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STUDIES ON THE ANTIGLUCOCORTICOID ACTION OF PROGESTERONE IN RAT THYMOCYTES*: EARLY *IN VITRO* EFFECTS

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SUMMARY

Progesterone was found to exhibit antiglucocorticoid properties in rat thymocytes, as indicated by its ability to compete effectively for triamcinolone acetonide (TA) binding sites as well as to inhibit the biochemical effects of TA. In a cell free system, progesterone competitively inhibited the binding of TA to glucocorticoid-specific binding sites and was more effective than the antiglucocorticoid cortexolone in this respect. Progesterone at 10^{-7}–10^{-6} M antagonized the inhibitory effect of 5×10^{-8} TA on RNA metabolism, although it was inactive by itself as an inhibitor of [^{3}H]-uridine uptake and incorporation into thymocyte RNA; this antagonism was not due to competition by progesterone for TA uptake into the cells. Progesterone was less efficient than cortisol or TA in depleting the cytosol receptors. However, unlike the glucocorticoid agonists which formed a temperature-dependent, salt extractable nuclear bound complex, progesterone was bound tightly to a fraction in the 27,000 *g* pellet which was unaffected by changes in the incubation temperature or salt extraction conditions. Additional binding of progesterone was observed in cells pre-saturated with TA, suggesting the presence in rat thymocytes of progesterone binding components other than the glucocorticoid receptors.

INTRODUCTION

It is generally believed that the mechanism of action of steroid hormones includes as an initial step the specific binding of the steroid to receptor proteins in cytosol fraction obtained from target tissues [1]. The resulting steroid-receptor complex then undergoes a temperature dependent translocation to the nucleus, where it interacts with specific nuclear sites [2–4]. The latter interaction is presumed to trigger transcriptional changes which ultimately lead to the observed biological response.

Certain steroid antagonists have been shown to compete with the biological action of steroid hormones [5–9]. Our studies on the antiglucocorticoid action of cortexolone§ [7, 10] indicate that the binding of cortexolone to the glucocorticoid receptor results in an altered conformation of the cytoplasmic complex. Although the cortexolone–receptor complex was translocated to the nucleus, it had decreased affinity for the nuclear acceptor site. The lack of glucocorticoid activity of the cortexolone-receptor complex could thus be explained as resulting from a marked decrease in binding at the nuclear level. Similarly, in rat kidney, the aldosterone antagonist spirolactone competed for aldosterone-specific cytoplasmic sites but failed to bind to chromatin acceptor sites [8]. However, studies by Rousseau *et al.* in culcured hepatoma cells suggested another mode of action for antihormones, involving competition with the active hormone at the cytoplasmic level and no nuclear uptake of the antagonist–receptor complex [11].

Progesterone has been reported to behave as a hormone antagonist in a number of steroid-receptor systems including rat thymocytes [11–15]. The present paper is concerned with the early *in vitro* action of progesterone in rat thymocytes, the nature of its interaction with the glucocorticoid receptors and the resulting antiglucocorticoid activity. In addition, we present evidence that progesterone interacts with components different from the glucocorticoid receptors in thymocytes, the nature and function of which is as yet unclear.

EXPERIMENTAL

Chemicals. [1,2,4-^{3}H]-triamcinolone acetonide (TA) (10.7 Ci/mmol) was purchased from Schwarz/Mann

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§ The abbreviations used are: TA, triamcinolone acetonide, 9α-fluoro-11β, 16α, 17, 21-tetrahydroxy 1,4-pregnadiene, 3, 20-dione-16, 17-acetonide; Progesterone, 4-pregnene-3, 20-dione; Cortisol, 11β, 17,21-dihydroxy-4-pregnene-3, 20-dione; Cortexolone, 17,21-dihydroxy-4-pregnene-3, 20-dione; TCA, trichloroacetic acid: CBG, corticosteroid-binding globulin.

BioResearch. [1,2-^{3}H]-progesterone (48 and 96 Ci/mmol), [1,2-^{3}H]-cortisol (44 and 53 Ci/mmol), [1,2-^{3}H]cortexolone (44.1 Ci/mmol) and [U-^{14}C]-D-sucrose (5 mCi/mmol) were obtained from New England Nuclear Corporation. [5-^{3}H]-uridine (23 Ci/mmol) was purchased from International Chemical and Nuclear Corporation. Radioactive steroids that were supplied as benzene or benzene–ethanol solution were evaporated to dryness in an atmosphere of N_2 and redissolved in absolute ethanol. Non-radioactive triamcinolone acetonide was a gift from Squibb Inc. Cortexolone was obtained from Steraloids Inc. Cortisol and progesterone were purchased from Sigma Chemical Corporation. Stock solutions of steroids (10^{-5}–10^{-2} M) were prepared in absolute ethanol and stored at 4°C. The complete Roswell Park Memorial Institute (RPMI) 1640 culture medium [16] was obtained in a powder form from Grand Island Biological Company and reconstituted before use. All chemicals and solvents used were of reagent grade.

Buffers. Buffers used were as follows: hypotonic Tris buffer—0.01 M Tris–HCl, pH 7.5, 0.4 mM Na_2EDTA; hypertonic Tris buffer—0.11 M Tris–HCl, pH 7.5, 1.43 M NaCl, 0.11 M KCl, 0.033 M $MgCl_2$; TESH buffer—0.01 M Tris–HCl, pH 7.5, 0.001 M Na_2EDTA, 0.012 M thioglycerol; TESH–KCl buffer—0.01 M Tris–HCl, pH 7.5, 0.001 M Na_2EDTA, 0.012 M thioglycerol, 3.3 M KCl.

Animals and treatment. Male, Sprague–Dawley rats (120–160 g) were obtained from Carworth Farms and A. R. Schmidt, or bred in our laboratory. Intact rats were used for the *in vitro* studies.

Preparation and incubation of cell suspensions. Unless otherwise specified all of the experimental procedures were carried out at 0° to 4°C. For binding studies, suspensions of rat thymocytes were prepared by mincing pooled thymi in a protein-free RPMI 1640 medium using the technique described by J. Rosen *et al.* [17]. Cell suspensions (3.8×10^7–4.2×10^7 cells per ml) were incubated for 30 min with 10^{-8}–10^{-5} M steroid at 0° or 37°C under an atmosphere of 95% air–5% CO_2 with constant shaking. At the end of the incubation, cells were cooled in ice for 3 min and centrifuged at 120 *g* for 3 min. The labelled cells were washed three times with an equal volume of incubation medium to remove nearly all the free and loosely bound steroid [18]. For viability studies, cells were prepared and incubated at 37°C for 15 h as described by Kaiser and Edelman [19].

Cell fractionation. Washed cell pellets were homogenized using all glass Potter–Elvehjem tissue grinders. Cells were disrupted by homogenization in 1 volume of TESH buffer. Following cell lysis, 1/10 volume of TESH–KCl buffer was added to give a final concentration of 0.3 M KCl and the suspension was homogenized again. Alternatively, cells were disrupted by homogenization in TESH buffer alone in the absence of KCl. The cell lysate was centrifuged at 27,000 *g* for 30 min. The resulting 27,000 *g* supernatant is being referred to as cytosol.

Subcellular distribution of radioactivity. Radioactivity in the 27,000 *g* supernatant and pellet was measured in aliquots of the respective fraction using Bray's scintillation solution with tritium counting efficiency of 30% [20]. To correct for counts associated with non-specific binding sites in the two subcellular fractions, cells were incubated with either 2×10^{-8} M labelled steroid alone or together with 10^{-5} M of the same unlabelled steroid. The amount of radioactivity recovered after three washes in subcellular fractions of cell suspensions incubated with tritiated steroid and 10^{-5} M unlabelled steroid was subtracted from that measured in samples incubated with the radioactive steroid alone. The value obtained is taken as the specifically bound radioactivity [10, 21].

Cytosol receptor depletion by steroids. A modification of the method described by Rousseau *et al.* was used to assess the cytosol receptor depletion activity of various steroids [11]. The total number of cytosol receptor sites in cells was measured following preincubation of cell suspensions at 0° or 37°C for 30 min without steroids. At the end of the incubation, cytosol fractions were prepared in the presence of near saturating concentrations of tritium labelled steroids (10^{-7} M), and incubated for 1–4 h at 0°C to achieve steady state binding. Parallel incubations contained 10^{-5} M of unlabelled steroid in addition to the tritium labelled steroid. At steady state (1 h incubation for progesterone and cortisol and 4 h for TA) free steroid was removed by adsorption to charcoal and the remaining bound steroid was counted [22]. The difference in binding between an incubation containing the labelled steroid alone and that containing [^{3}H]-steroid and 10^{-5} M unlabelled steroid was taken as specific binding.

To determine the cytosol-receptor depletion activity of the various steroids, cells were exposed to 2×10^{-8} M of [^{3}H]-steroids for 30 min at either 0°, or 37°C. The charcoal adsorption technique could not be used directly on the isolated cytosol to determine the concentration of cytosol receptors in cells exposed to the tritium labelled steroids. The reason for this is that saturation may not take place in intact cells, and dissociation of the steroid from the receptor may occur during preparation of the cytosol. Thus, to determine the concentration of the remaining cytosol receptors, this fraction was further incubated with near saturating concentrations of the same tritium labelled steroid used in the cell preincubations with and without an excess of unlabelled steroids. Specific binding was measured as described above.

Cell free binding analysis. Thymi were removed from rats and placed in a beaker containing ice-cold 0.9% NaCl solution. All of the experimental procedures were carried out at 0°–4°C. The thymi were cleaned on the back of a petri dish and minced well with scissors. A 10% homogenate was made using a Dounce homogenizer containing hypotonic Tris buffer, with radioactive steroids alone or together with the non-radioactive competing steroids. After

cell lysis, 1/10 volume of hypertonic Tris buffer was added and the suspension was further homogenized. Bound radioactivity was determined by the charcoal adsorption technique, and specific binding calculated as described in a previous publication [22]. The apparent K_I for progesterone or cortisol was calculated from double reciprocal plots. Bound radioactivity was assayed following incubations for 4 h with various concentrations of [^{3}H]-TA alone, or together with a single concentration of competing steroid. The apparent K_D was calculated from Scatchard plots [23] following incubations of the 27,000 *g* supernatant fraction with [^{3}H]-TA for 4 h or with [^{3}H]-progesterone for 1 h.

Other assays. Measurement of the biochemical response of thymocytes to TA and progesterone was done using the [^{3}H]-uridine uptake and incorporation assay as previously described [17]. Thymocyte viability was assayed by the trypan blue dye exclusion technique [19, 24]. Protein concentration was determined by the method of Lowry *et al.* using bovine serum albumin as a standard [25]. DNA was determined by the diphenylamine reaction with deoxyribose as a standard [26]. Radioactivity was assayed by liquid scintillation spectrometry using Bray's scintillation solution [20].

RESULTS

Competitive steroid interactions with the receptors

The inhibitory effects of equimolar concentrations of progesterone and cortexolone on the cell free binding of [^{3}H]-TA in rat thymus are compared in Table 1. Specific binding was measured following incubation at 0°C for 4 h (to obtain steady state binding) with 5×10^{-8} M of [^{3}H]-TA alone or together with 10^{-5} M of unlabelled TA. Binding was also measured in the presence of 10^{-8}–10^{-5} M of the two test steroids and expressed as percent of control. The results (Table 1) indicate that progesterone is a better competitor than cortexolone for TA binding at all concentrations used.

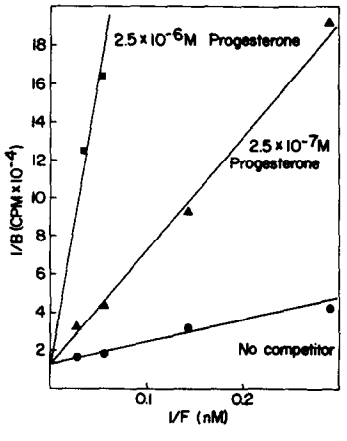

Fig. 1. Competitive inhibition of TA specific binding by progesterone. Specific binding of [^{3}H]-TA in the 27,000 *g* supernatant fraction of rat thymus was determined at various concentrations of [^{3}H]-TA and competing non-radioactive progesterone as indicated. Results were analyzed by a double reciprocal plot. B = bound steroid, F = free steroid.

Table 1. A comparison of the effects of equimolar concentrations of progesterone and cortexolone on the binding of [^{3}H]-triamcinolone acetonide ([^{3}H]-TA) in rat thymus

Competitor concentration	Competing steroid: Progesterone (% Control ± S.E.M.)	Competing steroid: Cortexolone (% Control ± S.E.M.)
Control†	100	100
10^{-8} M	94.0 ± 0.7	105.8 ± 3.3*
10^{-7} M	58.8 ± 1.8	97.3 ± 0.7*
10^{-6} M	22.5 ± 0.5	42.5 ± 1.2*
10^{-5} M	3.2 ± 0.6	10.7 ± 1.5*

† The 100% value assigned to the control binding activity was 3148 ± 106 c.p.m./mg protein in the progesterone competition studies, and 3087 ± 154 c.p.m./mg protein in the cortexolone competition studies.

* $P < 0.05$ as compared to progesterone.

Specific binding of [^{3}H]-TA in the soluble fraction of rat thymus was measured by the charcoal adsorption technique after incubation of cell free extracts with 5×10^{-8} M [^{3}H]-TA alone and together with 10^{-5} M nonradioactive TA, or a competing steroid as indicated. Control samples contained no competing steroids.

The inhibition by cortexolone of TA binding was of a competitive nature [10]. To determine whether progesterone resembles cortexolone in this respect we incubated various concentrations of [^{3}H]-TA with either no competitor or with 2.5×10^{-7} M or 2.5×10^{-6} M progesterone and assayed for specific binding. A double reciprocal plot of the results (Fig. 1) shows that progesterone is a competitive inhibitor of TA binding in rat thymus, suggesting that both TA and progesterone bind to the same macromolecule. The apparent K_I for progesterone was calculated to be 6×10^{-8} M and the apparent K_D for TA binding to be 1×10^{-8} M.

Relationship between binding and activity. A well-documented biochemical response to glucocorticoids in lymphoid tissues, which precedes lymphocytolysis is the decrease in uridine uptake and incorporation into RNA [27]. The effect on uridine metabolism was taken as a measure of an early response to glucocorticoid and lymphocytolysis as a measure of the delayed response, since significant cell death was not observed before 8 h of incubation with TA [19]. Both of these responses were measured at 37°C in suspensions of thymocytes and compared with cytoplasmic receptor binding carried out at 0°C under cell free conditions (Fig. 2). Good correlations were observed between occupancy of cytoplasmic binding sites by [^{3}H]-TA and the magnitude of the responses: inhibition of [^{3}H]-uridine metabolism and decreased survival of the cells, all of which reached a plateau at a concentration of 10^{-7} M TA.

A similar approach was used to compare the effect

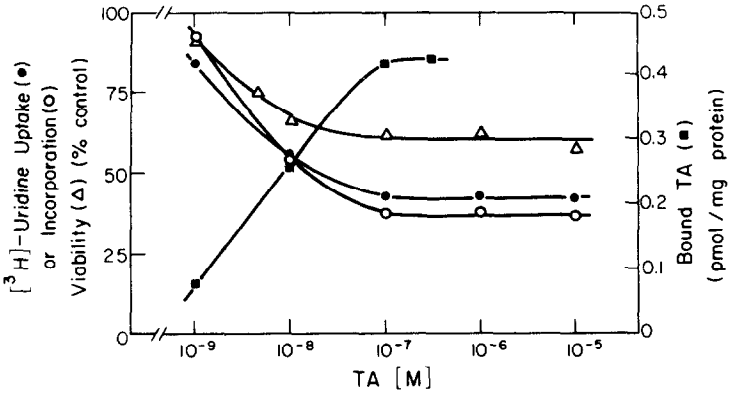

Fig. 2. Relationship between occupancy of cytosol binding sites and activity. The effect of increasing concentrations of TA on the uptake (●) and incorporation (○) of [^{3}H]-uridine into trichloroacetic acid (TCA) soluble and insoluble material was measured in thymocytes incubated *in vitro* at 37°C for 3 h. Viability (△) was determined following 15 h incubation at 37°C. Results are expressed as percent of control at various steroid concentrations. Specific binding of [^{3}H]-TA in the soluble fraction of rat thymus was assayed by the charcoal adsorption technique after incubation of cell-free extracts with various concentrations of [^{3}H]-TA for 4 h at 0°C. Results are expressed as bound steroid per mg protein (■) vs. free steroid concentration. Free steroid was calculated by subtracting the bound steroid from the total steroid added.

of various concentrations of progesterone on the binding of 5×10^{-8} M TA to thymus cytosol and on the [^{3}H]-uridine uptake response (TCA soluble radioactivity) to the same concentration of TA. A value of 100% was assigned to the 28% inhibition of [^{3}H]-uridine uptake produced by TA alone. The binding data are taken from Table 1. Figure 3 shows that the effect of progesterone on glucocorticoid binding parallels its effect on the biochemical response to glucocorticoids. The observation is valid at progesterone concentrations of 10^{-8}–10^{-6} M, where progesterone itself shows no effect on uridine metabolism. At concentrations higher than 10^{-6} M, progesterone alone enhances [^{3}H]-uridine uptake and incorporation which might explain the negative value obtained at a progesterone to TA ratio of 200:1. Similar results were obtained when [^{3}H]-uridine incorporation into TCA insoluble material was taken as a measure of the glucocorticoid effect (data not shown).

It was important to rule out an inhibitory effect of progesterone on the cellular uptake of TA. The data presented in Table 2 show that progesterone, at a 600-fold higher concentration than TA, inhibited TA uptake into thymocytes by only 13%. A comparable excess of unlabelled TA reduced the uptake of [^{3}H]-TA by 20%. These observations are consistent with a passive diffusion uptake of most steroids into target cells at physiologic concentrations [28].

Effects of agonist and antagonist steroids on the level of cytosol receptor

It is widely accepted that steroid hormones produce their effects at the nuclear locus of target cells [1]. Thus, it is possible that steroid antagonists which bind to the receptor but lack activity by themselves, have a different capacity to promote nuclear association of the receptor complex. To test this possibility, we compared the effects of the potent synthetic glucocorticoid, TA, with the less active, natural steroid cortisol, and the antagonist progesterone, on the depletion of cytosol receptors at 37°C. Following incubation of isolated thymocytes at 37°C alone or with 2×10^{-8} M of the various labelled steroids, the remaining cytosol receptor activity was assayed in the 27,000 *g* supernatant fraction using saturating concentrations of labelled steroids as described under Experimental. Control samples incubated without the steroids and assayed for specific binding with either progesterone, cortisol, or TA, showed similar levels

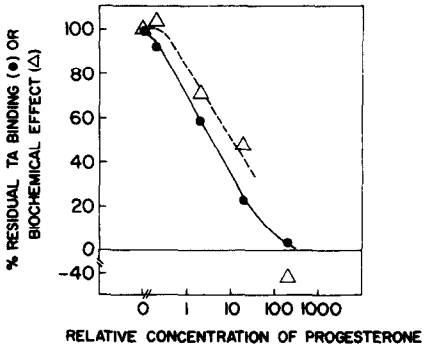

Fig. 3. The effect of progesterone on the biochemical response to TA and on the binding of [^{3}H]-TA. The biochemical response measured was the effect of TA on [^{3}H]-uridine uptake in rat thymocytes (△——△). A 100% value was given to the inhibition of [^{3}H]-uridine uptake (TCA soluble radioactivity) observed in the presence of 5×10^{-8} M TA alone. Competition by progesterone for specific TA binding (●——●) was measured in the 27,000 g supernatant fraction following incubation at 0°C for 4 h with 5×10^{-8} M [^{3}H]-TA alone and in presence of 10^{-5} M nonradioactive TA, or different concentrations of progesterone. Specific binding of [^{3}H]-TA in the absence of progesterone was taken as 100%.

Table 2. Effect of progesterone on the uptake of [^{3}H]-triamcinolone acetonide ([^{3}H]-TA) into rat thymocytes

Competing steroid	[^{3}H]-TA uptake (pmol/10^7 cells)	% Change
None	6.9	—
TA	5.5	−20
Progesterone	6.0	−13

Rat thymocytes (4×10^7 cells/ml) were incubated at 37°C for 30 min with [^{3}H]-TA (3.3×10^{-8} M) and [^{14}C]-sucrose (10^{-5} M) alone or together with unlabelled competing steroids (2×10^{-5} M). At the end of the incubation the cells were chilled in ice for 5 min and separated from the medium by centrifugation at 750 *g* for 5 min. Cell pellets were homogenized and radioactivity determined ([^{3}H] and [^{14}C]). Aliquots of medium were also counted for radioactivity. The [^{14}C] counts in the cell pellet were multiplied by the ratio of [^{3}H]/[^{14}C] in the medium to determine the amount of extracellular tritium labelled steroid present in the cell pellet [10]. Corrections were made for the [^{14}C] counts in the [^{3}H] channel using an internal standard.

of cytosol specific binding. Figure 4 shows the results of a series of experiments in which cells were preincubated with steroids, and the remaining cytosol binding activity compared to the control sample and expressed as percent of control. It can be seen that the cytosol binding activity that remained following exposure to TA was 10% of the control level. Following exposure to cortisol, 71% binding activity remained in the cytosol, while 86% remained following incubation with progesterone. When similar experiments were done at 0°C, a condition under which nuclear translocation of the steroid-receptor complexes is very slow [9], about 10% of the cytosol receptor activity was depleted by each of these steroids.

Association of receptor complexes with the 27,000 g pellet

In order to determine whether depletion of cytosol receptors is due to nuclear association of the steroid-receptor complexes, the effect of temperature on the specific binding of receptor complexes to the 27,000 *g* pellet, following 30 min incubation with 2×10^{-8} M [^{3}H]-steroids, was studied. In this part of the experiment, cells were extracted under low salt conditions which are not expected to extract chromatin associated complexes. The data in Table 3 show that in cells incubated with either TA or cortisol at 37°C, there was a marked increase in the level of specific radioactivity associated with the low salt pellets in comparison to the binding at 0°C. In contrast, the amount of the progesterone associated with these pellets was very high at both 0° and 37°C.

There is considerable evidence that active steroid-receptor complexes bound to chromatin can be extracted with buffers containing 0.15 M–0.4 M of KCl [1, 22]. It can be seen in Table 3 that the buffer containing 0.3 M KCl caused a marked decrease in the level of radioactivity associated with the 27,000 *g* pellet following 37°C incubation with TA or cortisol, but had no effect on progesterone binding to the pellet.

Given the high level of binding of progesterone to components in thymocytes which do not behave like steroid receptors, we tested for binding of progesterone to other cellular components, different from the glucocorticoid receptors. Cells were incubated at 0°C

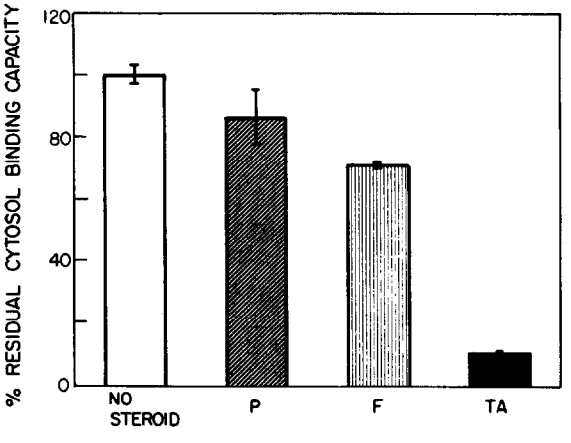

Fig. 4. Effect of triamcinolone acetonide (TA), cortisol (F) and progesterone (P) on the concentration of cytosol receptors. Thymocytes (4×10^7 cells/ml) were incubated in RPMI 1640 medium for 30 min at 37°C with 2×10^{-8} M of tritium labelled steroids as indicated. Control samples containing no steroid were run in parallel incubations. At the end of the incubation period, cells were cooled on ice and washed three times with fresh medium. Cytosol fractions were prepared in the presence of saturating concentrations of [^{3}H]-steroids and cytosol receptor concentration determined as described under Experimental. Results are expressed as percent of receptor concentration in samples incubated without steroids. The size of the bars and the vertical lines represent mean ± S.E.M. of three separate experiments. The residual cytosol binding with TA and cortisol are significantly different from controls ($P < 0.05$).

Table 3. Effects of temperature and salt concentration on the level of radioactivity associated with the 27,000 *g* pellet

[^{3}H]-steroid	Incubation temperature (°C)	Extraction condition	Specifically bound steroid (fmol/μmol deoxyribose)
TA	0°	Low salt	60
		High salt	−10
	37°	Low salt	860
		High salt	260
Cortisol	0°	Low salt	30
		High salt	20
	37°	Low salt	210
		High salt	40
Progesterone	0°	Low salt	1990
		High salt	1980
	37°	Low salt	1530
		High salt	1630

Rat thymocytes (4×10^7 cells/ml) were incubated in RPMI 1640 medium with 2×10^{-8} M of [^{3}H]-TA, [^{3}H]-cortisol, or [^{3}H]-progesterone alone or with a 500-fold higher concentration of the respective cold steroids. After 30 min incubation at the indicated temperature, cells were centrifuged and washed three times with cold fresh medium. Cell pellets were extracted by homogenization in hypotonic TESH buffer (low salt), or hypotonic TESH buffer followed by hypertonic TESH buffer to give a final concentration of 0.3 M KCl in the extraction buffer (high salt). Specifically bound radioactivity associated with the 27,000 *g* pellet was determined by subtracting the radioactivity determined in presence of an excess of cold steroid from that observed in the absence of cold steroid. Results are the average of two to three experiments.

for 30 min with a saturating concentration of unlabelled TA (10^{-7} M). Subsequently, 2×10^{-8} M of either [^{3}H]-TA, [^{3}H]-cortisol, or [^{3}H]-progesterone alone or together with 10^{-5} M of the same unlabelled steroids were added to the cells and the incubation continued for an additional 15 min. Subcellular fractions were prepared under low salt conditions following three successive washes with fresh medium to remove free and loosely bound steroids. We observed no specific binding of [^{3}H]-TA or [^{3}H]-cortisol in either the 27,000 *g* supernatant or pellet, but we did observe specific binding of [^{3}H]-progesterone: 1300 ± 20 fmol/mg protein in the 27,000 *g* supernatant and 1100 ± 50 fmol/μmol deoxyribose in the 27,000 *g* pellet.

Cytosol binding of glucocorticoids and progesterone

Table 4 summarizes two types of binding studies: one which is analyzed by a Scatchard plot to give an apparent K_D value and the number of binding sites for TA and progesterone, and the other which is analyzed by a double-reciprocal plot to give an apparent K_I value for cortisol and progesterone in competition with TA and the number of their binding sites. A comparison of the Scatchard analysis with the double-reciprocal competition analysis shows that progesterone binds to more sites than could be accounted for by the availability of glucocorticoid receptor sites. The apparent K_D and K_I for the progesterone binding sites were of a similar magnitude, suggesting similar affinities of progesterone to both the glucocorticoid site and the additional site(s).

DISCUSSION

The studies reported in this paper demonstrate that in rat thymus, *in vitro*, progesterone behaves as a glucocorticoid antagonist. This is indicated by the obser-

Table 4. A comparison between apparent K_D and K_I for cytosol binding of glucocorticoid analogs

Glucocorticoid analog	Apparent K_D* (M)	Number of sites** (fmol/mg protein)	Apparent K_I (M)	Number of sites (fmol/mg protein)
Triamcinolone acetonide	9×10^{-9} (5) $\pm 0.1 \times 10^{-9}$	397 ± 57 (238–577)	....	—
Progesterone	6.4×10^{-8} (3) $\pm 0.5 \times 10^{-8}$	920 ± 98 (755–1106)	6×10^{-8}†	290†
Cortisol	—	—	8×10^{-8}	577

* Mean ± S.E.M. Numbers in parentheses are number of separate experiments.

** Mean ± S.E.M. Numbers in parentheses represent range.

† Calculated from Fig. 1.

Apparent K_D was calculated from Scatchard plots, and apparent K_I from double reciprocal plots. In the competition analyses, competing steroid concentrations were: for cortisol = 2.5×10^{-7} M; for progesterone = 2.5×10^{-6} M and 2.5×10^{-7} M.

vation of the competitive inhibition by progesterone of TA binding to cytoplasmic sites (Fig. 1), which correlated with its inhibition of the expression of the early effect on uridine metabolism (Fig. 3). An effect by progesterone on the uptake of TA into thymocytes (Table 2) could not account for the marked inhibition of the TA effect on [^{3}H]-uridine uptake and incorporation into RNA.

A good correlation was observed between occupancy by TA of cytoplasmic binding sites and glucocorticoid activity, i.e. the early biochemical effects on uridine metabolism and the later thymolytic effect (Fig. 2). This observation together with the previous one concerning the progesterone antagonism of TA action, suggest that the availability of cytoplasmic binding sites plays a major role in the expression of glucocorticoid activity in normal thymocytes.

In comparing the effects of TA, cortisol and progesterone on the temperature dependent depletion of thymocyte cytosol binding components, it was observed that whereas TA and, to a lesser extent, cortisol depleted the cytosol of specific glucocorticoid binding sites, progesterone was inactive in this regard (Fig. 4). The differences in cytosol receptor depletion activities of equimolar concentrations of the agonists, TA and cortisol, could be rationalized by their different affinities to the cytosol receptors (Table 4, Ref. 10). At 2×10^{-8} M TA is expected to occupy ~75% and cortisol ~20% of the cytoplasmic sites in thymus homogenates, which correlate well with the values of cytosol receptor depletion: 90% for TA and 29% for cortisol. Progesterone which binds to the glucocorticoid receptors with an apparent affinity similar to cortisol ($K_I = 6 \times 10^{-8}$ M) caused only 14% depletion of cytosol binding sites, an effect which was not significantly different from controls. However, the method used for determining the concentration of cytosol receptors could not rule out their intracellular redistribution. Cells preincubated with steroids at 0° or 37°C were broken by hypotonic shock using a low salt buffer. While this technique is necessary to prevent extraction of chromatin bound receptor complexes, it causes some nuclear damage that could result in redistribution of free nuclear receptor complexes. Thus, our results might not distinguish between a defect in the nuclear uptake of the progesterone–receptor complex and its failure to bind to chromatin. In an attempt to resolve this problem, the association of various steroid–receptor complexes with the 27,000 *g* pellet was studied. Unlike TA and cortisol (Table 3), the progesterone-receptor complex did not show a temperature dependent translocation to the nucleus to yield a bound complex that was salt extractable. However, unlike cortexolone, which showed only minimal nuclear binding [10], progesterone bound tightly and extensively to the 27,000 *g* pellet at both 0° and 37°C. This observation is difficult to explain in view of the low cytosol receptor depletion activity of progesterone and its lack of glucocorticoid activity. These data, however, are compatible with the suggestion that progesterone might be bound to a second cytoplasmic component different from the glucocorticoid receptor that binds very tightly to a fraction in the 27,000 *g* pellet. This is further supported by the observation of additional progesterone binding at 0°C in cells which were preincubated with saturating concentrations of TA, and the excess number of binding sites observed in the Scatchard analysis, as compared to double-reciprocal plots (Table 4). The nature and function of this second binding component(s) has not been defined. It is possible, however, that the second progesterone binder detected in our experiments is related to a 20α-hydroxysteroid dehydrogenase which metabolizes progesterone and was recently identified in thymic extracts from mice by Weinstein *et al.* [29]. Alternatively, it could be an intracellular CBG-like protein [30].

The observation (Table 3) that at 0°C more radioactivity is associated with the 27,000 *g* pellet than at 37°C could have resulted from a higher intracellular concentration of progesterone at 0°C. It was reported that certain mammalian cells can actively transport steroids outward in a temperature dependent process [31]. Progesterone was shown to be among the steroids using this transport system in mouse fibroblasts which is a target tissue for glucocorticoids and behaves in many respects like rat thymocytes [21].

Our studies [7, 10] with the antiglucocorticoid cortexolone as well as studies by others [5, 8] have suggested that steroid hormone antagonists might bind to a different, "inactive," conformation of the cytoplasmic receptor. Sedimentation profiles of [^{3}H]-cortexolone as compared with [^{3}H]-TA receptor complexes [7] and [^{3}H]-spironolactone as compared to [^{3}H]-aldosterone–receptor complexes [8] strongly support this hypothesis. We could not obtain similar sedimentation profiles of [^{3}H]-progesterone–receptor complexes in rat thymocytes due to the instability of these complexes.

Progesterone has been reported to exert antiglucocorticoid activity in a number of systems *in vitro* [11–15], and in the present study it was found to be a potent glucocorticoid antagonist in rat thymocytes. In man, progesterone was found to be devoid of glucocorticoid activity, although several analogs of this steroid showed such activity [32]. Still, at very high concentrations, such as could be achieved in the foetoplacental unit, progesterone has been reported to be immunosuppressive [33]. In spite of the observation of pure antagonism by progesterone of glucocorticoid binding and early biochemical effects *in vitro*, progesterone derivatives were ineffective in antagonizing cortisol induction of liver tyrosine aminotransferase *in vivo* [34]. The failure to obtain antagonism *in vivo* could have resulted from the complexity of the system, due to multiple interactions between progesterone and cortisol at the level of the plasma proteins in addition to the cellular receptor sites [35].

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