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CYTOSOL PROGESTERONE RECEPTORS OF THE RAT UTERUS: ASSAY AND RECEPTOR CHARACTERISTICS

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SUMMARY

A reliable assay has been developed for the progesterone receptor of rat uterus. Cytosol was prepared in a buffer containing 10 mM Tris with 30% v/v glycerol at pH 7.4 and was incubated with [^{3}H]-progesterone in the presence or absence of unlabeled progesterone, R5020 (17,21-dimethyl-19-nor-4, 9-pregnadiene-3, 20-dione), or cortisol at 4°C overnight. This incubation procedure results in complete binding and/or exchange of the [^{3}H]-steroid–receptor complex with no detectable degradation up to 48 h. Separation of free [^{3}H]-progesterone from that bound to receptor was accomplished by brief (~30 sec) exposure to dextran-coated charcoal at 4°C. Nonspecifically bound [^{3}H]-progesterone was estimated by competition analysis as previously described [*Steroids* **24** (1974) 599]. The progesterone receptor thus measured exhibited minimal affinity for cortisol and corticosterone and high affinity for R5020. In addition, the receptor was thermolabile, unstable when incubated overnight at 4°C in the absence of steroid, and sensitive to a sulfhydryl-blocking reagent. Scatchard analysis of [^{3}H]-progesterone binding to the receptor yielded $K_D = 3.3 \pm 0.5$ nM and $n = 9.9 \pm 1.1$ pmol/uterus in the estradiol-primed ovariectomized adult rat. The relative binding activities of steroids for the receptor were R5020 > progesterone > deoxycorticosterone > corticosterone ≫ dexamethasone or cortisol. This specific progesterone receptor was absent in cytosol from lung and diaphragm. Injection of estradiol-17β resulted in an increase in the amount of cytosol progesterone receptor 24 and 48 h later. Cytosol receptor depletion was observed 1 h after progesterone injection, implying translocation to the nucleus. Thus, a cytosol progesterone receptor exists in the rat uterus and is readily distinguishable from the corticosteroid-binding-globulin-like protein.

INTRODUCTION

The presence of a specific progesterone receptor has been demonstrated in uterine cytosols of several mammals, i.e. hamster [1, 2], rabbit [3–6], guinea pig [7–9], and mouse [10]. Specific progesterone binding macromolecules were studied by sucrose density gradients, Sephadex chromatography, equilibrium dialysis and charcoal adsorption and were identified by one or more of the following characteristics: lack of appreciable cortisol competition, requirement of sulfhydryl groups for binding, high affinity for progesterone ($K_D = 10^{-10}$–10^{-9} M), and/or having a component with a sedimentation coefficient of 6–8 *S*.

In contrast, no reliable technique has been described for the assay of progesterone receptors in rat uterine cytosol, although there have been numerous attempts utilizing the above techniques [10–15]. The principal problems encountered by these investigators have been the instability of the progesterone receptor and interference in the assays by [^{3}H]-progesterone binding to lower affinity proteins, especially a corticosteroid-binding-globulin-like (CBG) protein described by Milgrom and Baulieu [11]. This protein shared the following characteristics with a similar protein in plasma (CBG): thermolability (60°C, 20′); higher relative binding activity for cortisol than for progesterone; $K_A = 1.8$ and 1.5×10^8, respectively; and a sedimentation coefficient of approximately 4*S* [16].

Past efforts to measure progesterone receptors in rat uterine cytosol can be grouped in the following manner: (a) no specific progesterone receptor was measured [11, 12, 14, 15] and (b) receptor was detected, but in miniscule amounts or with unacceptable variations [10, 13, 17]. When cytosol progesterone receptor was detected, the buffer always contained glycerol or sucrose* and the incubation temperature was not raised above 4°C. Thus, Faber *et al.* [13] detected a minor 6–7*S* component in sucrose gradients that was estradiol (E_2)-dependent and not inhibitable by corticosterone. Feil *et al.* [10] reported a 7 *S* component that was maximal at proestrus and Feil *et al.* [10] and Terenius [17] used the charcoal binding assay to demonstrate a cytosol progesterone receptor that was not inhibitable by cortisol.

In this paper we have utilized these observations to develop and validate an assay for the progesterone receptor of rat uterine cytosol.

* Note that the sucrose contained in sucrose gradients was not sufficient to stablize the cytosol progesterone receptor, possibly due to its absence in early steps of tissue processing.

EXPERIMENTAL PROCEDURE

Animals. Female rats (21 or 60 days old) of the Sprague–Dawley strain were obtained from Texas Inbred Mice Co., Houston, Texas and maintained in a temperature and light (7:00–19:00 h) controlled room with Wayne Lab Blocks and water available *ad lib.* The immature rats were used within four days of receipt. Adults were usually treated according to the following protocol: ovariectomy on day 0, injections of 1 μg E_2/rat s.c. (0.2 ml 1% ethanol in 0.9% NaCl) on days 3 and 4 at 9:00 h, and sacrifice 24 h later. Adults were decapitated to eliminate the possibility of stress-induced adrenal steroid release [18, 19].

Methods. After decapitation, uteri were rapidly trimmed, blotted, weighed and placed directly into ice cold 30% TG buffer (10 mM Tris 30% v/v glycerol at pH 7.4). Three or more uteri pooled/cytosol preparation were homogenized by a motor-driven pestle in a ground glass homogenizer, stopping frequently to maintain ice-cold conditions. The final tissue dilution was 50 mg tissue/ml homogenization buffer. Cytosol was then prepared by centrifuging in a Beckman Model J-21B at 25,000 *g* for 30 min at 4°C. A 250λ aliquot of the supernatant was added to 20λ of cold buffer containing [^{3}H]-progesterone plus or minus excess unlabeled steroid in a 12 × 75 mm tube on ice. Saturation analyses were performed with concentrations of [^{3}H]-progesterone ranging from 1–30 nM and 2 μM unlabeled steroid. Single concentration analyses of receptor quantity were performed with 16–20 nM [^{3}H]-progesterone in the presence or absence of 2 μM steroid. The determinations of total or nonspecific binding were always done in duplicate to yield a single average value for each binding parameter from a single experiment. After vortexing, tubes were incubated overnight on ice in the refrigerator.

Incubation was terminated by brief (< 30 sec) exposure to dextran-coated charcoal (1.0 g charcoal + 0.05 g dextran/100 ml 10% TG buffer). This step was accomplished by placing tubes (24 only/spin) in the appropriate Beckman JS-7.5 adapters (precooled), rapidly adding 250λ charcoal suspension to all the tubes, shaking the adapters 2–3 times, and immediately centrifuging at 2400 *g* for 6 min. This procedure was repeated as often as necessary to process all the tubes, storing processed tubes on ice until sampled. A 250λ aliquot of each supernatant was added to 4 ml ACS scintillation cocktail (Amersham-Searle) for radioactivity determinations on a Beckman LS-233 scintillation counter (efficiency for tritium: 29%).

Specifically bound [^{3}H]-progesterone was determined by the subtraction of nonspecific binding ([^{3}H]-progesterone bound in the presence of excess unlabeled progesterone) from total [^{3}H]-progesterone binding (that bound in the absence of excess unlabeled progesterone) [20]. [^{3}H]-progesterone binding that is inhibited by cortisol (i.e., CBG-like binding) was evaluated by determining the inhibition of [^{3}H]-progesterone binding in an additional set of tubes containing 2 μM cortisol. Excess R5020* was also used to estimate the quantity of specifically bound [^{3}H]-progesterone. This latter procedure eliminates the need for those tubes containing excess cortisol (since R5020 does not compete for [^{3}H]-progesterone bound to CBG if this binding does occur) and is recommended for routine assays because of its simplicity. However, the use of R5020 necessitates initial confirmation that glucocorticoid receptors are not involved, since R5020 binds to these proteins with a slightly higher affinity than does progesterone [21]. Lack of cortisol competition is a valid criterion to use.

The hydroxylapatite (HAP) assay was done by incubating 500λ cytosol as described above. Incubation was terminated by adding 200λ of the HAP solution (30% TG buffer containing 60% v/v Bio-Rad hydroxylapatite suspension) to each tube, followed by vortexing and incubating on ice for 15 min. The HAP was then pelleted by centrifuging at 800 *g* for 10 min and washed 3 times with buffer at 4°C. Following extraction with 1.25 ml 100% ethanol at 30°C for 15 min, the HAP was pelleted again and the ethanol decanted into scintillation vials containing 5 ml scintillation cocktail (4.93 g Packard's Permablend I/liter toluene). Protein concentrations were determined by the method of Lowry *et al.* [22].

Chemicals. Unlabeled progesterone, cortisol, deoxycorticosterone, and dextran were obtained from Schwarz-Mann. N-ethylmaleimide, estradiol-17β, hydrocortisone acetate, dexamethasone and corticosterone were purchased from Sigma Corp. Charcoal (activated U.S.P. powder) was procured from Mallincrodt, and the HAP was purchased from Bio-Rad Laboratories. [1,2-^{3}H(N)]-Progesterone (55.7 Ci/mmol) was procured from New England Nuclear. Nafoxidine was the gift of the Upjohn Co. [6,7-^{3}H]-R5020 (51.4 Ci/mmol) and unlabeled R5020 were donated by Roussel-Uclaf. All other chemicals were of reagent grade.

RESULTS

Progesterone receptor assay: preliminary characterization

In order to determine the effects of charcoal on progesterone binding, uteri from estradiol-primed castrated rats were cut into four approximately equal sections to assure sample uniformity. The pooled cytosols were prepared in the following buffers at pH 7.4: T (10 mM Tris), TE (10 mM Tris with 1.5 mM EDTA), 10% TG (10 mM Tris, 10% v/v glycerol), and 30% TG. After 1 h incubation at 4°C with 16 nM [^{3}H]-progesterone plus or minus 1.0 μM unlabeled

* Abbrevation: R5020 = 17,21-dimethyl-19-nor-4,9-pregnadiene-3,20-dione.

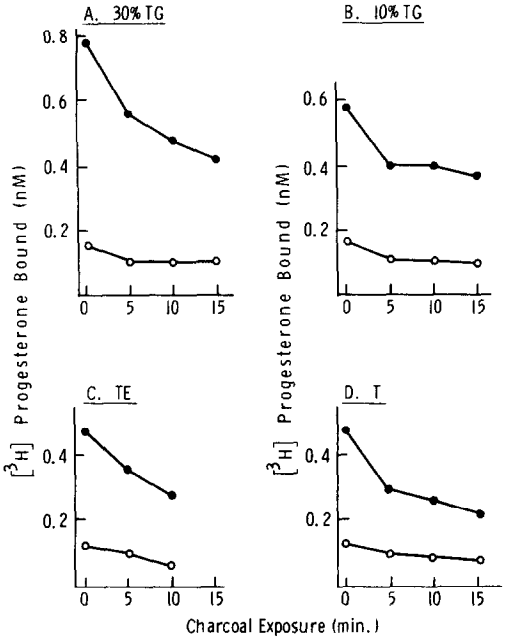

Fig. 1. Effect of buffers and charcoal treatment time on [^{3}H]-progesterone binding in cytosol. Uteri from E_2-primed castrated adults were cut into four approximately equal segments. The pooled cytosols were prepared in the following buffers at pH 7.4: T (10 mM Tris), TE (10 mM Tris with 1.5 mM EDTA), 10% TG (10 mM Tris, 10% v/v glycerol), and 30% TG. After incubating for 1 h at 4°C with 16 nM [^{3}H]-progesterone plus or minus 1.0 μM unlabeled progesterone or R5020, tubes were exposed to charcoal (1%)-dextran(0.05%) in the respective buffers (except 30% TG) for varying times.

steroid, tubes were exposed to charcoal in the respective buffers (except that 10% TG was substituted for 30% TG) for 0, 5, 10 or 15 min at 4°C.

With increased exposure time to charcoal, nonspecifically bound [^{3}H]-progesterone decreased only slightly. However, that amount specifically bound (identical whether measured by the addition of either 1.0 μM progesterone or R5020) decreased 40% within 10 min. Similar decreases in the specific progesterone binding component were obtained with estradiol-primed immature rats (21–28 days of age), with normal cycling rats, and with ovariectomized adults (primed with 0, 1, or 2 injections of estradiol). These data constitute the reason for performing the charcoal binding assay with the shortest possible exposure to charcoal (see Methods). Cortisol (1.0 μM) did not inhibit [^{3}H]-progesterone binding in any animal model under any test conditions, indicating that [^{3}H]-progesterone binding to CBG is negligible in this assay.

The use of different buffers did not affect the level of nonspecifically bound [^{3}H]-progesterone (Fig. 1), but the total amount bound (and, hence, that specifically bound) was markedly different: 30% TG > 10% TG > TE ≥ T. This difference in specific binding was not paralleled by any increase in cortisol inhibitable counts, and therefore was not attributable to CBG-like binding. Thus, although specific [^{3}H]-progesterone binding was detected after 1 h at 4°C with all buffers tested, glycerol provided receptor and receptor-binding stability, especially at shorter charcoal exposure times.

Effect of 0.7 nM NEM on binding to cytosol

The ability of NEM (N-ethylmaleimide) to destroy specific [^{3}H]-progesterone binding was used to differentiate between the progesterone receptor and CBG-like binding. Specific binding decreased 91% whereas nonspecific binding decreased only 21%. Thus, the specific binding component measured by this method requires sulfhydryl groups, a characteristic lacking in the CBG-like component previously measured in rat uterus and plasma [17, 23, 24].

Effect of incubation temperature on the measurement of cytosol receptor

In order to determine the optimum incubation temperature for the progesterone receptor measurement, cytosol was incubated at 4, 22, 30 or 37°C for 30 and 60 min. Specifically bound [^{3}H]-progesterone did not differ significantly at 4°C and 22°C, but was decreased at 30° and 37°C (Fig. 2). These results agree with those obtained in nuclei of rat uteri [25].

Effect of incubation time on [^{3}H]-progesterone binding at 4°C

The kinetics of binding and the extent of degradation at 4°C were examined by incubating cytosol for 1–48 h with 20 nM [^{3}H]-progesterone ± 2 μM unlabeled progesterone. Specific binding was complete by 4 h and remained stable for at least 44 h thereafter (Fig. 3A). The apparent lack of degradation was highly reproducible and was quite different from the pattern described at elevated temperatures in nuclei [25]. Thus, an incubation time of 18–24 h (overnight) at 4°C was chosen for subsequent experiments

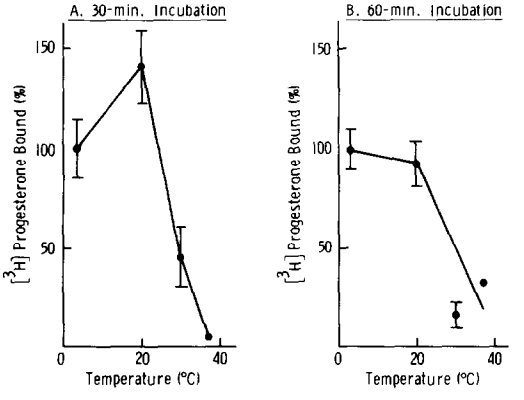

Fig. 2. Effect of incubation temperature on [^{3}H]-progesterone binding to the cytosol. Cytosol was incubated for 30 or 60 min at 4, 22, 30 or 37°C in the presence of 20 nM [^{3}H]-progesterone plus or minus 2.0 μM progesterone. Bound and free steroid were separated by the charcoal binding assay. Data are plotted as the per cent of specific binding obtained at 4°C (1.39 pmol/ml at 30 min; 1.46 pmol/ml at 60 min) in each experiment. Points and bars, respectively, represent the mean ± S.E.M. of 3–4 observations, except for 37°C (single observation).

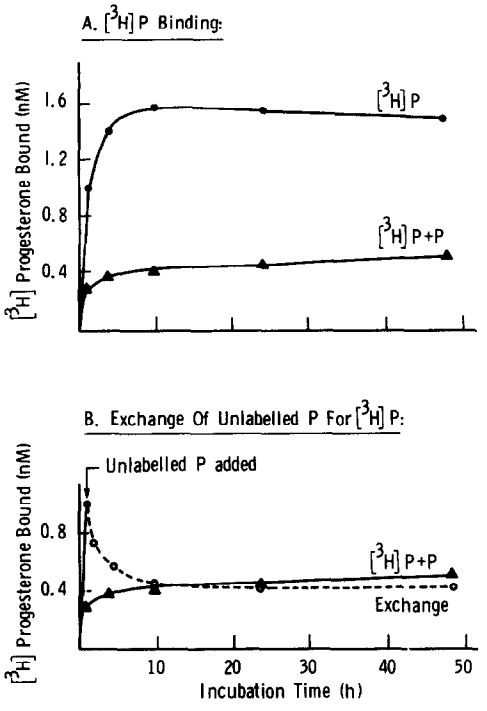

Fig. 3. [^{3}H]-Progesterone binding and exchange in cytosol. A. Cytosol was incubated with 20 nM [^{3}H]-progesterone ± 2 μM progesterone at 4°C for 1–48 h. B. A separate set of tubes was incubated 1 h with [^{3}H]-progesterone alone and then received 2 μM unlabeled progesterone for the subsequent 1–47 h (exchange).

to take advantage of this long plateau of receptor stability.

Effect of preincubation in the absence of steroid

The stability of [^{3}H]-progesterone binding in the cytosol at 4°C allowed an examination of the stability of the receptor in the absence of steroid. Cytosol was prepared and then divided into two pools. 250λ Aliquots were preincubated on ice for 1 h or 24 h and then [^{3}H]-progesterone ± excess unlabeled progesterone was added to examine binding during a subsequent 48 h incubation period. Specifically bound [^{3}H]-progesterone was 48% lower ($P < 0.001$) in the samples preincubated 24 h in the absence of steroid (data not shown). Conversely, nonspecific binding decreased only 18% ($P < 0.05$). The receptor lability in the absence of bound steroid is important in saturation experiments, where total binding of [^{3}H]-progesterone at sub-saturating concentrations will be artificially lowered. The net result will be an increase in the apparent K_D measured under these conditions. However, relative differences in the K_D for various [^{3}H]-steroids will remain detectable.

Exchange of unlabeled progesterone for [^{3}H]-progesterone in cytosol

In order to examine the exchange of unlabeled progesterone for [^{3}H]-progesterone bound to the receptor, cytosol was incubated with 20 nM [^{3}H]-progesterone in the presence or absence of 2 μM unlabeled progesterone (Fig. 3A). An additional set of tubes was incubated 1 h with [^{3}H]-progesterone alone, after which 2 μM unlabeled progesterone was added and the incubation was continued (Fig. 3B). Exchange was complete within 8 h and there was no subsequent difference in binding in the exchanged and the nonspecific samples.

Hydroxylapatite (HAP) assay for the progesterone receptor

To confirm that the charcoal binding assay detected the majority of the progesterone receptor in rat uterine cytosol, an attempt was made to modify the hydroxylapatite (HAP) assay [26, 27] for the cytosol progesterone receptor. After incubation of cytosol with [^{3}H]-progesterone and appropriate competitors, 500λ aliquots of the cytosol were exposed to 100, 200 or 500λ HAP suspension (60% HAP v/v in 30% TG) and the assay was continued (see Methods). Nonspecifically bound [^{3}H]-progesterone (Fig. 4A) increased in proportion to the amount of HAP in the range tested, approximating the level of nonspecific binding observed in the charcoal binding assay at 100–200λ HAP. Additionally, there was a preferential loss of cortisol-inhibitable binding (i.e. CBG-like binding) as the amount of HAP was decreased (Fig. 4B). The loss in the cortisol-inhibitable binding component accounted for the majority of the loss in specific [^{3}H]-progesterone binding (Fig. 4A) when the quantity of HAP was lowered from 500 to 200λ. Thus, a HAP assay utilizing 100–200λ HAP suspension/500λ cytosol is suitable for measuring the rat uterine progesterone receptor. The preferential loss of cortisol-inhibitable [^{3}H]-progesterone (i.e. CBG-like binding) with decreased HAP also supports the conclusion that the binding component studied herein is the specific progesterone receptor.

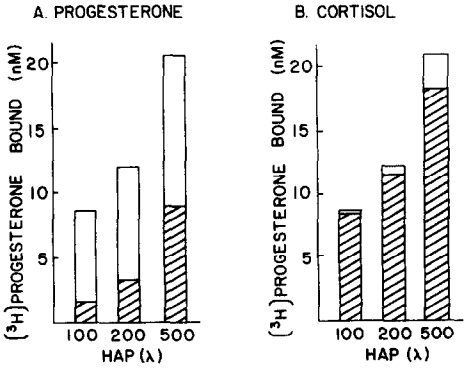

Fig. 4. Hydroxylapatite assay for the cytosol receptor. Cytosol was incubated overnight at 4°C in the presence of 20 nM [^{3}H]-progesterone ± 2 μM progesterone or cortisol. Bound and free steroid were separated by adding 100, 200 or 500λ of a 60% hydroxylapatite suspension in buffer, washing and extracting with ethanol. Stipled areas represent nonspecific binding in the presence of progesterone (A) or cortisol (B).

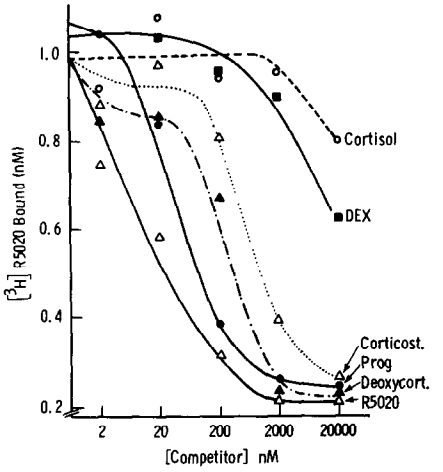

Fig. 5. Competition of [^{3}H]-R5020 binding in the cytosol. Cytosol was incubated overnight at 4°C in the presence of 20 nM [^{3}H]-R5020. Concentrations of unlabeled competitors ranged from 2 nM to 2×10^4 nM. Bound and free steroid were separated by the charcoal binding assay. The total binding of [^{3}H]-R5020 is plotted along the ordinate as a function of competitor concentration for each test steroid. The cytosol protein concentration was 0.2–0.5 mg/ml. Symbols: △——△ R5020, ●——● progesterone, ▲···▲ deoxycorticosterone, △···△ corticosterone, ■——■ dexamethasone, ○——○ cortisol.

Relative binding activities (RBA) of steroids for the progesterone receptor

To determine hormone specificity of the receptor, cytosol was incubated with 20 nM [^{3}H]-progesterone or [^{3}H]-R5020 with competing steroids present singly at concentrations ranging from 2 nM–2×10^4 nM. The results obtained with [^{3}H]-R5020 are shown in Fig. 5 to demonstrate the specificity of binding of this progesterone analog to the receptor. Similar binding curves were obtained with [^{3}H]-progesterone when these competitors were examined. The RBA of each of the test steroids was calculated as the relative steroid concentration when the [^{3}H]-steroid binding was inhibited 50% (Table 1). These specificities agree very well with those of other small mammalian species [1, 5, 8, 13, 28]. Additionally, the RBA's relative to [^{3}H]-R5020 are similar to those for [^{3}H]-progesterone when the difference in reference steroid is considered.

Saturation analysis of the cytosol progesterone receptor

In order to study the saturation characteristics of the progesterone receptor, cytosol was incubated in the presence of varying concentrations of [^{3}H]-progesterone $\pm$ 2 μM progesterone or R5020 (Fig. 6A). The specific binding was similar whether measured by excess progesterone or R5020. The Scatchard plot of the progesterone and R5020-inhibitable components is presented in Fig. 6B. When data from all such plots ($n = 4$) were combined by averaging the values obtained for K_D and n in each separate experiment, the specific binding of [^{3}H]-progesterone demonstrated K_D of 3.3 ± 0.5 nM and n of 9.9 ± 1.1 pmol/uterus. This apparent K_D is, of course, greater than the true value due to the receptor degradation at low concentrations of steroid (see above). Scatchard analysis of the binding of [^{3}H]-R5020 yielded K_D of 0.62 ± 0.06 nM and n of 6.15 ± 0.45 pmol/uterus ($n = 2$). The K_D's of the two steroids were thus significantly different ($P < 0.02$, Student's t-test).

An alternative method for determining the values of K_D and n has been developed by Cornish-Bowden and Eisenthal[29–30]. This method uses a direct linear plot and eliminates most assumptions of the Scatchard plot and other parametric (least squares) analyses of hyperbolic functions. In addition, the method permits the determination of K_D and n without calculations. More important, highly aberrant values exert little influence on the estimates of K_D and n via the nonparametric technique of the linear plot. When the experimental data of Fig. 6A were plotted by this method (Figs. 6C and 6D), the values for K_D and n were determined to be 5.5 nM and 6.6 pmol/uterus, respectively. The only disadvantage of this method is that it is unsuitable for the analysis of multiple binding populations, making a linear Scatchard plot a prerequisite for its use [29, 30].

Table 1. Relative binding activities of steroids for cytosol progesterone receptors

Steroid	RBA vs [^{3}H]P*	RBA vs [^{3}H]R†
Progesterone	100%	30%
R5020	1600	100
Deoxycorticosterone	55	8
Corticosterone	8	3
E_2	5	—
Dexamethasone	5	<0.1
Cortisol	<1	<0.1
Nafoxidone	0	—

Cytosol from E_2-primed ovariectomized rats was incubated with 20 nM [^{3}H]-progesterone in the presence of competing steroids (2 nM–2×10^4 nM). The relative binding activities were calculated from the concentrations of the competing steroids at the 50% level of competition. * 2–3 Determinations. † 1 Determination.

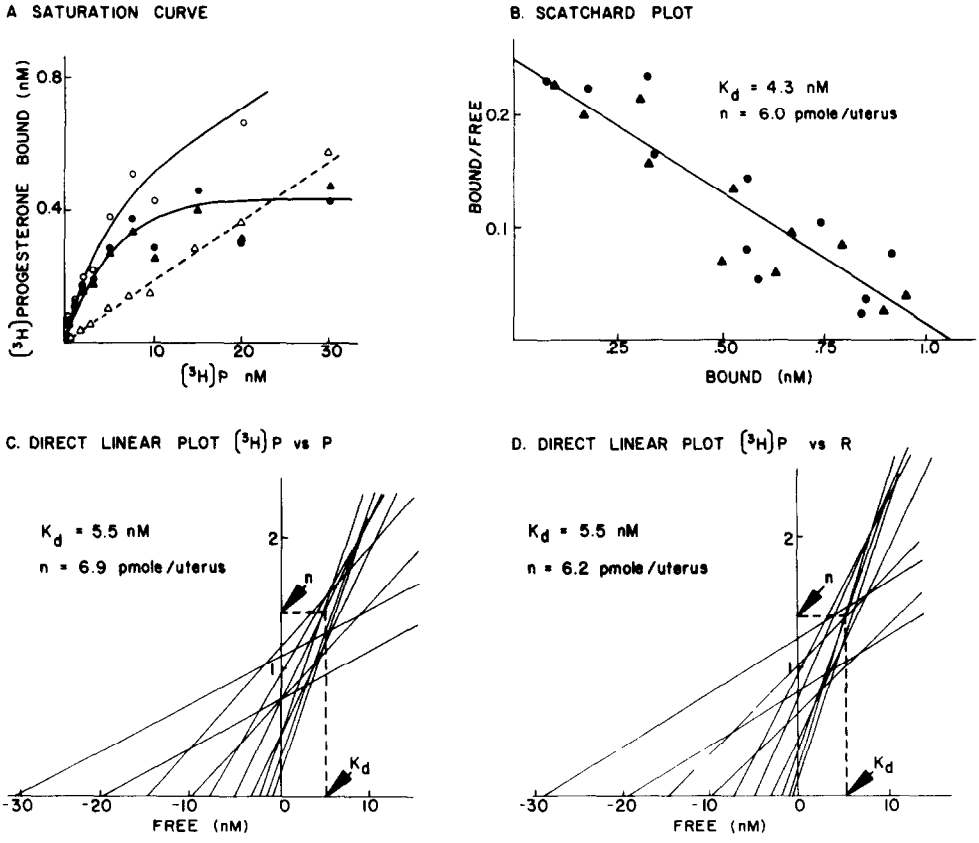

Fig. 6. Analysis of [^{3}H]-progesterone binding. Cytosol was incubated overnight at 4°C in the presence of varying concentrations of [^{3}H]-progesterone ± 2 μM progesterone or R5020. Bound and free steroid were separated by the charcoal binding assay method. For data plotted as the saturation curve (A) and the Scatchard plot (B) the symbols are O---O total [^{3}H]-progesterone bound, ●——● specifically bound (progesterone inhibitable), ▲——▲ specifically bound (R5020 inhibitable), △-·-△ nonspecifically bound. Lines were fitted in the Scatchard analysis by linear regression. The direct linear plots (C and D) were constructed by drawing lines through the corresponding values for [^{3}H]-progesterone on the abscissa and ordinate, respectively [28]. The arithmetic median of the intersections of these lines (——) along each axis corresponds to the best estimates for K_D and n (arrows).

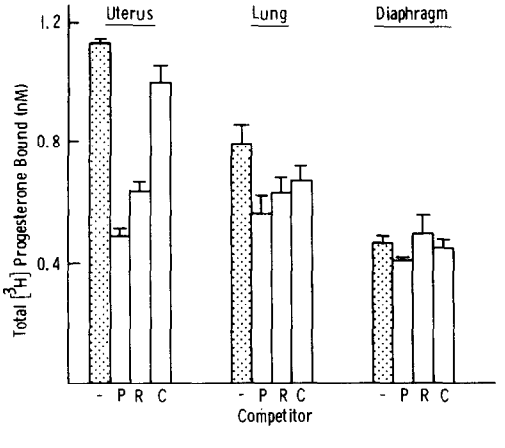

Fig. 7. Tissue specificity of [^{3}H]-progesterone binding in cytosol. Cytosol was prepared from the uterus, lung, and diaphragm of four rats and was incubated in the presence of 20 nM [^{3}H]-progesterone ± 2μM progesterone, R5020, or cortisol overnight. Bound and free steroid were separated by the charcoal binding method. Bars represent the mean ± S.E.M. for four observations.

Tissue specificity of [^{3}H]*-progesterone binding to cytosol*

In order to confirm the specific nature of [^{3}H]-progesterone binding to the cytosol receptor, cytosols were prepared from the diaphragm, lung, and uterus and were incubated overnight at 4°C in the presence of 20 nM [^{3}H]-progesterone plus or minus 2 μM progesterone, R5020, or cortisol. Significant ($P < 0.001$) competition of total [^{3}H]-progesterone binding by unlabeled progesterone and R5020 occurred only in the uterus (Fig. 7).

Effect of estradiol (E_2) *injection on uterine cytosol progesterone receptors*

Since the number of progesterone receptors is regulated by E_2 in other systems [1, 2, 13, 31–33], the effect of E_2 injection was studied in this assay. Cytosol receptors were measured in 3-day ovariectomized rats 24 h after 0, 1, and 2 E_2 injections (spaced 24 h apart). The number of cytosol progesterone receptors/g uterus and /uterus increased significantly

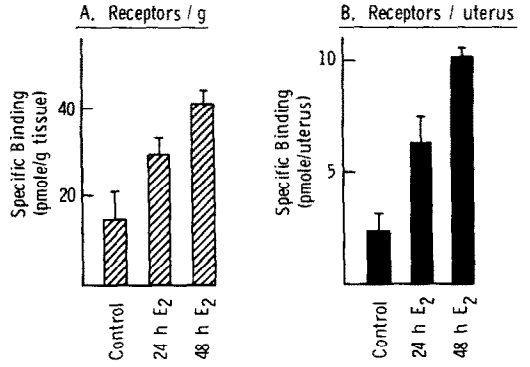

Fig. 8. Effect of estradiol on [^{3}H]-progesterone binding in the rat uterus. Adult rats were ovariectomized for 3 days and then received 0, 1, or 2 injections of E_2 (1 μg/rat s.c.) at 24 h intervals. After sacrifice 24 h after the last injection, pooled cytosols were incubated at 4°C overnight in the presence of 20 nM [^{3}H]-progesterone ± 2 μM R5020. Receptors were quantitated/g uterus (A) or /uterus (B) assuming that the receptor and steroid interact mole for mole. The cytosol protein concentration was 0.2–0.5 mg/ml. Bars represent mean ± S.E.M. for four observations.

($P < 0.02$ and $P < 0.01$, respectively, one-tailed F-test) 48 h after the initial E_2 injection (Fig. 8).

Depletion by the cytosol receptor after progesterone injection

To further determine whether the specific binding measured in this paper conforms to expected receptor behavior, rats were injected s.c. with 500 μg progesterone or with the vehicle (0.5 ml 30% ethanol in saline) 1 h prior to sacrifice. Cytosol was incubated at 4°C overnight in the presence of 20 nM [^{3}H]-progesterone ± 2 μM progesterone, cortisol, or R5020. As shown in Table 2, cytosol progesterone receptors/g uterus and/uterus decreased significantly ($P < 0.01$ and $P < 0.05$, respectively) following progesterone injection. These data suggest that translocation of receptor–progesterone complex from the cytoplasm to the nucleus occurred following progesterone injection. This translocation phenomenom will be described more fully elsewhere.

DISCUSSION

These results confirm the presence of a specific progesterone-binding macromolecule which is probably a progesterone receptor in the cytosol of the rat uterus. This receptor is readily distinguishable from the CBG-like protein described by Milgrom and Baulieu [11] and is extremely unstable at elevated temperatures. In addition, an assay has been developed for this cytosol progesterone receptor which allows reproducible measurements of the receptor in rats under various physiological states.

The progesterone receptor of rat uterine cytosol was identified by its similarity to progesterone receptors of other species. Thus, this receptor demonstrates thermolability [1, 32], sensitivity to a sulfhydryl-blocking agent [1, 32], high affinity for progesterone [1, 8], rapid exchange kinetics [1, 34] and a similar number of receptors to that of other species [1, 31]. In addition, the specificity of this receptor for unlabeled steroids in the competition assay (Table 1) was identical to that previously described in other systems [1, 5, 8, 13, 28]. The consistent parallel between R5020 and progesterone competition of [^{3}H]-progesterone binding is further proof of the identity of this progesterone receptor. The dissimilarity between the [^{3}H]-progesterone binding component described herein and the CBG-like protein [11] is evident from several criteria. This specific progesterone receptor lacks appreciable competition by cortisol and corticosterone (Table 2), depends on sulfhydryl groups for binding characteristics, binds R5020 with a high affinity (Table 1), and is negligible in the non-target tissues lung and diaphragm (Fig. 7). Further differentiation of the two binding components was observed when the cortisol-inhibitable component was selectively decreased in the HAP assay (Fig. 4B). However, a minor cortisol-inhibitable component (CBG) was occasionally observed in the experiments described in this paper. Therefore, the assays were controlled by including tubes with [^{3}H]-progesterone in the presence of 2 μM cortisol or R5020.

The demonstration that estrogen priming caused increased quantities of specific progesterone binding in the uterus (Fig. 8) and that an injection of pro-

Table 2. Depletion of cytosol progesterone receptors by progesterone injection

	Receptors/g		Receptors/uterus	
Injection	pmol	P	pmol	P
Vehicle	28.93 ±3.25	<0.01	7.20 ±0.92	<0.05
Progesterone	15.88 ±3.38		4.39 ±0.97	

E_2-primed ovariectomized rats were injected with 500 μg progesterone or the vehicle (0.5 ml 30% ethanol in saline) 1 h prior to sacrifice. Cytosol was incubated at 4°C overnight in the presence of 20 nM [^{3}H]-progesterone ± 2 μM progesterone, cortisol, or R5020. Data are expressed as the mean ± S.E.M. Significances were calculated by Student's one-tailed t-test.

gesterone causes depletion of these sites from the cytosol is further evidence that the progesterone receptor is measured by our assay techniques.

The rates of binding of [^{3}H]-progesterone to unoccupied receptor sites at 4°C is rapid and reaches completion by 5–10 h (Fig. 3). Likewise, the rate of dissociation and exchange of progesterone is relatively rapid and reaches completion by 10 h (Fig. 3). Therefore, this assay procedure cannot differentiate between occupied and unoccupied receptors [35], but it does permit the evaluation of the total quantity of receptor.

In conclusion, we have developed an assay for the progesterone receptor of the rat uterine cytosol. The assay depends on the use of a Tris–glycerol (30%) buffer for the preparation of cytosol and the rapid separation of bound from free steroid by charcoal at 4°C. These conditions permit the simultaneous measurement of occupied and unoccupied progesterone receptor sites and should prove useful in the further evaluation of the subcellular compartmentalization of the progesterone receptor.

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