EFFECTS OF PROGESTERONE ON THE BINDING OF ESTRADIOL-RECEPTOR TO RABBIT UTERINE CHROMATIN

M. L. Chatkoff and J. A. Julian

Departments of Obstetrics and Gynecology and Biochemistry
University of Texas Health Science Center at San Antonio, Medical School

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SUMMARY

Three day progesterone treatment of ovariectomized rabbits increased in vitro uterine estrogen-receptor binding to uterine chromatin. The increased binding was traced to changes in chromatin but not the cytosol. Both the number of chromatin acceptor sites and the binding affinity were higher in treated animals. Furthermore, chromatin acidic protein to DNA ratios from treated rabbits were higher by approximately the same factor as for binding sites. A mechanism of synergistic interaction is suggested.

INTRODUCTION

A synergistic effect of progesterone on estrogen-induced increase in RNA and protein concentrations has been described in the mouse uterus (1,2). The molecular mechanism of estrogen-progesterone interaction is unknown. Although estrogen binds to specific receptors in target tissue cytoplasm (3), progesterone does not appear to compete with estrogen for these sites (4).

A second location of interaction between steroid hormones and their target organs resides in target cell nuclei. Both estrogen-receptor complexes and progesterone-receptor complexes bind to a chromatin component in target tissues (5), suggesting an interactive site. Progesterone enhancement of estrogen action at the chromatin level could be the result of an increase in the number of available chromatin binding sites or to an increase in the affinity of chromatin for the hormone-receptor complex, or both. Experiments were designed to investigate these possibilities.

METHODS

Preparation of Chromatin. Eighteen ovariectomized mature female New Zealand white rabbits were divided into two equal groups. One group was given 2.5 mg of progesterone (Upjohn) in 0.5 ml cottonseed oil I.M. for

three successive days beginning on the fourth day following castration. The other group was untreated. All rabbits were killed on day seven post castration. Three sets of uterine nuclei were prepared from each group following the method of Steggles et al (6). Chromatin was purified by the method of Spelsberg and Hnilica (7).

Receptor-Chromatin Binding Assay (RCBA). 105,000 g supernatant containing estrogen receptor was isolated from an additional eighteen one-week castrate rabbit uteri which were either non-treated or progesterone treated as above. The cytosol (10 mg/ml) was labeled with (6,7-H^3)-estradiol-17β (Schwartz Bioresearch, Sp. Act. 48,000 Mc/MMole) at a concentration of 1.5 X 10^{-8}M for 1.5 hr. at 4°C. Free estradiol was removed by incubation with dextran-coated charcoal (15 min. at 4°C). Increasing amounts of H^3-estradiol-uterine receptor complex were incubated for one hour at 4°C with 30 to 50 µg of chromatin. The remaining assay procedure followed that of Steggles et al (5) except that total and free estradiol-receptor concentration was expressed in Moles per g of DNA added. Total receptor added was determined by (5 to 20 percent) sucrose gradient centrifugation of an aliquot of cytosol and H^3-estradiol added in the RCBA. The pMoles bound in the 8S peak were corrected to pMoles/µg DNA by assaying an aliquot of the chromatin suspension added in the RCBA. In calculating Lineweaver-Burke plots, receptor bound on the filters was adjusted to pMoles/µg DNA by extracting DNA from the dried filters (in 0.5 N PCA 30 min. at 90°C) and assaying according to Burton. All combinations of untreated and progesterone treated cytosol and chromatin were assayed (Fig. 1, legend).

Analysis of Protein/DNA Ratios. Total protein, basic and acidic proteins and DNA concentrations were determined using separate aliquots for each assay. Basic and acidic proteins were extracted according to Spelsberg and Hnilica (7) and assayed by the method of Lowry et al (8) using bovine serum albumin as a standard for total and acidic protein and calf thymus histone (Schwartz/Mann) as a standard for basic protein. DNA was assayed by the method of

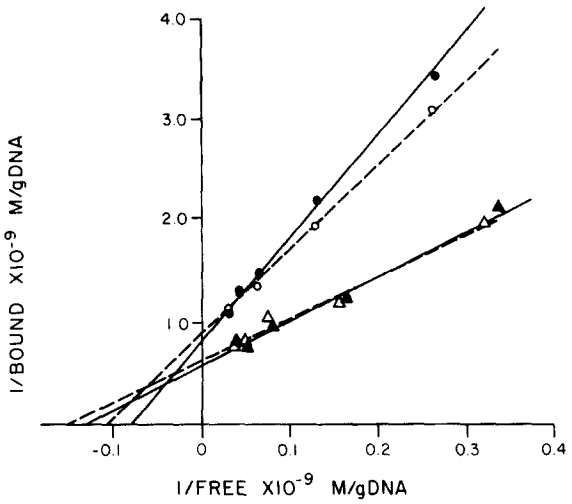

Figure 1. Lineweaver-Burke estrogen-receptor chromatin binding plots for combinations of progesterone treated and untreated chromatin and cytosol.

1a) —▲—▲—▲—▲—▲— progesterone treated chromatin and untreated cytosol

1b) --o--o--o--o--o-- untreated chromatin and untreated cytosol

2a) --Δ--Δ--Δ--Δ--Δ-- progesterone treated chromatin and progesterone treated cytosol

2b) —●—●—●—●—●— untreated chromatin and progesterone treated cytosol

Assays (a) and (b) were performed simultaneously with a single cytosol sample. A similar pairing was used for assays 2a and 2b and for all other assays in Table I.

Burton (9) using calf thymus DNA (Calbiochem Sodium Salt, A grade) as a standard. In all assays the standards were treated in the same manner as the samples.

Calculations. Lineweaver-Burke plots were calculated using a weighted least squares regression analysis. The weighting function, proportional to the square of the amount bound, compensates for magnification of errors at low concentration caused by the mathematical transformation to reciprocals. Measurement errors were found to be independent of the amount bound and therefore did not contribute to the weighting factor.

RESULTS

Four-way comparisons of Lineweaver-Burke plots show a significant dif-

TABLE I

COMPARISON OF LINEWEAVER-BURKE LINE SLOPES, ESTROGEN ACCEPTOR SITES, AND DISSOCIATION CONSTANTS FOR COMBINATIONS OF NON-TREATED AND PROGESTERONE TREATED CHROMATIN AND CYTOSOL

	UNTREATED CHROMATIN			PROGESTERONE TREATED CHROMATIN			RATIOS OF PROGESTERONE TREATED TO UNTREATED CHROMATIN		
	Line Slope	Estrogen Acceptor Sites M/g DNAx10^{-9}	K_D M/g DNAx10^{-9}	Line Slope	Estrogen Acceptor Sites M/g DNAx10^{-9}	K_D M/g DNAx10^{-9}	Line Slope Ratio	Estrogen Acceptor Site Ratio	K_D Ratio
Untreated Cytosol	9.0 ±1.1	0.95 ±.13	8.3 ±.3	4.1 ±.1	1.35 ±.23	5.6 ±1.1	.46 ±.06	1.39 ±.08	0.65 ±.10
Progesterone Treated Cytosol	7.0 ±2.3	0.99 ±.13	7.2 ±2.8	3.0 ±0.9	1.45 ±.06	4.5 ±1.3	.45 ±.03	1.51 ±.16	0.68 ±.10
Ratio of Treated to Untreated Cytosol	0.85 ±.31	1.09 ±.22	0.82 ±.30	0.74 ±.21	1.15 ±.21	0.77 ±.13			

ference in kinetics between chromatins but not cytosols (Fig. 1). Progresterone treated chromatin demonstrated both a greater number of acceptor sites and a higher affinity for receptor. Additional binding assays (three assays of each of the four combinations) confirmed these findings (Table I). A good linear fit was obtained for all 12 assays (mean correlation coefficient 0.99). Significant differences in line slopes between progesterone treated and non-treated chromatin were found ($P < .001$; all assays irrespective of cytosol treatment). Cytosol line slope differences were not significant ($P > .1$). Significantly increased available chromatin acceptor sites (mean increase of 45 percent) with progesterone treatment was found ($P < .005$), but binding site differences between cytosols were not significant ($P > .1$). The dissociation constants were significantly smaller ($P < .05$) for treated than non-treated chromatin but not between treated and non-treated cytosol ($P > .1$).

An analysis of variance (which ignores the pairing of assays) also showed a significant difference ($P = .02$) between chromatins but not between cytosols ($P > .6$) (Table II). The lack of an interactive effect between cytosols and chromatins strengthens the reliability of the conclusions.

No significant difference in the kinetics of binding was found when *progesterone receptor was present during incubation of non-treated chromatin with estrogen receptor (Fig. 2).

Protein measurements of the six chromatins from the binding assay plus two additional preparations indicated significantly increased acidic proteins per gram of DNA (an average of 54 percent) by progesterone treatment ($P < .005$) (Table III). Total protein to DNA ratios, however, showed no significant corresponding increase.

The average ratio of binding sites for treated to untreated chromatin was 1.45. The average ratio of acidic protein concentrations was 1.54. The difference between three pairs of these two ratios was not significant ($P > .2$).

DISCUSSION

The results indicate that treating ovariectomized rabbits for three days

*confirmed by sucrose gradient analysis

TABLE II

ANALYSIS OF VARIANCE OF CHROMATIN ESTROGEN ACCEPTOR BINDING SITES

Source	M.S.	D.F.	F-Ratio	P
Total	0.11	11.		
Between	0.19	3.		
Chromatins	0.53	1.	7.76	.02†
Cytosols	0.02	1.	0.23	0.65
Chromatin x Cytosol Interaction	0.00	1.	0.06	0.80
Within	0.07	8.		

Means	Untreated M/g DNA x 10^{-9}	Progesterone Treated M/g DNA x 10^{-9}
Chromatin	0.96	1.39
Cytosol	1.14	1.21

†significant at between the 5 and 1% levels.

TABLE III

PROTEIN COMPOSITION OF UNTREATED AND PROGESTERONE PRIMED CHROMATIN

	Basic Protein mg/mg DNA	Acid Protein mg/mg DNA	Basic/Acidic
Untreated Chromatin	1.54 1.47 1.35 1.33 1.43±.05	0.48 .48 .37 .47 .45±.03	3.07 3.10 3.68 2.81 3.17±.18
Progesterone Treated Chromatin	1.44 1.22 1.34 1.35 1.33±.04	.74 .60 .75 .67 .70±.04	1.95 2.02 1.78 2.04 1.95±.06

with progesterone enhances estrogen-receptor binding to uterine chromatin *in vitro* by increasing both the number of available acceptor sites and the affinity of binding. Although values of .96 and 1.39 X 10^{-9}M/g DNA were obtained for the average number of acceptor sites per g DNA (Table II) in untreated

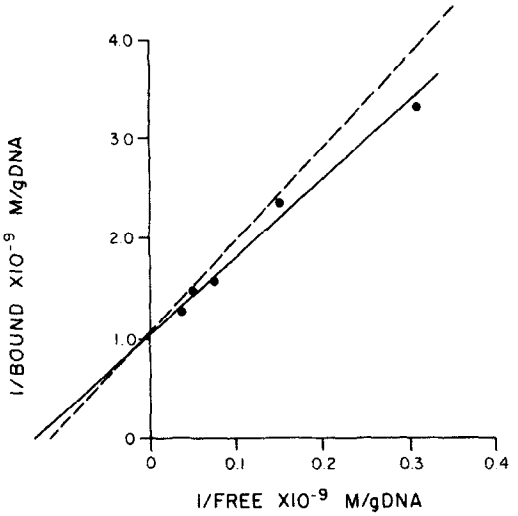

Figure 2. Lineweaver-Burke comparative analysis of progesterone-receptor competition for estrogen-receptor chromatin sites.

a) — — — — — — average kinetics for untreated chromatin and untreated cytosol, calculated from line slopes and binding sites in Table I.

b) —●—●—●—●— identical assay to a) except that the incubation of ^{3}H-estradiol with cytosol was carried out in the presence of an equal concentration of progesterone. After charcoal assay to remove free steroid, the cytosol containing both steroid-receptor complexes was incubated with chromatin.

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and progesterone treated chromatin respectively, these may not reflect in vivo quantities. It has been demonstrated that kinetic parameters are functions of the incubation conditions (10). By pairing the treated and non-treated chromatin assays the results expressed as ratios may correspond to in vivo values. Neither direct nor indirect competition by progesterone for estrogen binding sites is found upon incubation of non-treated chromatin in the presence of estrogen and progesterone. This finding strengthens the conclusion that the interaction occurs through alteration of the number of binding sites and not by direct interaction.

The failure to find a significant cytoplasmic effect on receptor-chromatin binding does not entirely rule out such a possibility. Results were expressed in a form independent of cellular levels of estrogen receptor. Furthermore,

standard errors between cytosol groups were significantly larger than between chromatin groups, so that a small cytosol effect may have gone undetected.

Another possible explanation of the results is if progesterone treatment altered endometrial to myometrial cell number ratios. Apparent changes in the average chromatin composition and binding could result if the chromatins from the respective tissues differed. However, it has been repeatedly demonstrated that progesterone does not induce a significant increase in whole uterine DNA content and therefore it is presumed that cell ratios remain approximately constant.

Ovariectomy reduces chromatin acid protein by more than 50 percent (11, 12). In contrast to previous whole nuclear assays (13), progesterone treatment of ovariectomized rabbits increased chromatin acid protein toward non-castrate levels. However, progesterone-induced acid proteins may not be identical to those lost following castration.

The approximately proportional increase in binding sites and acid protein levels in treated animals strengthens the hypothesis that the synergistic interaction of progesterone with estrogen is mediated by progesterone induction of additional nuclear acidic protein leading to increased nuclear acceptor sites for estrogen-receptor complexes.

REFERENCES

1. Martin, L. and Finn, C. A. in Basic Actions of Sex Steroids on Target Organs. Hubinont, P. O., Leroy, F. and Geland, P., eds. New York, S. Karger, pp. 163-181 (1971).
2. Bronson, F. H. and Hamilton, T. H. Biol. Reprod. 6, 160 (1972).
3. Toft, D. and Gorski, J. Proc. Nat. Acad. Sci. (U.S.) 55, 1574 (1966).
4. Toft, D., Shyamola, G. and Gorski, J. Proc. Nat. Acad. Sci. (U.S.) 57, 1740 (1967).
5. Steggles, A. W., Spelsberg, T. C., Glassner, S. R. and O'Malley, B. W. Proc. Nat. Acad. Sci. (U.S.) 68, 1479 (1971).
6. Steggles, A. W., Spelsberg, T. C. and O'Malley, B. W. Biochem. Biophys. Res. Comm. 43, 20 (1971).
7. Spelsberg, T. C. and Hnilica, L. S. Biochem. Biophys. Acta 228, 202 (1971).
8. Lowry, O. H., Rosebrough, N. J., Farr, A. L. and Randall, R. J. J. Biol. Chem. 193, 265 (1951).
9. Burton, K. Biochem. J. 62, 315 (1956).
10. McGuire, W. L., Huff, K. and Chamness, G. C. Biochem. 11, 4562 (1972).
11. Teng, C.-S. and Hamilton, T. H. Proc. Nat. Acad. Sci. (U.S.) 60, 1410 (1968).
12. Chatkoff, M. L. and Julian, J. A., unpublished.
13. Smith, J. A., Martin, L., King, R. J. B. and Vértes, M. Biochem. J. 119, 773 (1970).