer, with and without ammonium sulfate precipitation, in an effort to further characterize the cytosol estrogen binder. Using TG buffer, there was a high capacity (8 pmol) [^{3}H]estradiol binder present in the cytosol, but ammonium sulfate precipitation failed to recover this binding material indicating that it was not Re. Ruh and Toft (1984) reported the existence of a high capacity, nonspecific steroid binder from chick cytosol termed sex steroid binder (SSB). They indicated that [^{3}H]estradiol binding to SSB was highly variable unless measured in the presence of glycerol, which appeared to stabilize the binding activity somewhat. Thus, the [^{3}H]estradiol binder we observed in the presence of TG buffer was probably SSB, while the low levels of estradiol binding detected after ammonium sulfate precipitation or by the PLP method appear to represent cytosol Re.

It is of some importance that the progesterone effect on Re is similar in both mammalian and avian systems, because the mode of reproduction is very different in these two groups. Mammals have a reproductive pattern which is specialized to provide an environment for the developing embryo. Multiple embryos are usually produced concurrently and the length of time that these embryos are retained is often considerable [e.g., the 16 day gestation period of hamsters is the shortest among eutherian mammals (Rowlands and Weir, 1984)]. In contrast, birds have a reproductive pattern which is specialized to produce heavily yolk-laden cleidoic eggs. Eggs in a given clutch are arranged in a hierarchical fashion, and rapid processing of eggs (often less than 24 hr) facilitates the synchronization of incubation and feeding events (Follet, 1984). The general pattern of progesterone secretion also differs between birds and mammals. In mammals, there is a surge of progesterone secretion by the follicles just prior to ovulation, and a high level of progesterone secretion by the corpus luteum after ovulation. When pregnancy occurs, progesterone is secreted continuously throughout the gestation period by the corpus luteum of pregnancy and/or by the placenta (Heap, 1972). In birds, there is a single period of progesterone secretion by the follicles, occurring about 6 hr prior to ovulation, no corpus luteum is formed after ovulation, and there is little progesterone secretion until 6 hr prior to the next ovulation (Furr *et al.*, 1973; Shodono *et al.*, 1975; Etches and Cunningham, 1976). That the effect of progesterone action on nuclear Re is identical between the most highly specialized viviparous vertebrates (mammals) and the most committed of oviparous vertebrates (birds) suggests that down-regulation of nuclear Re is a fundamental and conservative mechanism of progesterone action. Furthermore, it appears that this mechanism is not restricted to species which exhibit prolonged egg or embryo retention. Information on the regulation of Re by progesterone in lower vertebrates is necessary to establish the generality of the down-regulation process.

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