IN VITRO EVIDENCE FOR A PROGESTERONE AND
CORTICOSTEROID BINDING PROTEIN
IN THE HUMAN UTERUS

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ABSTRACT

When $^3$H-progesterone was incubated with the 273 000 g supernatant fraction from human uteri, labelled steroid was bound to a macromolecular component. Concomitant incubation with unlabelled corticosteroids, progesterone, testosterone and oestradiol-17$\beta$ depressed binding of $^3$H-progesterone, whereas cholesterol and the synthetic progestins chlormadinone and medroxyprogesterone acetate did not competitively inhibit $^3$H-progesterone binding. These findings are consistent with the existence of a transcortin-like progesterone binder in uteri of lower species such as the rat, guinea pig and rabbit.

Extensive research has demonstrated the presence of progesterone binding proteins in the uterus of various mammalian species (Milgrom & Baulieu 1970a, b; Milgrom et al. 1970; McGuire & Bariso 1971, 1972; McGuire & DeDella 1971; McGuire et al., submitted). Two binders have been described. One, termed a receptor, binds specifically to progestational agents; it has been hypothesized to have a role in the biochemical action of progesterone. The other, less specific in its binding, resembles plasma transcortin in its specificity to steroid binding. Important to the relevance of these binders are their extension to human biology. Wiest & Rao (1970) have reported the existence of the receptor in man. Our data indicate the presence of the second binder in the human uterus.
Materials and Methods

[1,2-3H] Progesterone (50 Ci/mm) was obtained from New England Nuclear Corporation. Unlabelled progesterone, cortisol, oestradiol-17β, cholesterol and testosterone were purchased from Steraloids. Unlabelled medroxyprogesterone acetate and chlormadinone were obtained from Dr. Arvin Shroff of the Division of Organic Chemistry, Ortho Research Foundation. All of these compounds were found to be pure by thin layer chromatographic analysis. A Packard Model 3375 Tri-Carb Liquid Scintillation Spectrophotometer and an Intertechnique Model SL-30 Liquid Scintillation Counter were used for all radioactivity determinations. Quenching was corrected for by the use of an external standard.

Human uteri and samples of abdominal muscle were obtained from both pre- and post-menopausal patients (ages 28 to 67) immediately following hysterectomy for various gynaecological disorders. Immediately following hysterectomy the tissues, including both myometrium and endometrium in the case of the uterus, were homogenized in 2/5 volume of phosphate buffer (0.2 m Na₂HPO₄, 0.2 m NaH₂PO₄, 0.001 m EDTA, pH 7.5) at 4°C. The homogenate was first centrifuged at 12,000 x g for 30 min, and the resulting supernatant was then centrifuged at 273,000 x g for 1 h.

Binding of 3H-progesterone in vitro and competition by non-labelled compounds for that binding was studied using sucrose density gradient analyses. 0.5 ml of the high speed supernatant fraction (cytosol) was incubated with 0.1 μCi 3H-progesterone plus unlabelled compounds where noted at a concentration of 5 μg/ml at 4°C for 1 h. Two hundred μl of the incubation media was then layered on a 5-20% linear sucrose density gradient and centrifuged at 273,000 x g for 16 h. Twelve drop fractions were collected from a hole in the bottom of the tube into counting vials, scintillation fluid was added and radioactivity in the fractions was determined.

The following experiments were designed to study the nature of the transcortin-like progesterone binding macromolecule. Proteolytic enzymes (Worthington Biochemical Corporation) were used in an attempt to affect 3H-progesterone binding in uterine cytosol. Pronase, ribonuclease and α-amylase were used at a concentration of 10 ng/ml and incubated with the uterine cytosol at 4°C for 15 min prior to the addition of 3H-progesterone. Uterine cytosol was also heated in a boiling water bath for 15 min prior to adding isotope. Finally the sedimentation properties of bovine serum albumin, chymotrypsinogen and aldolase were analyzed on identical sucrose density gradients in an attempt to estimate the molecular weight of the binder.

Results

Incubation experiments using the cytosol fraction of human uteri, but not abdominal muscle, indicate binding of progesterone to a macromolecular component of uterine cytosol. In sucrose density gradient ultracentrifugation analyses of the incubated uterine cytosol fraction only one region in the gradient contained bound 3H-progesterone (Fig. 1). That radiolabelled component sediments with a coefficient of approximately 4–5 s based on standards (Fig. 2). Unlabelled progesterone and cortisol both competed for 3H-progesterone binding, cortisol more strongly (Fig. 3), but the synthetic progestins chlormadinone and

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Sucrose density gradient patterns of tissue cytosol fractions demonstrating the region where $^3$H-progesterone is bound in the uterus (Fig. 1). The progesterone binder appears to have an $s$ value of 4–5 as judged by standards (Fig. 2). At a concentration of 5 $\mu$g/ml, unlabelled progesterone and cortisol both compete for $^3$H-progesterone binding in uterine cytosol (Fig. 3), but no competition was seen with the synthetic progestins, medroxyprogesterone acetate and chlormadinone (Fig. 4). The pattern of bound progesterone found using the cytosol fraction of abdominal muscle was markedly different from that found with uterine cytosol, and neither unlabelled progesterone nor cortisol appeared to compete for $^3$H-progesterone binding (Fig. 5). Two-tenths ml of cytosol fraction containing isotope and potential competitors was layered on 5–20 $%$ sucrose density gradients and centrifuged for 16 h at 205 000 g, 4°C.
medroxyprogesterone acetate did not (Fig. 4). Testosterone and oestradiol-17β both competed weakly, but in separate studies cholesterol did not compete. Treatment of uterine cytosol with pronase but not ribonuclease or α-amylase resulted in decreased ³H-progesterone binding to the uterine component studied here, as did heating cytosol in a boiling water bath for 15 min.

The sedimentation pattern for cytosol from the abdominal muscle, shown in Fig. 5, produced only little bound ³H-progesterone. Unlabelled progestins did not effect the amount of radioactivity in abdominal muscle cytosol fraction. We thus assume that in the abdominal muscle labelled hormone is bound non-specifically and that no similar binder exists in that non-target tissue.

**DISCUSSION**

This study provides evidence for the existence of a macromolecule in the 273 000 g supernatant fraction of the human uterus which binds to progesterone and cortisol but not to synthetic progestins. It sediments at a rate consistent with 4–5 s proteins. Although many of its characteristics are similar to those of plasma transcortin, it does not appear to exist in non-target tissues and thorough washing of uterine tissue does not eliminate steroid binding in the uterine cytosol. This suggests that the protein is not of plasma origin. Steroid binding to the protein occurs in the cold, indicating no requirements for an energy dependent process, and binding appears to be of a non-covalent type, since the steroids are completely extractable with organic solvents. Steroid binding *in vitro* obviously does not depend on cellular organization, since it can be demonstrated in the supernatant of uterine homogenate, but one cannot exclude the possibility that this binder is membrane bound *in vivo*. Many of its features appear identical with those of a uterine transcortin-like binder found in the uterus of rats, rabbits and guinea pigs (*Milgrom & Fielgelson 1970a, b; McGuire & Bariso 1971, 1972*).

The physiological role of the progesterone and corticosteroid binding protein reported here is not clear for either man or lower mammals. It is not known whether it has any role in the mechanism of action for progesterone or whether it has any relationship to the progestogen receptor. This situation of two different binding proteins is not unique to target tissues for progestogens, however. Both a class specific binder with high affinity and specificity as well as a non-specific binder (lower affinity and not specific for any one class of hormones) exist in target tissues for androgens (*Fang et al. 1969*) and glucocorticoids (*Beato & Fielgelson 1972*).

Clearly the uterine binder studied here and in lower mammals has many features identical with plasma corticosteroid binding globulin (transcortin or
CBG). Milgrom & Baulieu (1970a,b) have speculated that if the uterine protein is CBG, then transcortin may not merely be a plasma reservoir or carrier as generally thought but rather it may have a role in the passage of progesterone into the uterus as a transmembrane carrier. Facts consistent with this hypothesis are that oestrogen priming increases 1) the permeability of the uterus to globular proteins (Peterson & Spaziani 1969; Kalman 1955), 2) the in vivo uterine response to progestational compounds (Clauberg 1930) and 3) the number of progestogen receptors (Milgrom et al. 1972; Toft & O'Malley 1972) and transcortin-like progesterone binders (Milgrom & Baulieu 1970a,b) in the uterus of the rat. Finally, other plasma binding proteins, such as transferrin (Fletcher & Huehns 1968), apparently function as transport proteins.

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REFERENCES

Clauberg C.: Zbl. Gynäk. 54 (1930) 2757.

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