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OESTROGEN-INDUCED PROGESTERONE RECEPTOR IN HUMAN UTERUS

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SUMMARY

High affinity progesterone binding proteins having the characteristics of steroid receptors were found in the endometrial and myometrial cytosols of human uteri obtained after hysterectomy from fertile females and estrogen-treated postmenopausal women. Only traces, if any, of this type of binding protein were present in the uterine cytosols of untreated postmenopausal women. The steroid-binding properties of the progesterone receptors in both uterine compartments were very similar. Both proteins were capable of binding a great variety of potent progestins, derivatives of 19-norprogesterone and norethisterone, whereas cortisol was not bound by either receptor.

The following physicochemical characteristics were determined at +4°C for the myometrial progesterone receptor(s): Sedimentation rate 3·8S and 7·5S (hypoionic medium), 3·8S (hyperionic medium, 0·3 M KCl); equilibrium association constant $1–4 \times 10^9$ l/mol; rate of association $6·7 \times 10^4$ l/mol $\times$ s^{-1}; rate of dissociation $4·4 \times 10^{-5}$ s^{-1}; isoelectric points at pH 4·8 and 5·2. The binding process was heat and acid labile had a pH optimum of 8·3. An involvement of SH-groups in the steroid binding process was indicated by a loss of binding ability in the presence of p-hydroxymercuribenzoate. Furthermore, some divalent cations interfered with hormone binding, possibly by interacting with SH-groups on the protein.

In the myometria of estrogen-treated postmenopausal women, the binding site concentration varied from 0·35 to $3·6 \times 10^{-12}$ mol/mg of cytosolic protein. In fertile females, the concentration was 0·06–$0·55 \times 10^{-12}$ mol/mg protein. In normally menstruating women, the myometrial binding site concentration seemed to correlate directly with plasma estradiol-17β and indirectly with plasma progesteone concentrations, as in some animal species.

INTRODUCTION

It is now widely accepted that steroid hormones are bound in their target tissues by cytoplasmic receptor proteins of high affinity and specificity. The steroid receptor complex is then "activated" in the cytoplasm and subsequently translocated to the nucleus, where its binding to chromatin initiates physiological changes *via* a modification of gene expression (for review, see [1–3]). Although this sequence of events has been demonstrated in more detail for androgen and estrogen receptors in their respective target organs, it is also believed to hold true for progesterone in mammalian tissues. Over the past few years, the presence of high affinity cytoplasmic receptors for progesterone in the uteri of various mammalian species has been reported and some of the properties of these receptors have been described [4–21].

In this communication, we summarize our recent studies on the physicochemical properties, binding characteristics and the regulation of the biosynthesis of progesterone receptors in human uterine tissue. Our results show that as in several animal species, the human uterus possesses an estrogen-inducible progesterone binding protein with molecular characteristics typical of a steroid receptor. There are progesterone receptors in both the endometrial and myometrial compartments which closely resemble one another and are capable of binding potent progestins but not cortisol, in marked contrast to the human progesterone binders described in some reports [22–24]. Furthermore, there is a strong correlation between the *in vitro* binding and *in vivo* biological activity of a number of progestins, supporting the concept that the action of these steroids is mediated through their binding to cytoplasmic receptors.

EXPERIMENTAL

Patients

Uterine tissue was obtained from normal fertile (aged 37–48 yr) or postmenopausal (aged 55–70 yr) women, who underwent hysterectomy because of uterine prolapse or related reasons. In addition, a group of postmenopausal patients (aged 45–69 yr) were treated with estrogen for one week prior to the operation as described previously [25]. Immediately after hysterectomy, endometrium and myometrium were separated from one another. The myometrium was then cut longitudinally and the tissue specimens chilled. Further processing of the samples was commenced within the following 10–15 min.

Animals

In comparative studies, progesterone receptors were induced in rabbit, guinea-pig and sheep uteri with estradiol-17β as previously described [15, 26]. The properties of these receptor proteins have been reported in detail elsewhere [15, 26].

Steroids

The sources of labeled and non-labeled steroids are given in ref. [26].

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Buffers

In most cases, TEND-G (50 mM Tris–HCl, 1·5 mM EDTA, 3 mM NaN_3, 2 mM dithiothreitol and 25% glycerol (v/v), pH 7·5 at 23°C) was used. Where glycerol is omitted from the buffer it was termed TEND. When the effect of SH-reagents and cations on the binding process was studied, EDTA and dithiothreitol were not included in the buffer (TN-G).

Isolation of myometrial and endometrial progesterone receptors

A detailed description of the methods used in the preparation of the myometrial receptor has been given elsewhere [25, 26]. The same technique was used for isolating the endometrial receptor: After homogenization of the tissue samples in 4 vol. of TEND-G, the material was centrifuged at 105,000 *g* to yield a soluble supernatant fraction (referred to as cytosol). Ammonium sulfate precipitation of unbound or ligand-bound cytosolic progesterone receptors was then performed by adding solid $(NH_4)_2SO_4$ to give a fractional saturation of 35% [25]. The precipitate obtained following centrifugation at 20,000 *g* for 30 min contained the bulk of the high affinity progesterone-binding sites ([25], and Results and Discussion). The precipitate was dissolved in a small volume of TEND-G. Unless otherwise stated, ammonium sulfate precipitated receptors from both myometrium and endometrium were used in all characterization studies.

Density gradient centrifugation

Samples were layered on either linear 10 to 35% glycerol (v/v) or 5 to 20% sucrose (w/v) gradients made up in 50 mM Tris–HCl, 1·5 mM EDTA and 3 mM NaN_3 (pH 7·5 at 23°C) with or without 0·3 M KCl. The tubes were centrifuged for 12–17 h at 45,000 rev./min in a Spinco SW 50·1 rotor at 0–2°C.

Gel exclusion chromatography

Calibrated Sephadex G-200 columns (dimensions 2·5 × 47 cm.) were equilibrated with TEND-G with or without 0·3 M KCl. Elution was effected by a constant hydrostatic pressure, using the up-flow technique with a flow rate of 10–12 ml per h.

In order to decrease the ionic strength of the receptor protein fraction after $(NH_4)_2SO_4$ precipitation, the sample was passed through a column of Sephadex G-25 (2 × 20 cm.) which was equilibrated with TEND-G. On some occasions, the Sephadex G-25 column was equilibrated against and eluted with TN-G [27].

Isoelectric focusing

This was performed at +4°C with a 110 ml LKB 8101 apparatus with double-cooling jackets, in 2% ampholine (pH 3-10) using a 10–50% glycerol gradient as stabilizer. The column was prefocused at 300V for 24 h, whereafter the sample in TEND-G was applied in the upper third of the column. Approximately 24 h after sample application, when a constant milliamperage was reached, 2 ml fractions were collected and the pH and radioactivity in each fraction determined.

Receptor specificity studies

Ammonium sulfate precipitated endometrial and myometrial receptor proteins were assayed for their hormone binding specificity using the methods described in detail by Kontula *et al.*[26]. Aliquots of receptor protein fractions were incubated at +4°C with radioactive progesterone (0·15–1·5 pmol) along with varying amounts of unlabeled progesterone or other steroids (63 in all), over a range of 0·03–300 pmol. After incubation, bound and unbound hormones were separated using a Dextran-coated charcoal technique or by density gradient centrifugation. The relative binding affinity (RA) of each ligand was computed relative to progesterone. Comparable results were obtained using these two separation techniques, but owing to its convenience and better reproducibility the Dextran-coated charcoal method was chosen for routine use. Correlation coefficients between the steroid specificity of the human progesterone receptors and those of progesterone receptors from various animal sources were calculated by linear regression analysis.

Determination of binding kinetics and binding energy

The intrinsic association constant at equilibrium (at +4°C) and the number of high affinity binding sites were determined as described previously [26]. The energy of binding was calculated using the formula

$$\Delta F = -RT \ln K_{\text{steroid}}$$

where K_{steroid} is the intrinsic equilibrium association constant of the receptor for the steroid in question. This was calculated from the RA data and the $K_{\text{progesterone}}$ according to the method of Korenman[28].

The rate of association was determined using the method described in detail by Best-Belpomme *et al.*[29], Sanborn *et al.*[30] and Schrader and O'Malley[31]. Briefly, the method involves the initial labeling of the ammonium sulfate purified receptor with [1, 2, 6, 7-$^3H_4$]-progesterone, stopping the reaction at specific times by adding a 1000-fold excess of unlabeled progesterone, and finally determining the receptor-bound radioactivity by the Dextran-coated charcoal technique immediately after the addition of unlabeled steroid. The initial rate of association is then plotted and calculated, as described by Best-Belpomme *et al.*[29].

The rate of dissociation was estimated by adding a 1000-fold molar excess of unlabeled progesterone to tubes containing aliquots of the receptor protein fraction (ammonium sulfate precipitated) which had been labeled previously with saturating amounts of [^{3}H]-progesterone. At various times after the addition of the unlabeled steroid, the receptor-bound [^{3}H]-progesterone was determined by the Dextran-coated charcoal assay. The first-order dissociation

rate constant was determined, as described in refs. [29–31]. Possible denaturation of the receptor protein over the period of incubation was monitored simultaneously. Since no significant loss in the amount of receptor-bound [^{3}H]-progesterone took place over 16 h in the absence of added unlabeled progesterone, the decay curves obtained are considered to represent the dissociation rates rather than rates of denaturation.

Studies on the nature of the binding site(s)

These studies involved the use of various enzymes and SH-reagents. Furthermore, the effect of some cations on the binding of [^{3}H]-progesterone was investigated, as described in greater detail elsewhere [27].

Determination of the biological activity of progestogens

The estimation of *in vivo* progestational activity of steroids was performed by measuring histological changes in rabbit glandular endometrium after systemic administration of the progestin, using the criteria of McPhail[32]. Comparison of the biological potencies of various steroids was carried out relative to progesterone, as described by Kontula *et al.*[26].

Determination of plasma free steroids

This was carried out by radioimmunoassay ([33], and unpublished).

RESULTS AND DISCUSSION

Physiocochemical properties of human uterine progesterone receptor

When the steroid binding characteristics of unfractionated myometrial cytosols were studied, some of the macromolecule-bound [^{3}H]-progesterone could be displaced with unlabeled cortisol. Density gradient analyses revealed that cortisol was capable of decreasing the binding of progesterone only to the lighter protein component sedimenting at about 4S (see below). When $(NH_4)_2SO_4$ was added to the cytosol to produce a fractional saturation of 35%, 75–80% of the original high affinity binding sites for progesterone were recovered in the precipitate [25]. This procedure gave a 10-fold increase in the specific activity (counts/min/mg protein) over that in the unfractionated cytosol. Furthermore, at the same time most of the transcortin-like material (or other steroid-binding plasma proteins) remained in the soluble fraction and subsequently, the receptor lost its ability to bind cortisol (see [25]). Our studies on the physicochemical properties and hormone binding characteristics of the estradiol-induced progesterone receptor(s) in human uterus were performed on this relatively crude, 10-fold purified preparation which was, however, practically free of transcortin-like material.

Although addition of 25% glycerol (v/v) and 2 mM dithiothreitol to the buffers used greatly increased the stability of the human progesterone receptor [25], we repeatedly failed to purify this material beyond the $(NH_4)_2SO_4$-precipitation step using classical protein purification techniques. We invariably lost most of the binding activity upon further chromatography. The use of some newer and more rapid purification techniques, in particular, affinity chromatography, may help solve some of these stability problems.

TABLE 1. Physicochemical Properties of the Estrogen-Induced Progesterone Receptor in Human Postmenopausal Myometrium

Parameter	Value
Sedimentation rate	
No KCl	3.8S; 7.5S
0.3 M KCl	3.8S
Equilibrium association constant	1-4 x 10^9 liters/mole
Rate of association	6.7 x 10^4 liters/mole x sec^{-1}
Rate of dissociation	4.4 x 10^{-5} sec^{-1}
Binding site concentration	0.35 - 3.6 x 10^{-12} moles/mg protein
Isoelectric point (pI)	4.8; 5.2
pH optimum for binding (at +4°C)	8.3

The physico-chemical properties of the human myometrial progesterone receptor induced with estradiol in postmenopausal women are summarized in Table 1. Some of these parameters were also estimated for receptors from uteri of fertile women during the various phases of the menstrual cycle, and no disagreement was found with the values given in Table 1. Some of the physicochemical properties of the receptor are discussed in more detail below.

Sedimentation rate

When the $(NH_4)_2SO_4$ purified receptor was labeled *in vitro* with [1, 2, 6, 7-^{3}H$_4$]-progesterone and centrifuged on a density gradient, [^{3}H]-steroid-macromolecule complexes with sedimentation rates of 7·5S and 3·8S in hypoionic, and 3·8S in hyperionic (0·3 M KCl) gradients were observed (Fig. 1). The two peaks noted on density gradient centrifugation in hypoionic medium possessed similar hormone specificities and

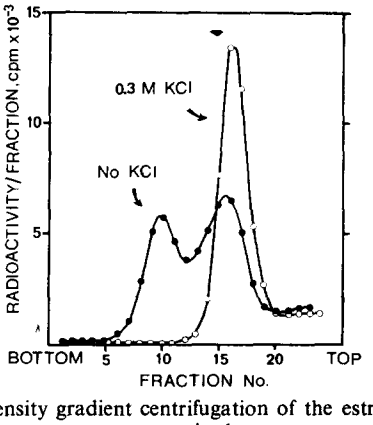

Fig. 1. Density gradient centrifugation of the estrogen-induced progesterone receptor in human postmenopausal myometrium in the presence (O——O) and absence (●——●) of 0·3 M KCl. Aliquots (0·25 ml) of ammonium sulfate precipitated receptor fractions in TEND were labeled at +4°C with [1, 2, 6, 7-^{3}H]-progesterone, layered over 5–20% sucrose density gradients and centrifuged as indicated in the Experimental section. The arrow points to the position of BSA.

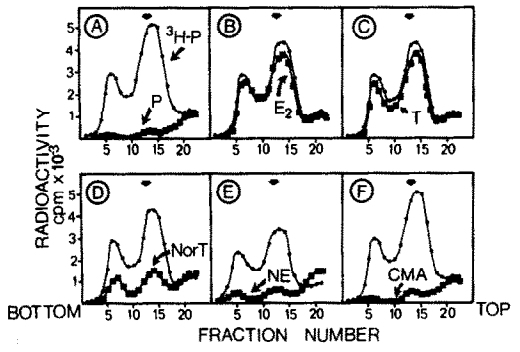

Fig. 2. Sucrose density gradient centrifugations of the myometrial progesterone receptor in the presence of [1, 2, 6, 7-^{3}H]-progesterone and various non-labeled steroids. Myometrial receptor (0·3 ml, ammonium sulfate fraction) in TEND was labeled with 1·5 pmol of [^{3}H]-progesterone (^{3}H-P) in the absence (●——●) or presence (■——■) of 37·5 pmol of the following non-labeled steroids: 2A, progesterone (P); 2B, estradiol (E_2); 2C, testosterone (T); 2D, nortestosterone (NorT); 2E, norethynodrel (NE); and 2F, chlormadinone acetate (CMA). Other details as in the legend for the Fig. 1 and in the text.

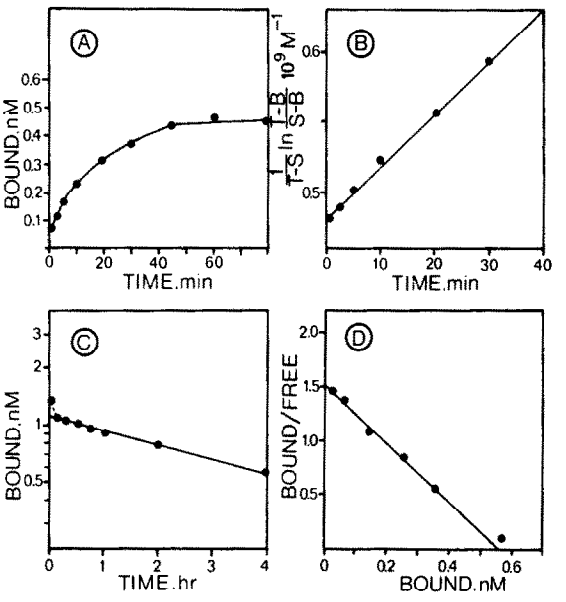

Fig. 3. Kinetics of the interaction between [1, 2, 6, 7-^{3}H]-progesterone and human myometrial receptor. 3A and 3B. Association rate reactions: The binding of [^{3}H]-progesterone (0·9 nM) to the receptor (4·25 nM) is plotted as the amount of hormone bound *vs.* the reaction time (3A) or as a second-order association rate plot (3B) according to Best-Belpomme *et al.*[29]. S = concentration of binding sites (4·25 nM), T = total hormone added (0·92 nM) and B = amount of bound [^{3}H]-progesterone at a given time (t). 3C. Analysis of the dissociation rate of myometrial receptor–progesterone complex. An aliquot of the receptor (1·4 nM) was saturated with [^{3}H]-progesterone. After labeling, a 1000-fold excess of unlabeled progesterone was added and the amount of radioactive hormone–receptor complex measured at various times by Dextran-coated charcoal assay. 3D. Scatchard analysis of [^{3}H]-progesterone binding by the myometrial receptor protein.

binding affinities, as illustrated in a series of displacement studies (Fig. 2, see [26]). Both peaks were capable of binding steroids known to be active progestins. Furthermore, when a steroid displaced radioactive progesterone from the receptor(s), both peaks were equally diminished. No significant binding of cortisol could be shown by either of these peaks, thus ruling out the possibility that the lighter component was a transcortin-like protein. Whether the 7·5S and 3·8S peaks represent two separate receptor components is not known. It is also possible that during $(NH_4)_2SO_4$ precipitation, an irreversible "salt activation" of the receptor has taken place, leading to the formation of a non-aggregating 3·8S component.

There are reports showing that human uterine cytosol also contains a 4S component which binds progesterone and cortisol, but not synthetic progestins [22–24, 34]. In none of these reports was a specific receptor demonstrated which bound progestins in proportion to their biological activity. If this type of progesterone-binding protein were present in our cytosol preparations, it must have been separated during the $(NH_4)_2SO_4$ precipitation step (see above) from the proteins we believe to be true human uterine progesterone receptor(s).

Binding kinetics and binding energy

This data is summarized in Table 1 and Figs. 3 and 4. The intrinsic association constants of the receptors measured at equilibrium varied to some extent in different preparations and were 1–4 × 10^9 l/mol. Only traces, if any, of this kind of high affinity binding were detected in uteri of non-treated postmenopausal women. The following values were obtained for the initial rate of association and the first-order rate of dissociation, when measured at +4°C: 6·7 × 10^4 l/mol × sec^{-1} and 4·4 × 10^{-5} sec^{-1}, respectively. These values are similar to those reported for progesterone receptors in chick oviduct [31]. It should be noted, however, that the determinations we performed were carried out in the presence of 20% glycerol, which might have affected the rate constants to some extent [13]. When the equilibrium association constant was calculated from the kinetic parameters (rate of association/rate of dissociation), a value of 1·5 × 10^9 l/mol was obtained which compares favorably with the values obtained by Scatchard analysis (see above).

BINDING ENERGY CONTRIBUTIONS

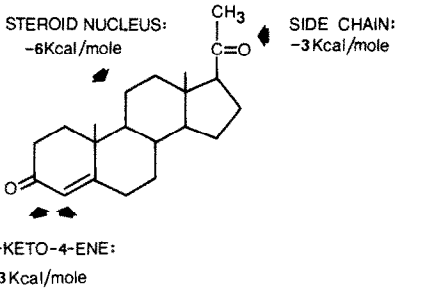

Fig. 4. Calculated binding energy contributions by various regions of progesterone molecule to the interaction between the hormone and the myometrial receptor. Details of the calculations are given in Kontula *et al.*[26].

The estimated change in the free energy of progesterone binding to the myometrial receptor protein was −12 Kcal/mol [26]. By comparing the binding of different structurally related pairs of steroids, we estimated the binding energy contributions of the various regions of the progesterone molecule to the interaction with the receptor. As illustrated in Fig. 4, each of the two functional groups in the steroid molecule, the 3-keto-4-ene grouping and the acetyl side-chain, contribute −3 Kcal/mol to the free energy of binding. Accordingly, the energy of interaction of the steroid skeleton with the receptor protein is only of the order of −6 Kcal/mol, indicating a fairly loose fit of this nucleus to the protein. In fact, this latter value is very close to the binding energy measured for interaction of steroids with serum albumin [35]. Some hydrophobic substituents (e.g. 18-methyl, 6α-methyl) contribute substantially to the binding [26], possibly by interacting with a specific attachment site on the receptor molecule. This agrees with results showing that a single $-CH_2$ group in close contact with a protein molecule already generates a binding energy of −2 Kcal/mol [36].

Apparent molecular weight and molecular (Stokes) radius determinations

The ammonium sulfate purified myometrial receptor formed two peaks of bound radioactivity, when passed through Sephadex G-200 columns (Fig. 5). When gel exclusion chromatography of the receptor was carried out in TEND-G containing 0·3 M KCl, some changes in the peak sizes were observed, possibly due to decreased aggregation of the receptor during molecular sieving in a high ionic medium. On the basis of these salt-induced changes in the peak sizes, it was assumed that the steroid-binding protein eluted at 175–180 ml (Fig. 5) represented the component sedimenting at 3·8S on density gradient centrifugation whereas the binding peak appearing at the void vol. of the column was possibly due to the non-specific aggregation tendency of the receptor. Accepting the validity of these assumptions, a Stokes radius of 3·4 nm was calculated for the 3·8S component according to the method of Ackers and Steere[37], corresponding to an apparent molecular weight of 54,000. Studies on the steroid-binding specificities of the two peaks from Sephadex G-200 revealed that the aggregate form of the binding protein was specific for progesterone, whereas the other peak also contained some non-specific binding activity (results not shown). The decrease in the size of the leading peak effected by the inclusion of 0·3 M KCl in the elution buffer was followed by a concomitant increase in the second peak. This was seen, not only as an increase in the amount of bound radioactivity (Fig. 5), but also as enhanced specific progesterone-binding activity of the latter peak, as revealed by steroid competition studies.

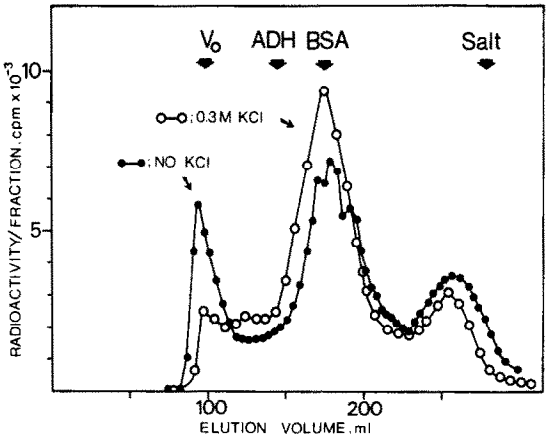

Fig. 5. Sephadex G-200 gel exclusion chromatographies of myometrial progesterone receptors. Aliquots of the ammonium sulfate purified receptor fraction were labeled with [1, 2, 6, 7-^{3}H]-progesterone and eluted with TEND-G with (○——○) or without (●——●) 0·3 M KCl. Vo = void volume of the column, ADH = elution position of yeast alcohol dehydrogenase, BSA = elution position of BSA, and salt = total column volume as measured by the elution of salt or free [^{3}H]-progesterone.

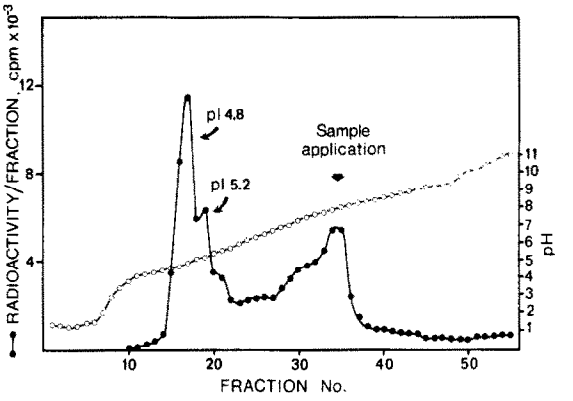

Fig. 6. Isoelectric focusing of the myometrial progesterone receptor. After $(NH_4)_2SO_4$ precipitation, the ionic strength of the receptor fraction was decreased by passing it through Sephadex G-25. The receptor was then labeled with [^{3}H]-progesterone and isoelectric focusing performed as described in the Experimental section.

Isoelectric focusing of the myometrial receptor is demonstrated in Fig. 6. A major peak focused repeatedly at pH 4·8, but some minor components were also usually seen, pointing to possible heterogeneity of the myometrial receptor component(s). Unfortunately, isoelectric focusing could not be used for purification purposes because of the greater instability of the myometrial receptor at pH's below 6·9. In contrast, the receptor seemed to have a slightly alkaline pH optimum for binding (8·3 at +4°C) and was, furthermore, relatively stable up to pH 9 [25].

Nature of the binding site

The progesterone receptor(s) studied were proteinaceous in nature, as judged by the complete loss of the binding ability after incubation with proteolytic enzymes, whereas no loss of binding capacity occurred after treatment with DNase or RNase [25]. The integrity of SH-groups was necessary either for the actual

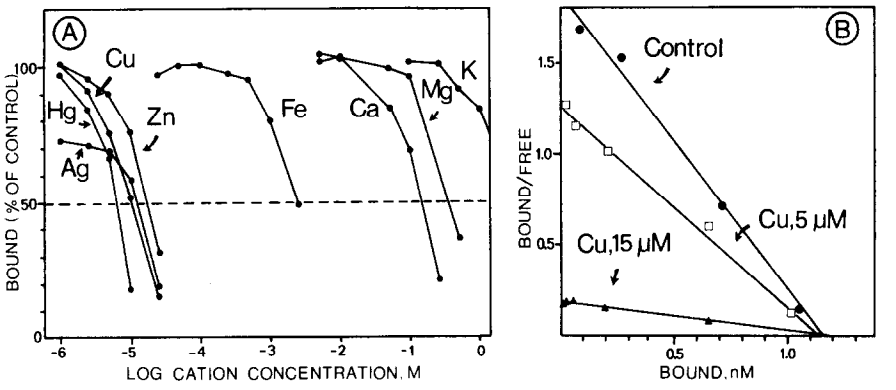

Fig. 7. Action of a variety of mono- and divalent cations on the binding of [1, 2, 6, 7-^{3}H]-progesterone by the human myometrial receptor. A. Dose-response curves for the cations tested. B. Scatchard analysis for the association of [^{3}H]-progesterone with the receptor in the presence of cupric ions. The equilibrium association constants were as follows: control (●——●), $1{\cdot}64 \times 10^9$ l/mol; Cu(II) 5 μM (□——□), $1{\cdot}07 \times 10^9$ l/mol; and Cu(II) 15 μM (▲——▲), $1{\cdot}4 \times 10^8$ l/mol.

binding process or for maintaining the tertiary structure of the receptor in an active binding form. This was demonstrated by the susceptibility of the binding activity to SH-reagents such as p-hydroxymercuribenzoate [25].

Some cations also seemed to interfere effectively with the binding of [^{3}H]-progesterone to the cytosolic receptor, as illustrated in Fig. 7. We found that the presence of cupric ions decreased the equilibrium association constant of the receptor for progesterone, whereas no changes in the number of binding sites took place (Fig. 7B). As the action of cations was partly abolished by the addition of dithiothreitol to the incubation medium, we assumed that the cations interfered with normal SH-group functioning in the receptor molecule. It is not known, however, whether cations such as cupric ions exert their effect by competing directly with the steroid for the SH-groups at the binding site or whether their action on SH-groups changes the tertiary structure of the progesterone receptor leading to a decrease in its binding affinity. These problems are currently under study in our laboratory. It is of special interest that Cu(II) ions are capable of inhibiting the binding of progesterone by its myometrial receptor *in vitro* in concentrations similar to those present in human endometrium in the presence of copper-releasing IUDs [27, 38]. It is tempting, therefore, to suggest that an inhibition of progesterone action at its target sites is of importance for the contraceptive effectiveness of the metal-bearing IUDs.

Binding specificity studies

Tolerated and non-tolerated changes in the ligand structure. Sixty-three steroids were tested as to their ability to compete with progesterone for the binding sites on the estrogen-induced endometrial and myometrial receptors in human uterus [25, 26]. The results of these studies are summarized in Fig. 8 which correlates tolerated (8A) and non-tolerated (8B) changes in progesterone structure with effective binding by the receptors. A more detailed discussion of these results has been published elsewhere [26]. In short, the following conclusion can be drawn: (1) the presence of keto-groups at C-3 and C-20 is essential for binding; (2) the acetyl side-chain can be replaced by a 17α-ethinyl-17β-hydroxyl function with no major loss of binding activity; (3) an introduction of hydro-

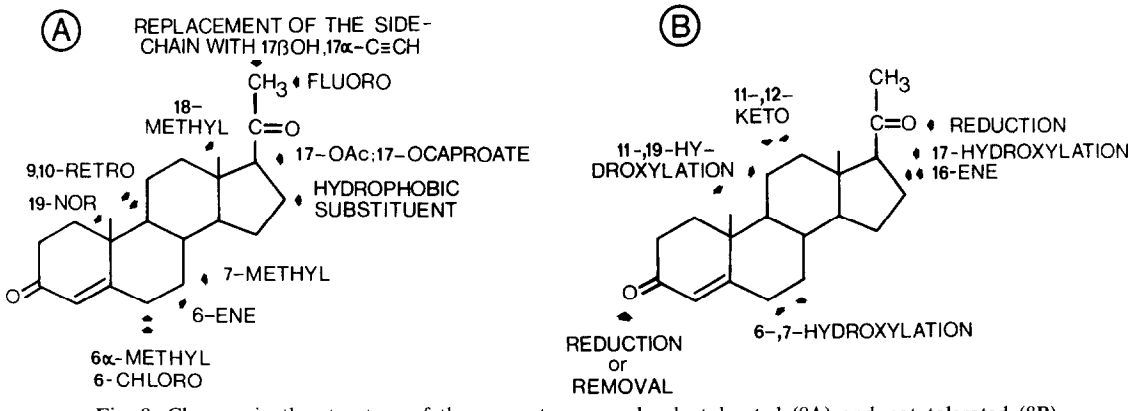

Fig. 8. Changes in the structure of the progesterone molecule tolerated (8A) and not tolerated (8B) in regard to active binding by the human endometrial and myometrial receptors. Conclusions are based on data obtained for 63 ligands (see [26]).

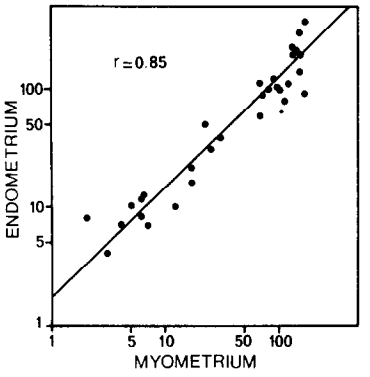

Fig. 9. Comparison of the hormone specificities of progesterone receptors in human endometrium and myometrium. Compounds with an RA greater than 1 (RA for progesterone = 100) were used in this calculation. The correlation coefficient $r = 0{\cdot}85$.

philic substituents into the progesterone molecule is usually not tolerated, whereas additional hydrophobic groups may even increase binding; (4) planar structure of the steroid skeleton is a basic requirement for effective binding; (5) removal of the angular CH_3-group at C-10 increases binding of the hormone.

Comparison of the hormone specificities of endometrial and myometrial receptors. Estrogen-induced endometrial and myometrial receptor proteins displayed very similar hormone-binding specificities towards all the 63 steroids investigated (Fig. 9). The binding activities of these two receptors had a correlation coefficient of 0·85, when ligands with an RA value greater than 1 (RA for progesterone = 100) were compared. Both progesterone receptors were capable of binding steroids known to be active progestins very effectively, but they did not bind cortisol. This is in agreement with two very recent reports which demonstrate that estrogen-induced endometrial and myometrial receptors from hamster and sheep uteri have similar hormone specificities and bind progestogenic steroids [16, 39].

When the *in vitro* binding affinities of the endometrial and myometrial receptors towards a variety of progestins were compared with the *in vivo* biological activities of the same steroids, a fairly good correlation was seen in most cases [26]. Some disagreement observed between the RA and biological activity of a few steroids was usually possible to explain by metabolic transformations known to take place in human organism [26]. When the structure of a given ligand was modified in order to change the extent of *in vitro* binding by either the endometrial or myometrial receptor, a concomitant change in the *in vivo* biological action of the altered ligand was seen. These data stregthen our contention that the binding proteins studied were uterine progesterone receptors. The results also support the concept that the biological action of synthetic progestogens involves their initial binding to the cytoplasmic progestin receptors. Unfortunately, we did not have any antiprogestational compounds with a high RA at our disposal to obtain further proof for these proposals.

Search for animal models

We have compared a variety of physicochemical properties and ligand specificities of estrogen-induced progesterone receptors isolated from rabbit, sheep and guinea-pig uteri with those of the human uterine receptor(s) [25, 26]. All the studies were made with $(NH_4)_2SO_4$ purified preparations. The physicochemical characteristics of these estrogen-induced animal receptors were all very similar, if not identical [15, 16, 26]. Detailed hormone specificity studies revealed, however, clear-cut differences in the guinea pig receptor properties as compared to the others [15, 26]. These studies are summarized graphically in Fig. 10.

As can be seen in this Fig., the rabbit and sheep myometrial receptors have binding properties very similar to the human receptor. The sheep progesterone receptor seems to be the most selective of these three proteins, whereas the rabbit receptor appears to tolerate structural variations slightly better than the human. The only steroids which seemed to display clearly lower affinity for the sheep receptor than for

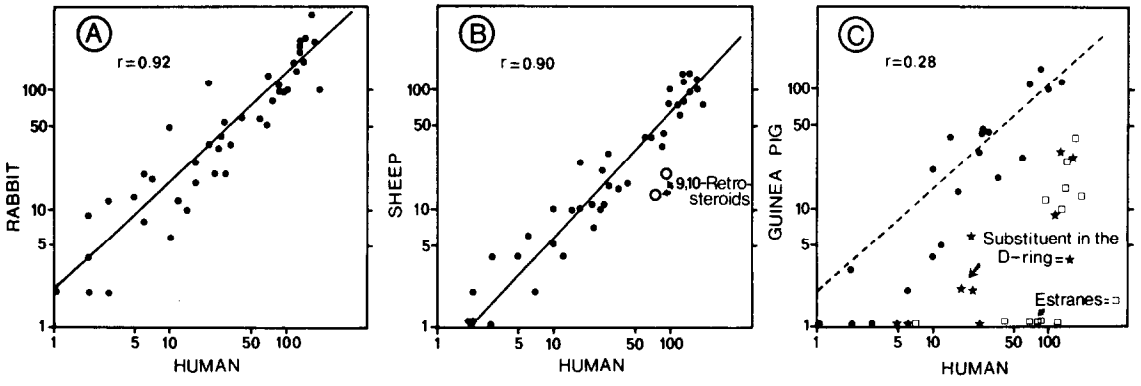

Fig. 10. Comparison of the steroid-binding affinities of myometrial progesterone receptors from various animals with the human myometrial receptor. 10A. Human vs. rabbit, correlation coefficient $r = 0{\cdot}92$; 10B. Human vs. sheep, correlation coefficient $r = 0{\cdot}90$. 10C. Human vs. guinea-pig, correlation coefficient $r = 0{\cdot}28$. If D-ring substituted pregnane and estrane derivatives are excluded, the correlation coefficient between the human and guinea-pig receptors is $r = 0{\cdot}86$ (dotted line).

the human receptor were two 9β,10α-retrosteroids, as indicated in Fig. 10B. Such systematic differences were not found between the human and rabbit myometrial binders. As shown in Fig. 10C, there are two groups of progestins, which are emphatically rejected by the guinea-pig receptor, but which are well accepted by the human receptor. The first group of steroids consists of pregnane derivatives with a substituent in the D-ring, *e.g.* 17α-acetoxyprogesterone and 16α-ethylprogesterones (for details, see [15, 26]). The second group of progestins consists of 17α-ethinyl-17β-hydroxyestranes which are bound by the guinea-pig receptor to approximately one-tenth of the extent to which they are bound by the human receptor.

Thus, the steroid specificity data clearly demonstrate that estrogen-induced progesterone receptors are not similar in all mammalian species. This possibly explains the well-known differences in the biological activities of synthetic progestins in a variety of animal species. On the basis of the present results it can be concluded that rabbit and sheep seem to be appropriate models for assessing the biological activity of synthetic progestins intended for human use. It is also suggested that an *in vitro* binding assay using rabbit or sheep myometrial receptor would be a simple and useful tool to screen potentially active progestogens before extensive *in vivo* studies. If a new steroidal agent is emphatically rejected by one of these two receptors, it is very unlikely that this compound could have progestational activity in humans. This requires, of course, that the compound is obviously not converted to one with a higher RA before it enters its target tissue.

Factors regulating the receptor concentration in the human uterus

Estrogen priming. It has been repeatedly shown in animal experiments that the concentration of the progesterone receptor in mammalian uteri is under steroidal control. Estradiol-17β and diethylstilbestrol are positive effectors, inducing the synthesis of new uterine progesterone receptors, whereas progesterone seems to control the rate of degradation of its own receptor [5, 6, 13, 21, 40]. Undoubtedly, estrogens also control the synthesis of human uterine progesterone receptor(s), as can be seen from Fig. 11. Thus, estrogen treatment for one week [25] led to the appearance of high affinity progesterone binding sites in the uteri of all the postmenopausal women treated in concentrations ranging from 0·35 to 3·6 (mean 1·3) pmol/mg of cytosolic protein. In contrast, only traces, if any, of high affinity binding receptors for progesterone were found in the uteri of non-treated postmenopausal women.

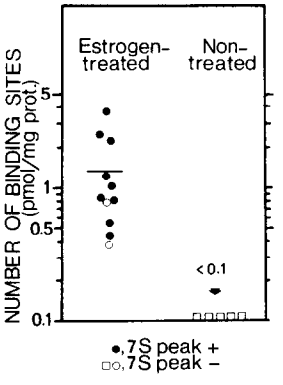

Fig. 11. Concentration of high-affinity binding sites for progesterone in postmenopausal women after 1 week's treatment with estrogen. Control subjects received no estrogen treatment. Closed circles (7S peak +) refer to cases, where density gradient analyses revealed the presence of both 3·8S and 7·5S receptor peaks. Similarly, open symbols (7S peak −) depict the cases where no evidence of the existence of 7·5S peak was obtained upon density gradient centrifugation.

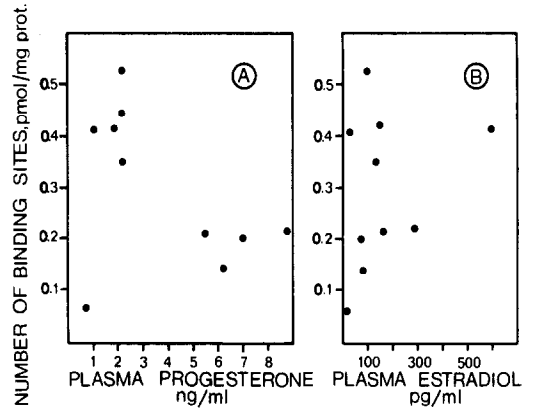

Fig. 12. Relation of the receptor concentration in human myometrial cytosol to plasma progesterone (12A) and estradiol-17β (12B) levels. Uterine samples were obtained from fertile women at various stages of the menstrual cycle. Receptor concentration was measured by Scatchard analysis and plasma steroids by radioimmunoassay.

It has been reported that in some animal species the density gradient sedimentation pattern of uterine progesterone receptor(s) varies with the menstrual cycle and in response to administered exogenous estrogen [5, 6, 21]. After administration of estrogen to guinea pigs, a 6·7S binding component in addition to the 4·5S component, appears in the uterus, and subsequently, the decay of the 6·7S binding protein is faster than that of its lighter counterpart [6]. Since we have used rather long-term estrogen-treatment with all the postmenopausal women studied, we were unable to detect possible time differences in the appearance of the two receptor components in human myometrium. As can be seen in Figs. 1 and 11, this estrogen treatment lead to the appearance of both 3·8S and 7·5S receptor components in the myometria of almost all the women. The 7·5S peak was undetectable in uteri from non-treated postmenopausal patients. The reason for the absence of the 7·5S component in the uteri of two of our estrogen-treated women is not known.

Binding site concentration in relation to the phase of the menstrual cycle. The results of these studies are summarized in Fig. 12, where the human myometrial binding site concentration of the progesterone receptor in samples obtained at different stages of the men-

strual cycle is plotted against plasma progesterone (12A) and estradiol-17β (12B) levels. Although our small material does not allow any definitive conclusions to be drawn, certain similarities with changes in animal model systems are evident. Thus, high plasma progesterone levels seem to be accompanied by lower myometrial receptor concentrations, pointing to a possible negative control by progesterone on the concentration of its own receptor, which also was seen in the animal systems [5, 6, 21, 40]. As far as the relationship between the binding site concentration of the myometrial receptor and plasma estradiol-17β levels is concerned, no clear-cut pattern was seen in our material, probably owing to the great variations in the plasma progesterone levels. However, as the experiments reported above demonstrate, estradiol-17β also exerts some control over the synthesis of the human myometrial progesterone receptor, at least when plasma progesterone levels are low as is the case in postmenopausal women.

In accordance with results obtained from animal models [5, 6, 21], the equilibrium association constant of human myometrial progesterone receptor did not seem to vary during the menstrual cycle, but was approximately 4×10^9 l/mol in all the samples (results not shown).

CONCLUSIONS

We have demonstrated that estrogen treatment leads to the appearance of progesterone binding component(s) in human postmenopausal endometrium and myometrium. The properties of the binding proteins found in both compartments of the human uterus are very similar. In addition, their properties seem to be characteristic of steroid receptors. Both endometrial and myometrial receptors are able to bind a great variety of progestational steroids, but not cortisol. Furthermore, there seems to be a good correlation between the *in vitro* binding affinity and *in vivo* biological activity of different steroidal agents. As in several animal species, estradiol-17β increases the concentration of progesterone receptor in human uterus, whereas progesterone possibly controls the rate of disappearance (degradation?) of its own receptor.

Rabbit and sheep uteri possess progesterone receptors very similar to those found in human endometrium and myometrium. These animals may serve, therefore, as good models for future attempts to develop new progestational agents for use in humans.

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