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DIFFERENTIATION OF BINDING PROTEINS FOR D-NORGESTREL IN HUMAN UTERINE TISSUE AND PLASMA

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SUMMARY

Binding proteins of high affinity and limited capacity for D-norgestrel have been detected in human uterine cytosol and in plasma. Polyacrylamide gel electrophoresis and competitive binding experiments with dextran coated charcoal indicated that the uterine and plasma proteins are different. Binding occurs in plasma to two proteins, one of which has characteristics similar to those of sex hormone binding globulin whereas the uterine binding macromolecule has affinity for progesterone but not for cortisol.

INTRODUCTION

Norgestrel, a potent synthetic progestational steroid, has been widely used as a contraceptive agent[1, 2] and is selectively retained in human endometrium[3]. Victor and his associates[4] concluded that the carrier protein for D-norgestrel* in plasma is identical to sex hormone binding globulin. In contrast, Uniyal and Laumas[5] have suggested the protein responsible for binding of norgestrel in plasma is neither sex hormone binding globulin nor cortisol binding globulin. Therefore, the identity of the protein concerned is a source of controversy and has provided an impetus to study the interaction of norgestrel with binding proteins both in plasma and target tissue. Using polyacrylamide gel electrophoresis and competitive studies with dextran coated charcoal, a binding protein for D-norgestrel was detected in human uterine cytosol which was distinguishable from the binding protein in plasma.

EXPERIMENTAL

Chemicals. D-[15,16-(^{3}H)-Norgestrel (13β ethyl 17α-ethynyl-17β-hydroxygon-4-en-3-one), S.A. 56 KCi/mol provided by Schering Chemicals (U.K.) was purified by paper chromatography using Bush B1 solvent system[6]. Non-radioactive norgestrel and other steroids were obtained from Wyeth Laboratories, U.S.A. and Steraloids, Bucks., England, respectively. All reagents were of Analar grade.

Preparation of cytosol and plasma. Freshly obtained human uterine tissue was rinsed with cold 50 mM Tris–HCl buffer containing 1 mM EDTA, 1 mM mercaptoethanol and 10% glycerol, pH 7.4 (Buffer A) and homogenised in 4 vols (vol./wt.) of buffer using 3 × 10 s bursts with a cooling interval of 1 min between each run. A cytosol fraction was prepared by centrifugation of the homogenate at 105,000 *g* for 90 min at 4°C. Blood samples were collected from normal women and plasma was obtained by centrifugation. Prior to use, plasma was diluted (1:5) with buffer A.

Polyacrylamide gel electrophoresis (PAGE). 100 μl of cytosol or diluted plasma (1:5) was equilibrated with [^{3}H]-norgestrel (2 nM) for 2 h at 0°C. Steady state polyacrylamide gel electrophoresis was carried out by the method of Ritzen *et al.*[7]. The gel slices were directly placed into 5 ml of scintillator (15 g PPO + 1 g dimethyl POPOP in 1250 ml of Triton X 114 and 3750 ml of xylene) then counted using a Nuclear Chicago liquid scintillation spectrometer at a counting efficiency of 45%.

Measurement of binding of [^{3}H]-norgestrel to cytosol and plasma with dextran coated charcoal. One hundred microlitre of cytosol or diluted plasma (1:5) was incubated with [^{3}H]-norgestrel (0–15 nM) for 2 h at 0°C. Identical incubations containing 100 fold molar excess of unlabelled norgestrel were carried out to measure non-saturable binding. Following the incubation, protein bound and free steroids were separated by addition of 500 μl dextran coated charcoal suspension (0.025 g dextran and 0.25 g Norite A /100 ml Tris–HCl buffer pH 7.4).

Dextran charcoal competitive protein binding assays were performed as described elsewhere[8].

* Renamed as Levonorgestrel.

RESULTS AND DISCUSSION

On polyacrylamide gel electrophoresis the [^{3}H]-norgestrel bound cytosol protein complex moved in a single peak (Fig. 1) with a mobility of 0.48 relative to bromophenol blue. Incubation with [^{3}H]-norgestrel in the presence of 100 fold molar excess of unlabelled norgestrel displaced the radioactivity from this peak. Progesterone, however, was only partly effective in displacing the radioactivity from this protein (Fig. 1) whereas dihydrotestosterone and cortisol were ineffective in suppressing the binding.

Binding studies in which free and protein bound steroids were separated with dextran coated charcoal demonstrated the presence of a binding protein for norgestrel with high affinity and low capacity (Fig. 2). Analysis of the data by Scatchard plot suggested a dissociation constant (K_D) for the protein-D-norgestrel complex in the order of 2.4×10^{-9} M. Competition studies with dextran coated charcoal showed that progesterone was an effective competitor (Table 1) for [^{3}H]-norgestrel binding sites. Dihydrotestosterone, testosterone, oestradiol-17β and cortisol did not compete for norgestrel binding.

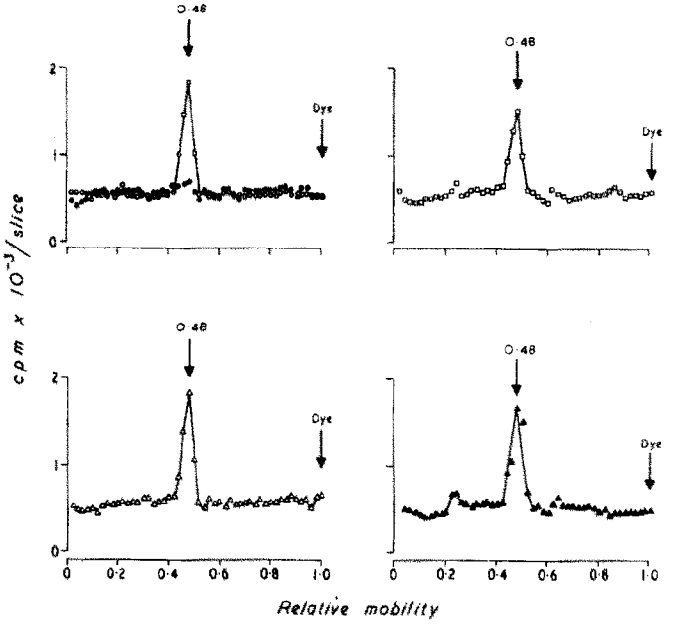

Fig. 1. Polyacrylamide gel electrophoretic profile of [^{3}H]-norgestrel bound cytosol complex. Cytosol (containing 1.4 mg protein) labelled with [^{3}H]-norgestrel (○), or [^{3}H]-norgestrel plus 100 fold molar excess of unlabelled competitors; norgestrel (●), progesterone (□), dihydrotestosterone (△) and cortisol (▲). Bromophenol blue marker is shown by dye arrow.

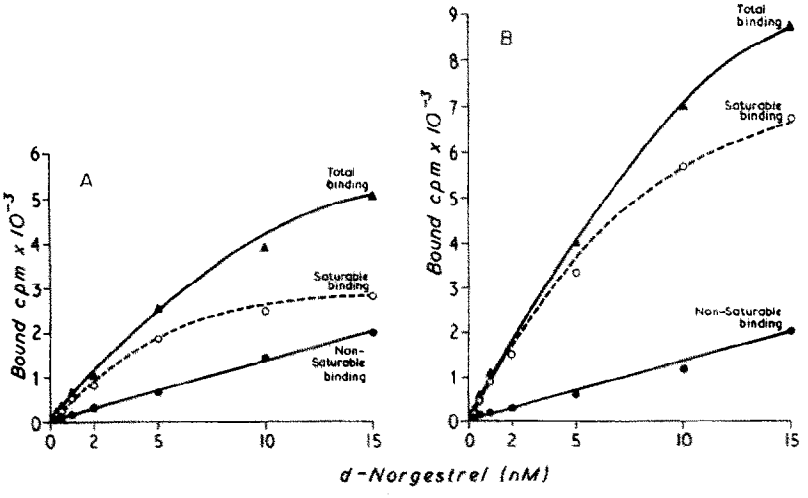

Fig. 2. Binding of [^{3}H]-norgestrel to human uterine cytosol (A) and plasma (B). Cytosol (1.4 mg protein) and plasma (1.1 mg protein) were incubated with [^{3}H]-norgestrel and free steroid from bound fraction was separated by dextran coated charcoal (detailed in 'Methods').

Table 1. Competitive inhibition of [^{3}H]-norgestrel binding to cytosol and plasma

Competitor*	% Inhibition ± S.D.†	
	Cytosol	Plasma
Norgestrel	85.52 ± 2.53	79.84 ± 1.87†
Dihydrotestosterone	4.92 ± 7.22	86.25 ± 4.53
Testosterone	3.0 ± 4.47	82.80 ± 5.72
Oestradiol-17β	4.74 ± 5.47	63.44 ± 1.01
Cortisol	2.0 ± 4.47	8.27 ± 1.38
Progesterone	65.4 ± 5.68	7.85 ± 0.75

* 100 fold molar excess. † Mean of five specimens analysed in triplicate.

Polyacrylamide gel electrophoresis of the norgestrel bound plasma complex revealed that the radioactivity was associated with two proteins having relative mobilities 0.24 and 0.59 respectively (Fig. 3). Incubation in the presence of unlabelled norgestrel displaced tritium from the first binding protein (mobility 0.24) whereas the size of the second peak remained unaffected. Similarly, incubation in the presence of dihydrotestosterone (100 fold molar excess) also suppressed the norgestrel binding to the first peak but not to the second peak. On the other hand, neither progesterone nor cortisol displaced radioactivity from either peak (Fig. 3).

Binding studies with dextran coated charcoal in plasma (Fig. 2) exhibited saturable binding and the K_D value obtained was in the range of 9.6×10^{-8} M. In contrast to the binding of [^{3}H]-norgestrel to cytosol, dihydrotestosterone, testosterone and oestradiol-17β were strong competitors of norgestrel binding to plasma. Progesterone and cortisol did not compete for norgestrel binding sites in plasma (Table 1).

The results reported here demonstrate the presence of a macromolecule with a high binding affinity for norgestrel in human uterine cytosol. The binding protein was distinguishable from sex hormone binding globulin by its electrophoretic mobility and displacement pattern with unlabelled steroids. This component does not show affinity for cortisol. The electrophoretic profile of the norgestrel bound protein complex and its affinity for steroids, distinct from those of sex hormone binding globulin and cortisol binding globulin, indicate that the norgestrel binding in uterine cytosol is not a contaminant from plasma proteins. The dissociation constant observed for D-norgestrel binding to cytosol (2.4×10^{-9} M) is comparable with K_D values obtained for progesterone (4.0×10^{-9}) as shown in the earlier studies[8].

The inference derived from competition experiments reveals that, of the steroids tested,

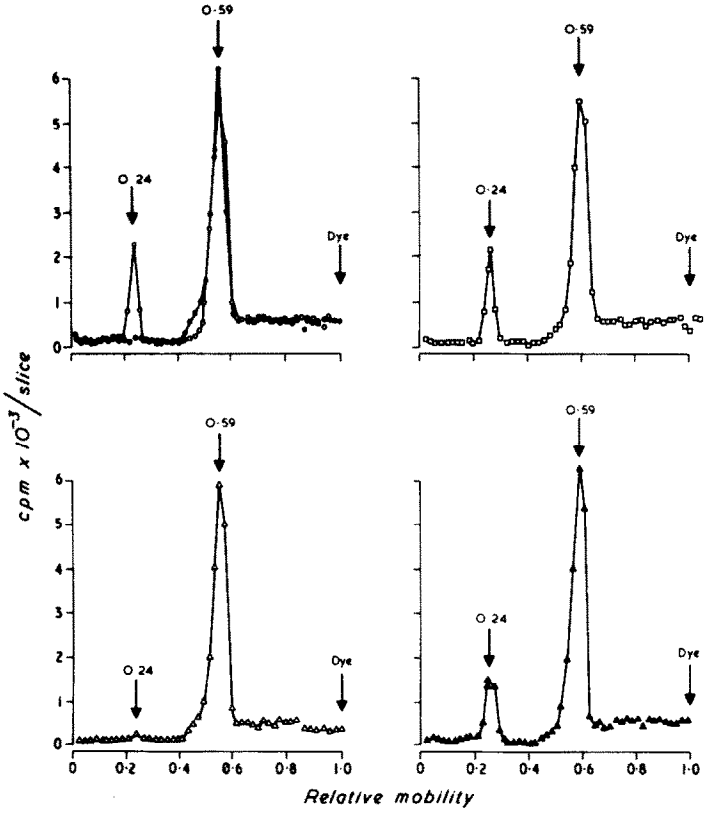

Fig. 3. Electrophoretic pattern of [^{3}H]-norgestrel bound plasma macromolecule. Plasma (containing 1.1 mg protein) equilibrated with [^{3}H]-norgestrel (○), or [^{3}H]-norgestrel plus 100 fold excess of unlabelled competitors; norgestrel (●), progesterone (□), dihydrotestosterone (△) and cortisol (▲). Dye arrow indicates migration of the bromophenol marker.

only progesterone showed affinity for norgestrel binding sites. These observations on cytosol confirm the earlier findings of Laumas and Uniyal[9] who reported the presence of a binding protein for norgestrel in the human uterus. However, further studies on nuclear uptake are needed to elucidate the possible role, if any, of this uterine cytoplasmic binding protein for norgestrel.

Markedly different, high affinity binding proteins for norgestrel occur in plasma. The proteins capable of binding D-norgestrel in plasma have also been detected by other workers but their results are conflicting. Victor *et al.*[4] by competitive binding experiments concluded that the plasma binding protein is identical to sex hormone binding globulin. Contrary to this, Uniyal and Laumas[5] demonstrated a norgestrel binding protein in plasma which is different from human serum albumin, sex hormone binding globulin and cortisol binding globulin. The dissociation constant (K_D 0.7×10^{-6} M) for this complex was much lower than that of the values observed in this study.

It is evident from the results of these electrophoretic studies that the norgestrel in plasma is associated with two protein moieties. The fast migrating macromolecule has a high capacity since inhibition of D-norgestrel binding could not be seen even in the presence of 100 × molar excess of unlabelled steroid. The affinity of this protein for norgestrel is low, and its electrophoretic mobility resembled that of albumin. The slow migrating protein with a limited capacity but high affinity for norgestrel and dihydrotestosterone, was similar in mobility to sex hormone binding globulin.

Competition studies showed that dihydrotestosterone, testosterone and oestradiol-17β, but not progesterone or cortisol, are strong competitors for norgestrel binding sites in plasma—a pattern typical of sex hormone binding globulin.

Thus, these results revealed the presence of two discrete binding proteins for norgestrel in plasma and resolved the discrepancy between the earlier conflicting reports. The relative role and retention of norgestrel in plasma in relation to its biological efficacy remains yet to be established.

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