A SPECIFIC GLUCOCORTICOID BINDING MACROMOLECULE OF RABBIT UTERINE CYTOSOL*

George Giannopoulos**

Department of Experimental Medicine, McGill University and the University Clinic, Royal Victoria Hospital, Montreal, Canada.

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SUMMARY. A high affinity (K_d=2.7 x 10^{-10}M at 0°) dexamethasone binding macromolecule has been identified in the cytosol fraction of rabbit uteri. Competition studies show high specificity for glucocorticoids since binding of labeled dexamethasone is inhibited by cortisol and corticosterone but not by progesterone, testosterone, or estradiol 17β. The binding component has a sedimentation coefficient of 8S and its concentration in uterine cytosol is about 0.2 pmoles per mg protein. Uptake of labeled dexamethasone by isolated uterine nuclei requires the presence of cytosol and is temperature dependent. The KCl-extractable nuclear complex sediments at 4S. Thus the dexamethasone binding components of the rabbit uterus have properties similar to those described for steroid hormone receptors present in target tissues. Specific dexamethasone binding could not be demonstrated in rat uterine cytosol.

INTRODUCTION. The uterus is a target organ for a variety of hormones including estrogens, progestins and androgens (1). Although glucocorticoids do not promote uterine growth (2), they inhibit the estrogen-induced fluid imbibition and cause a partial inhibition of long-term growth responses to estrogens (3,4). This inhibitory effect is also observed in hypophysectomized animals suggesting a direct action of glucocorticoids in the uterus (2).

An early event in the action of steroid hormones is binding to specific cytoplasmic receptors followed by translocation of the steroid-receptor complex to the nucleus where physiological changes are believed to be initiated via a modification of genetic expression (5). Such receptor molecules have been found in most steroid-sensitive tissues which have been investigated (5). The interaction of estrogens and progesterone with specific receptors in uteri of several species has been studied extensively (6). It has also been shown that the rat uterus contains specific binding proteins for testosterone (7). If the uterus is a target for glucocorticoids, one would expect it to contain glucocorticoid receptors. The present studies demonstrate the presence of a dexamethasone binding macromolecule in rabbit uterine cytosol. This macromolecule also has an affinity for cortisol and corticosterone but not for progesterone, testosterone and estradiol-17β, and it therefore has a high binding specificity for glucocorticoids. However, with the methods used specific dexamethasone binding could not

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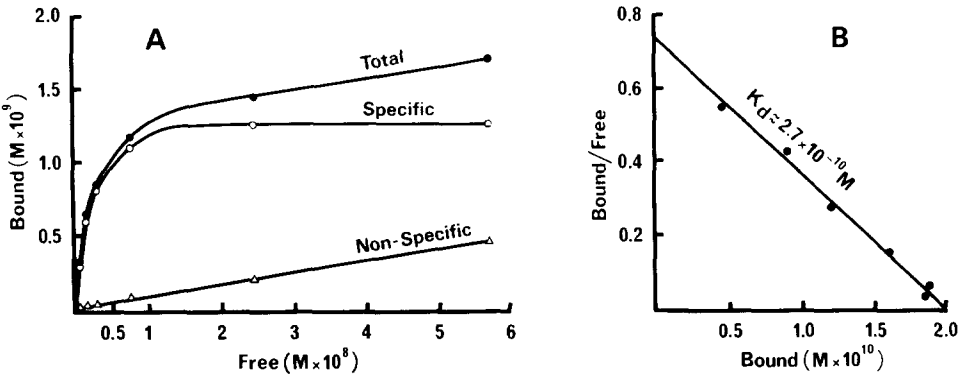

Fig. 1. A. Concentration-dependence of binding of ^{3}H-dexamethasone to rabbit uterine cytosol. Aliquots of cytosol were incubated for 2 hrs at 0° with increasing concentrations of ^{3}H-dexamethasone alone or together with an 100-fold concentration of non-labeled dexamethasone. ^{3}H-Dexamethasone binding was measured by a charcoal assay (8). Specific binding of ^{3}H-dexamethasone was calculated by subtracting the amount bound in the presence of 100-fold excess of non-labeled steroid (non-specific binding) from the total binding (in the absence of non-labeled steroid). B. Scatchard plot of ^{3}H-dexamethasone to rabbit uterine cytosol. Data were taken from the results shown in A.

be demonstrated in the cytosol fraction of the rat uterus.

METHODS. Mature intact female New Zealand White rabbits (body wt. 2.7-3.2 Kg) and mature hooded Long Evans female rats (body wt. 200-250 g) were used in these studies. The rats were either intact or adrenalectomized 15 days before being used. Uteri were minced and homogenized in 5 volumes (w/v) of 0.01M Tris-HCl buffer, pH 7.4, containing 0.0015M Na_2EDTA (Buffer A). The cytosol was prepared by centrifugation of the homogenate at 224,000 x g for 30 min. Aliquots of the cytosol were incubated for 2 hrs at 0° with ^{3}H-dexamethasone (22 Ci/mmole) alone or in combination with non-labeled steroid. Specific binding of ^{3}H-dexamethasone was then measured by a charcoal assay and the binding components were characterized by sucrose density gradient centrifugation. The charcoal assay and methods of sucrose gradient centrifugation, fractionation, and measurement of radioactivity have been described earlier (8). Specific uptake of ^{3}H-dexamethasone by isolated uterine nuclei was studied as described in the legend to Fig. 3.

RESULTS. When rabbit uterine cytosol was incubated with increasing concentrations of ^{3}H-dexamethasone and analyzed by adsorption of the free hormone on charcoal, saturation of a limited number of binding sites was observed (Fig. 1A). A plot of the binding data according to Scatchard (9) indicated a single class of high affinity binding sites with a dissociation constant of 2.7×10^{-10}M (Fig. 1B). The concentration of binding sites was calculated from the results of eight experiments to be approximately 0.2 pmoles per mg of cytosol protein.

To determine whether dexamethasone is bound to macromolecular components, the

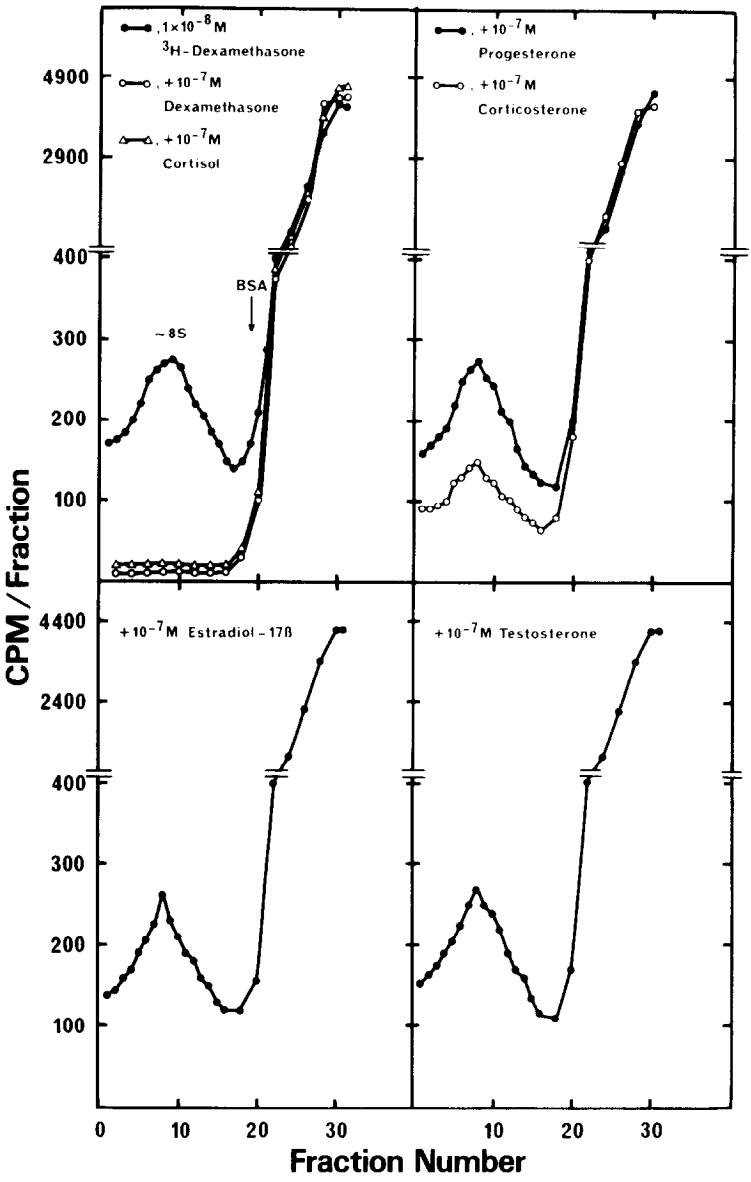

Fig. 2. Sedimentation of bound ^{3}H-dexamethasone on sucrose gradients and competition by various non-labeled steroids for binding of ^{3}H-dexamethasone to rabbit uterine cytosol. Aliquots of the cytosol were incubated for 2 hrs at 0° with 1 x 10-8M ^{3}H-dexamethasone alone or together with 1 x 10-7M of non-labeled steroid. Samples were layered on 10-30% sucrose gradients prepared in Buffer A and centrifuged for 16 hours at 55,000 rpm in a SW56 rotor. BSA, bovine serum albumin.

cytosol was incubated with 1 x 10^{-8}M ^{3}H-dexamethasone and aliquots were layered on 10-30% sucrose gradients containing Buffer A. A clear peak of ^{3}H-dexamethasone binding was observed (Fig. 2). The ^{3}H-dexamethasone binding material had a sedi-

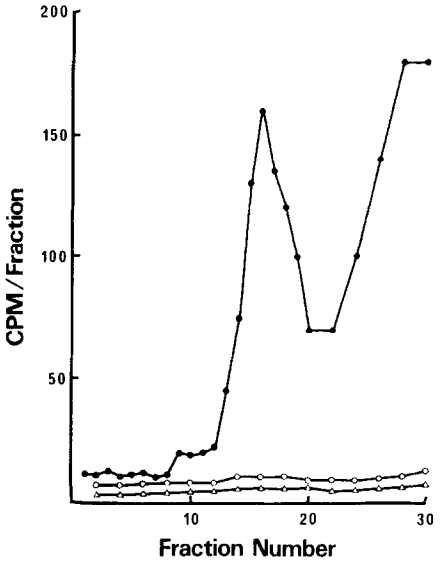

Fig. 3. Effect of uterine cytosol and temperature on the uptake of ^{3}H-dexamethasone of isolated uterine nuclei. Rabbit uteri were homogenized in 0.25M sucrose-0.01M Tris-0.001M $MgCl_2$ buffer pH 7.4 (Buffer B). The homogenate was filtered through four layers of cheesecloth and centrifuged at 700 x g for 10 min. The 700 x g supernatant was centrifuged for 30 min at 224,000 x g to prepare the cytosol. The 700 x g pellet was washed 3 times with Buffer B to obtain a washed nuclear pellet. Aliquots of the nuclei were suspended either with Buffer B containing 1 x 10^{-8}M ^{3}H-dexamethasone or with the uterine cytosol which had been incubated with 1 x 10^{-8}M ^{3}H-dexamethasone at 0° for 2 hrs. The nuclear suspensions were incubated for 30 min at 0° or 25°. Following incubation, the nuclei were centrifuged, washed 3 times with buffer, and extracted with Buffer A containing 0.4M KCl. Aliquots of the nuclear extracts were layered on 5-20% sucrose gradients prepared in Buffer A containing 0.4M KCl and centrifuged for 16 hrs at 55,000 rpm in a SW56 rotor. ●—● , nuclei incubated with uterine cytosol at 25°; o—o ,nuclei incubated with uterine cytosol at 0°; Δ—Δ , nuclei incubated with Buffer B at 25°.

mentation coefficient of approximately 8S. The specificity of dexamethasone binding to uterine cytosol and the relative affinity of the binding components for various steroids was examined by sucrose gradient and charcoal adsorption analysis. The stereo-specific nature of the binding sites was inferred from the observations that dexamethasone and cortisol completely inhibited binding of ^{3}H-dexamethasone to the 8S component whereas progesterone, testosterone and estradiol 17β did not compete for binding sites. Corticosterone also inhibited binding of ^{3}H-dexamethasone but it was less effective than dexamethasone and cortisol (Fig. 2). Similar results were obtained by charcoal analysis (data not shown).

When isolated uterine nuclei were incubated with buffer containing ^{3}H-dexamethasone, specific uptake of the hormone was not observed. Incubation of the nuclei with ^{3}H-dexamethasone in the presence of the uterine cytosol resulted in significant hormone uptake at 25° but not at 0°. Following extraction of

TABLE 1. SPECIFIC BINDING OF ^{3}H-DEXAMETHASONE TO RABBIT AND RAT UTERINE CYTOSOL.

Source of uterine cytosol	Bound ^{3}H-Dexamethasone* (pmoles/mg protein $\pm$ SEM)
Rabbit (intact)	0.19 $\pm$ 0.03
Rat (intact)	not detected
Rat (adrenalectomized)	not detected

*Aliquots of cytosol (5.8-7.9 mg protein per ml) prepared from rabbit or rat uteri were incubated for 2 hrs at 0° with 1 x 10^{-8}M ^{3}H-dexamethasone alone or in combination with 1 x 10^{-6}M non-labeled dexamethasone. Specific binding of ^{3}H-dexamethasone was measured by a charcoal assay (8). The values shown represent the mean of 6 experiments in duplicate or triplicate.

the nuclei with buffer containing 0.6M KCl and centrifugation of the extract on 5-20% sucrose gradients, ^{3}H-dexamethasone was associated with components sedimenting near 4S (Fig. 3). Thus specific uptake of ^{3}H-dexamethasone by uterine nuclei is temperature dependent and requires the presence of cytosol.

To date, attempts to demonstrate dexamethasone binding in rat uterine cytosol have not been successful. Specific binding of ^{3}H-dexamethasone could not be detected in cytosol fractions of uteri from intact or adrenalectomized adult rats (Table 1). Similarly, sucrose gradient analysis did not show association of ^{3}H-dexamethasone with macromolecular components or rat uterine cytosol (not shown).

DISCUSSION. The techniques of charcoal adsorption and sucrose gradient analysis have been employed to demonstrate the presence of specific, high affinity, glucocorticoid binding macromolecules in the cytosol of uteri from sexually mature rabbits. The concentration of specific binding sites in rabbit uterus and the properties of the uterine binding component, including its dissociation constant, steroid binding specificity and sedimentation coefficient, are similar to those reported for specific glucocorticoid receptors in fetal rabbit liver (8) and adult rat liver (10). It has also been shown that specific uptake of dexamethasone by isolated rabbit uterine nuclei is temperature-dependent and requires the presence of cytosol. Thus the uterine glucocorticoid binding macromolecules described here have properties similar to those reported for other steroid receptors in a variety of target tissues (5).

A specific physiological role for the glucocorticoid receptors in the rabbit

uterus cannot yet be assigned, although it may be speculated that the overall regulation of uterine growth and function is the result of many hormones, including the glucocorticoids, acting in concert. An important function of glucocorticoids may be associated with the onset of parturition. Administration of ACTH or glucocorticoids to immature fetal lambs leads to premature delivery (11) and bilateral fetal adrenalectomy prolongs pregnancy (12). Parturition induced by infusion of ACTH or glucocorticoids is associated with a fall in plasma and myometrial concentrations of progesterone, a rise in plasma concentration of free estradiol-17β and a rise in the concentration of prostaglandin F2α in maternal cotyledons and myometrium (13). It has also been shown recently that administration of glucocorticoids accelerate parturition in the rabbit (14). The possibility of a direct action of glucocorticoids in the uterus to promote metabolic changes, such as increased levels of prostaglandins, leading to parturition and the mechanism by which they exert these effects are areas of future study.

The failure in the present studies to detect specific dexamethasone binding in rat uterine cytosol does not necessarily imply that glucocorticoid receptors are not present in this tissue. It is possible that glucocorticoid receptors in rat uterus are present at very low concentrations or that they are very unstable and therefore not detectable with the methods used. A second possibility is that the rat uterus contains receptors with high affinity for corticosterone, the major glucocorticoid in the rat, but not dexamethasone. Such a corticosterone-specific binding protein has recently been reported to be present in rat kidney (15). Milgrom and Baulieu (16) have reported that rat uterine cytosol contains a protein which binds cortisol. This protein, however, is not specific for glucocorticoids since it also binds progesterone and appears to be very similar, if not identical, to serum transcortin. Therefore, it is unlikely that the rat uterine protein described by Milgrom and Baulieu (16) represents a specific glucocorticoid receptor.

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REFERENCES

(1) Lerner, L.J., Recent Progr. Hormone Res. 20, 435, 1964.

(2) Velardo, J.T., Ann. N.Y. Acad. Sci. 75, 441, 1959.

(3) Velardo, J.T., Hisaw, F.L. and Bever, A.T., Endocrinology 59, 165, 1956.

(4) Szego, C.M. and Roberts, C., Recent Progr. Hormone Res. 8, 419, 1953.

(5) Raspe, G. (ed), Advances in the Biosciences 7, Pergamon Press, Vieweg, 1971.

(6) Jensen, E.V. and DeSombre, E.R., in Biochemical Actions of Hormones (G. Litwack, ed), Vol II, p. 215, Academic Press 1972.

(7) Giannopoulos, G., J. Biol. Chem. 248, 1004, 1973.

(8) Giannopoulos, G., J. Biol. Chem. 248, 3876, 1973.

(9) Scatchard, G., Ann. N.Y. Acad. Sci. 51, 660, 1949.

(10) Beato, M. and Feigelson, P. J. Biol. Chem. 247, 7890, 1972.

(11) Liggins, G.C., J. Endocrinol. 42, 323, 1968.

(12) Drost, M. and Holm, L.W., J. Endocrinol. 40, 293, 1968.

(13) Liggins, G.C., Grieves, S.A., Kendall, J.Z. and Knox, B.S., J. Reprod. Fertil., Suppl. 16, 85, 1972.

(14) Nathanielsz, P.W. and Abel, M., J. Endocrinol. 57, 47, 1973.

(15) Feldman, D., Funder, J.W. and Edelman, I.S., Endocrinology 92, 1429, 1973.

(16) Milgrom, E. and Baulieu, E.E., Endocrinology 87, 276, 1970.