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Subcellular Compartmentalization of the Progesterone Receptor in Cat Uteri Following the Acute Administration of Progesterone[1]

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ABSTRACT

Cytosol and nuclear progesterone receptors in the cat uterus were measured by Scatchard analysis to determine the relationships between dose of progesterone administered and the time following administration with the content of receptor in these two cellular compartments. Cats were ovariectomized, treated for 7 days with estradiol and then injected via the saphenous vein with progesterone. One uterine horn was removed prior to, and the other uterine horn after the injection of progesterone. The amount of cytosol receptor translocated was found to be dose-dependent over the range of 0–200 μg of progesterone. A maximum of 40% of the cytosol receptor was depleted even when the amount of progesterone injected was increased. In non-estradiol-primed animals it was also found that approximately 40% of the cytosol receptor was depleted following a progesterone injection. Within 3 h of the injection of 300 μg of progesterone, the cytosol and nuclear receptor levels had returned to preinjection values. A second administration of progesterone at 1 or 3 h after the first injection of progesterone caused a partial depletion of the cytosol receptor and an increase in nuclear progesterone receptor concentration. These data suggest that the translocation of cytosol receptor and the appearance of nuclear receptor is dose-dependent until approximately 40% of the cytosol receptor is depleted following a single injection of progesterone, that the retention of nuclear receptor after an acute injection of progesterone is of short duration (<1 h), and that the replenishment of cytosol receptor is complete within 3 h.

INTRODUCTION

Progesterone (P) has an immediate anti-estrogenic effect on the uterine glands in cats, which is then followed by a unique progestational response (Boomsma et al., 1982). This progestational response in the uterus includes hyperplasia, hypertrophy and glycogen synthesis and storage. With prolonged P treatment (7–10 days), the glycogen deposits disappear. Presumably, the biological responses induced by P are mediated via the P receptor system.

The chronic administration of physiological doses of P to estradiol-primed, ovariectomized-adrenalectomized cats results in the total depletion of the P receptor within 10 days even though estradiol (E_2) treatment is continued (Boomsma et al., 1982). Since it is known that the continuous presence of P is necessary in most species to maintain pregnancy and that the presence of P receptor is required for the hormone response, this data was surprising. This may, however, only reflect our failure to completely understand the control of the P receptor system.

Although less representative of the physiological situation, additional information may be gained about the control of the P receptor system from studies where acute doses of P are given. Using the cat as a model system, we have begun to study the receptor fluxes observed following the i.v. injection of a bolus of P. Here we report on the movement of P receptor between the cytosol and nuclear compartment following the acute administration of P in variable physiological doses, and on the return to control levels of the cytosol and nuclear receptor over a period of time. We also monitored the peripheral plasma levels of P by radioimmunoassay (RIA) and compared the changes in hormone level to the levels of receptor.

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MATERIALS AND METHODS

Animals

Domestic cats (∿3.2 kg), bilaterally ovariectomized for at least 4 weeks, were treated with E_2 for 7 days by means of a tubular Silastic implant (1.0 cm, Dow Corning No. 601–331) placed subcutaneously in the midscapular region prior to the administration of P. Previous studies have shown that this results in a systemic level of E_2 of approximately 30 pg/ml (Verhage et al., 1979; Bareither and Verhage, 1980). Progesterone, in 2% ethanol-0.9% NaCl, was given i.v. via the saphenous vein.

Animals were anesthetized with ketamine hydrochloride (i.m.) and unilaterally hysterectomized prior to the administration of P. The contralateral uterine horn was removed at times up to 12 h following P administration. The animals were then given a lethal dose of sodium pentobarbital (i.v.).

Preparation of Tissue

Immediately after removal, the uterus was placed in Hank's buffered saline solution at room temperature and quickly trimmed of excess fat and mesentery. The tissue was then placed in ice cold buffer and immediately prepared for the receptor assays.

Receptor Assays

Tissue was homogenized in 4 vol buffer A (0.5 M sucrose, 10 mM Tris-HCl, 2 mM MgCl, pH 7.2) and centrifuged at 2000 × *g* for 10 min at 4°C, as previously described (Verhage et al., 1979). All subsequent steps were carried out at 0–4°C except where otherwise noted. The supernatant (6–8 ml) was incubated for 10 min with a pellet prepared from 5 ml of dextran-coated charcoal (0.5% Norit A and 0.05% Dextran T-70 in 1.5 mM EDTA and 10 mM Tris-HCl pH 7.4 [DCC]) in order to remove endogeneous steroids, then centrifuged at 2500 × *g* for 10 min and the resulting supernatant was centrifuged at 105,000 × *g* for 1 h. The supernatant was used for cytosol receptor determinations.

The crude nuclear pellet from the first centrifugation was washed twice by gentle rehomogenization in buffer B (buffer A + 30% glycerol), poured through organza and centrifuged at 12,000 × *g* after each wash, and resuspended in buffer B.

Aliquots (300 μl) of the cytosol or nuclear fractions were added to two parallel series of tubes, one containing the labeled steroid and the other containing the same concentration of the labeled steroid plus a 200-fold excess of unlabeled steroid. The ligand used in the cytosol assay was [^{3}H]P, and [17α-methyl-^{3}H] promegestone (R5020) was used to assay the nuclear receptor. In addition, all cytosol tubes contained a 200-fold higher concentration of cortisol than [^{3}H]P to minimize the binding of P to corticosteroid binding globulin. Cytosols were incubated at 0°C for 2 h and nuclei at 22°C for 4 h (Verhage et al., 1979).

Bound and free steroid in the cytosol was separated by adding 300 μl DCC to each tube. Following incubation for 5 min at 0°C the tubes were centrifuged at 2000 × *g* for 10 min and the radioactivity in the supernatant was determined. Nuclear incubations were terminated by centrifugation and the pellets were washed once with 1 ml of buffer C (buffer A + 0.2% Triton X-100). The pellets were then solubilized in 0.1 N NaOH at 90°C and counted in scintillation fluid composed of toluene, Triton X-100, and PPO-POPOP (RPI Scintillator) in a ratio of 2000:1000:126. The counting efficiency was approximately 34%.

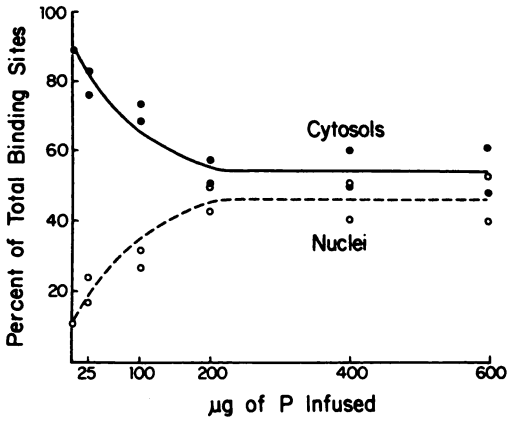

FIG. 1. Dose-dependent subcellular distribution of PcR in E_2-primed animals before, and 10 min after, the acute administration of P. The percentage of binding sites in the cytosol and nuclear compartment before the administration of P represents the arithmetic mean of eight determinations. Individual data points are shown for the determinations from tissue exposed to P.

Serum Progesterone Determinations

Venous blood was collected at the time of tissue removal, allowed to clot at room temperature for 1 h and then centrifuged. The serum was collected and stored at −20°C until the time of assay. Progesterone was measured by radioimmunoassay (Verhage et al., 1979).

Other Analytical Methods

Protein was measured by the method of Lowry et al. (1951) and DNA was assayed by the method of Burton (1956). The dissociation constants and number of binding sites were determined by the method of Scatchard (1949).

Steroids

[1,2,6,7-^{3}H] Progesterone (96.5 Ci/mmol), [17α-methyl-^{3}H] promegestone (R5020) (86.0 Ci/mmol) and unlabeled R5020 were obtained from the New England Nuclear Corp. (Boston, MA). Unlabeled P and cortisol were obtained from Sigma Chemical Co. (St. Louis, MO).

RESULTS

Dose-Dependent Translocation

Ten E_2-primed animals were given various doses (25–600 μg) of P to determine whether

the depletion of PcR and the accumulation of PnR was directly correlated with the amount of P injected. Each animal was unilaterally hysterectomized prior to the i.v. injection of P and 10 min after the P injection the contralateral uterine horn was surgically removed. The percentage of total P receptor (cytosol and nuclear) in the uterus 10 min after P administration in these ten determinations was 83.9 ± 2.1% (mean ± SEM) of that found before infusion. Approximately 90% of the total specific binding was found in the cytosol compartment in uterine cells obtained from E_2-primed animals (Fig. 1). Measurement of receptor levels 10 min after the administration of P demonstrated that there was a dose-dependent depletion of PcR. The maximum amount of depletion was approximately 40% of that measured in E_2-primed animals. The maximum depletion of PcR was attained after administration of 200 μg of P; a further 2- or 3-fold increase in the amount of P administered did not cause additional depletion of the PcR. Concurrent with the depletion of PcR, there was a dose-dependent increase in PnR. The plasma levels of P had peaked by 2 min after administration, and at all times the levels were reflective of the dose administered (Fig. 2).

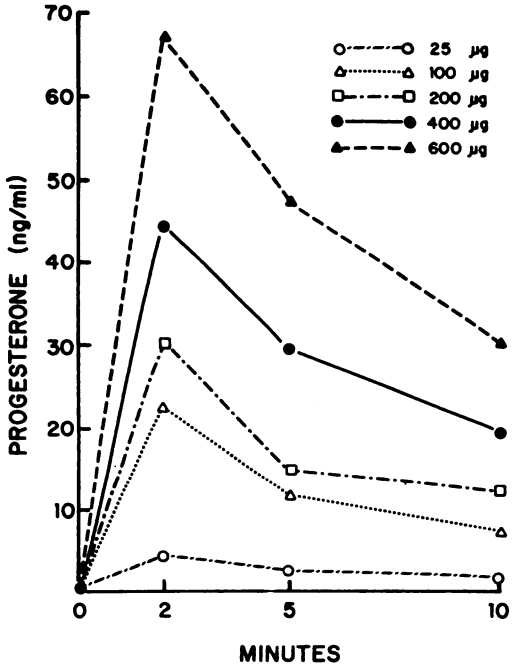

FIG. 2. Serum P concentrations determined by RIA for the animals whose subcellular receptor compartmentalization is shown in Fig. 1. Each value represents the arithmetic mean of the individual determinations from two animals.

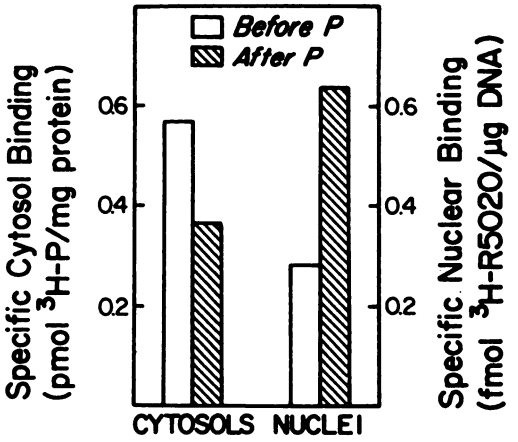

FIG. 3. Subcellular distribution of PcR and PnR in ovariectomized, non-E_2-primed animals before and 10 min after an i.v. injection of 400 μg P. Each bar represents the arithmetic mean of two determinations (two animals/determination).

It was possible that only partial depletion was attained because of a difference between the PcR present in non-E_2-primed animals and the PcR induced by E_2 treatment (Boomsma et al., 1982). Therefore, ovariectomized, non-E_2-primed animals were unilaterally hysterectomized and then injected with a bolus of 400 μg P. Ten minutes later the contralateral uterine horn was removed and receptor determinations made. Again, similar to what was observed with the E_2-primed animals, approximately 40% of the PcR available before P administration was depleted within 10 min of the administration of P (Fig. 3).

Changes in the Subcellular Distribution of Progesterone Receptors with Time Following the Acute Administration of Progesterone

Progesterone-induced changes in the subcellular distribution of P receptors were studied by the single i.v. injection of a 300- μg dose of P at time zero (Fig. 4). Ten minutes later all the animals were unilaterally hysterectomized and the contralateral uterine horn was removed 1, 3, 6 or 12 h after the injection of P. Following the initial translocation of PcR to the nuclear compartment at 10 min, there was a rapid decline in PnR binding within the first hour

reaching values not significantly different from control levels. Our data suggests that there may be an initial lag phase in the recovery of PcR; however, by 3 h the quantity of PcR had also returned to control levels. This decrease in nuclear binding and increase in cytosol binding with time following the acute administration of P was directly correlated with declining peripheral plasma levels of P (Table 1).

In another experiment, a second i.v. injection of 300 μg of P was administered at either 1 h or 3 h after the first injection to determine if the replenished PcR was biologically active or whether the cell entered a period refractory to additional P. E_2-primed animals were used in this experiment and the animals were unilaterally hysterectomized just before the second injection of P, and the contralateral uterine horn was removed 10 min after the second injection. Progesterone induced the immediate translocation of a significant proportion of the PcR present before P administration at both 1 h and 3 h (Fig. 5). The translocation of this PcR to the nuclear compartment was correlated with a second increase in serum P levels (Fig. 5).

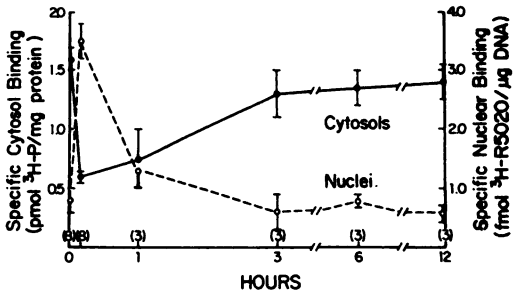

FIG. 4. Subcellular distribution of PcR and PnR. E_2-primed animals were injected with a single 300-μg dose of P at time zero. The zero time values are the same as those shown in Fig. 1 except that the results are expressed as pmol/mg protein. The data is expressed as mean ± SEM. The number of determinations is shown in parentheses.

DISCUSSION

Translocation was found to be dose-dependent within the cat uterus, and maximal when approximately 40% of the PcR had been depleted. A similar dose-dependent, limited translocation process has been described for rats (Walters and Clark, 1978), hamsters (Chen and Leavitt, 1979), rabbits (Isomaa et al., 1979), and guinea pigs (Saffran and Loeser, 1980). The failure to totally deplete PcR, even with excessive doses of P, could be due to a limited number of nuclear binding sites, to a pool of PcR which is unable to undergo nuclear translocation, or to some other unknown control mechanism. The possibility that the limitation is in the number of nuclear binding sites seems unlikely since the percentage of available PcR translocated in E_2-primed and nonprimed animals was equivalent but the total number of receptors translocated was much greater in E_2-primed animals. This conclusion is also supported by direct in vitro studies of nuclear binding which generally demonstrate a nonsaturability of nuclear binding sites (Chamness et al., 1974). Two different pools of PcR could exist: the PcR found in non-E_2-primed, ovariectomized animals (Boomsma et al., 1982) and the PcR induced by E_2 treatment. To test whether one of these pools was not translocatable, P was administered to nonprimed ovariectomized cats to determine whether the PcR present in these animals could be depleted: again, P induced the depletion of approximately 40% of the available PcR. These results were not surprising since we have shown that a typical progestational response can be induced in the cat uterus by the administration of P to nonprimed ovariectomized animals (Boomsma et al., 1982), suggesting translocation must occur. Thus these two pools do not account for the failure to totally deplete PcR following the acute administration of P. It is still possible, however, that there are two pools of PcR and that equal proportions of translocatable PcR's are found both in E_2-primed and nonprimed tissue. This concept is supported by the recent description of two forms of 8 S P receptor in chick oviduct (Dougherty and Toft, 1982).

The replenishment of PcR and the loss of

TABLE 1. Serum progesterone concentrations (ng/ml) determined by RIA for the animals whose subcellular receptor compartmentalization is shown in Fig. 4. Each value represents the mean ± SEM of the individual determinations from three animals.

Time	P (ng/ml)
10 min	16.9 ± 3.2
1 h	8.9 ± 1.8
3 h	3.2 ± 1.0
6 h	2.1 ± 0.8
12 h	0.9 ± 0.2

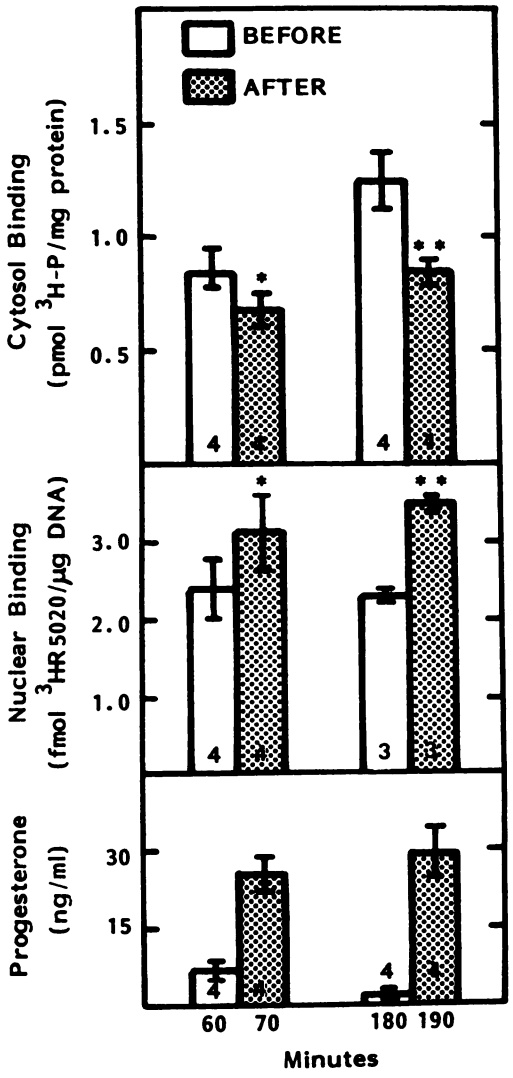

FIG. 5. Subcellular distribution of PcR and PnR before and after a second 300- μg injection of P. E_2-primed animals were first injected with 300 μg of P at time zero, and again at either 60 min or 180 min. One uterine horn was removed just before the second P injection, and the contralateral uterine horn was removed 10 min after the second injection of P. The data is expressed as mean ± SEM. The number at the base of each bar represents the number of individual determinations. *P<0.025 and **P<0.01 as determined by paired *t* test.

PnR with time following the administration of P in the cat was different from that observed in the rabbit (Isomaa et al., 1979) and guinea pig (Saffran and Loeser, 1980). In the cat, replenishment of the PcR occurred within 3 h, whereas in the rabbit replenishment had not yet occurred at 25 h (Isomaa et al., 1979). In the guinea pig replenishment had occurred at 24 h following the s.c. administration of 1 mg/kg of P; however replenishment was not observed at 24 h when 10 mg/kg of P was given (Saffran and Loeser, 1980). In the cat, the levels of PnR had declined to preinjection values within 1 h, whereas in the hamster (Chen and Leavitt, 1979), rabbit (Isomaa et al., 1979) and guinea pig (Saffran and Loeser, 1980) the PnR had not returned to control levels until 6–8 h after the administration of P. It is difficult to compare the studies in these other species with our results because of the different methods and amounts of P that were administered. Additionally, it is difficult to determine whether the effects observed by others are due to physiological or pharmacological levels of P since serum levels of the hormone were not reported. The longer retention time reported for the hamster (Chan and Leavitt, 1979) and guinea pig (Saffran and Loeser, 1980) may be due to the s.c. administration of P where, presumably, it takes more time for the serum levels of P to plateau. A longer retention time was also observed in the rabbit (Isomaa et al., 1979) where P was administered i.v.; however the dose administered to each animal was 30–40 times greater than in the present study.

In our study, the serum concentrations of P were determined and compared with the receptor fluxes. The doses of P administered in this study produced serum concentrations of P which were within the physiological range for this species (Verhage et al., 1976). Also, the serum levels of P peaked within 2 min of the i.v. injection of a bolus of P, and then declined very rapidly. We believe that the i.v. administration of doses of P which produced serum levels of P within a range found to be physiological in the intact animal is responsible for the short retention time of PnR, and the rapid replenishment of PcR, as observed in this study. Thus, our data may give a more accurate estimate of the actual retention time of any given PnR molecule. This contention is supported by a recent study by Horwitz and co-workers (Horwitz et al., 1982; Mockus et al., 1982) who were able to demonstrate quantitative translocation of PcR to the nuclear compartment in T47D human cancer cells if their determinations were made within 3 min of the addition of P to the incubation media. The recovery of PnR was no longer quantitative at longer time intervals because of the rapid processing of PnR.

In summary, our results demonstrate that the depletion of PcR after acute P administration is limited to approximately 40% of the total PcR even when the PcR is much reduced, as in the nonprimed, ovariectomized animal. Our concurrent measurements of serum P indicate, for the first time, that maximum translocation occurs before the serum concentrations of P reach pharmacological levels. Another significant finding of this study is the rapid (within 3 h) return of both the PcR and PnR to preinjection values. Administering a second injection of P at 1 h and 3 h demonstrates that P must be continuously present at elevated levels to assure the long-term presence of PnR.

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