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PROGESTIN RECEPTORS IN HUMAN TISSUES: CONCENTRATIONS AND BINDING KINETICS

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SUMMARY

Progestin receptor level was measured from myometrial cytosol samples obtained from normally menstruating women (group I), postmenopausal women (group II) and postmenopausal women after 1 week's treatment with estrogens (group III). The following receptor levels were found: Group I, 63–722 fmol/mg cytosol protein; group II, below 100 fmol/mg, and group III, 350–3600 fmol/mg. Estrogen treatment of postmenopausal women preferentially promoted formation of the 7S progestin receptor in the myometrial cytosol. By contrast, the binding of [^{3}H]-progesterone occurred only to the 4S component in the myometrial cytosol samples from normally menstruating women. During the menstrual cycle, progestin receptor level in the myometrial cytosol correlated directly to the cytosol estradiol-17β content and inversely to progesterone concentration in both the myometrial cytosol and serum.

Interaction of the partially purified myometrial progesterone receptor at +4°C with the following [^{3}H]-labeled progestins was studied: Progesterone (P), norethisterone (NET), 16α-ethyl-21-hydroxy-19-nor-4-pregnene-3,20-dione (ORG 2058), 17,21-dimethyl-19-nor-4,9-pregnadiene-3,20-dione (R 5020) and 13-ethyl-11-methylene-18,19-dinor-17α-4-pregnen-20-yn-17-ol (ORG 2969). The rate of association of ORG 2969, lacking the 3-keto group, was much slower ($k_a = 0.23 \times 10^5$ l × mol^{-1} × s^{-1}) than that of other progestins (k_a range 1.9–4.6 × 10^5 l × mol^{-1} × s^{-1}). The rate of dissociation was fastest for P ($k_d = 13.0 \times 10^{-5}$ s^{-1}, $t_{\frac{1}{2}}$ 90 min) followed by NET and ORG 2969 ($t_{\frac{1}{2}}$ 205 and 235 min). Much slower rates of dissociation were recorded for R 5020 and ORG 2058 ($t_{\frac{1}{2}}$ 620 and 1000 min). The apparent intrinsic dissociation constants (K_D) calculated from the kinetic parameters were: P 0.56, NET 0.3, ORG 2058 0.04, R 5020 0.04 and ORG 2969 2.5 nmol × l^{-1}. These values were about 5–15 times lower than those obtained in the equilibrium binding studies.

In the myometrial cytosol of estrogen-treated women, the potent progestins were bound to both 4S and 7S components, although they preferred association with the latter. The low rate of dissociation of ORG 2058 and minimal binding to non-receptor sites render it very suitable for progestin receptor measurements also from non-uterine tissues. This is exemplified by results obtained in assays of progestin receptors from human breast cancer tissue.

INTRODUCTION

According to the current consensus of opinion, the primary event in the action of a steroid hormone is its combination with a cytosol receptor protein in the target cell, after which a transformed receptor–hormone complex is translocated to the cell nucleus. Reports from many laboratories have now indicated that the cytosol receptor protein plays a central role in the regulation of response to hormonal stimuli of steroid-sensitive tissues [1, 2]. The nuclear translocation along with binding of the receptor–steroid complex to chromatin in turn facilitates gene transcription which is followed by events that finally lead to the expression of a steroid specific biological response [1, 2]. Although the *in vivo* biological action of a steroid is dependent upon a variety of factors such as metabolic clearance rate and bioactivation, the biological activity of different groups of steroids usually correlates at least modestly with the *in vitro* binding of the same compounds to their respective cytosol receptors. Studies from this [3, 4] and other [5] laboratories have indicated that this rule holds true also for progesterone and its derivatives.

Recent studies with estrogens have suggested that the ultimate biological activity of a given steroid may be more dependent on the time of its nuclear residence, i.e. binding to acceptor sites on the chromatin, rather than on the extent of initial nuclear uptake of cytosol receptor–steroid complexes [6, 7]. Furthermore, the components of the receptor–steroid complex seem to dissociate from the nuclear acceptor sites following different kinetics, the steroid moiety being released first [8]. These findings prompted us to investigate whether the disparity between the extent of the *in vitro* binding affinity and *in vivo* biological activity, sometimes observed for a few potent progestins, could possibly rely upon an altered interaction of the progestins with the cytosol receptor eliciting subsequently changes in the biological functions of the receptor complexes. In this communication, we show that some *in vivo* highly potent progestins indeed follow different kinetics in their *in vitro* interaction with the human uterine progestin receptor than does the physiological steroid, progesterone. These properties

of the progestins may, in part, be responsible for their enhanced biological activity.

EXPERIMENTAL

Patients and samples

Uterine tissue was obtained from normal fertile or postmenopausal women, or from estrogen-treated postmenopausal patients, most of whom underwent hysterectomy because of uterine prolapse or myomata uteri [9]. Breast cancer samples (primary tumors or metastases) were collected in connection with radical mastectomies or surgical removal of the tissue [10].

Steroids

The sources of non-labeled steroids are given in ref. [3]. [1,2,6,7-^{3}H]-Progesterone (S.A. 80 Ci/mmol) was purchased from the Radiochemical Centre, United Kingdom. Other tritium-labeled steroids were kindly donated by the following colleagues: ORG 2058 (16α-ethyl-21-hydroxy-19-nor-4-pregnene-3,20-dione, 30 Ci/mmol) and ORG 2969 (13-ethyl-11-methylene-18,19-dinor-17α-4-pregnen-20-yn-17-ol, 11 Ci/mmol) by Dr. E. de Jager, Scientific Development Group, Organon, Oss, The Netherlands; NET (17α-ethinyl-17β-hydroxy-4-estren-3-one, norethisterone, 40 Ci/mmol, custom-labeled in the New England Nuclear, Boston, Mass., U.S.A.) by Dr. T. Luukkainen, Helsinki, Finland and R 5020 (17,21-dimethyl-19-nor-4,9-pregnadiene-3,20-dione, 50 Ci/mmol) by Dr. J. P. Raynaud, Roussel Uclaf, Romainville, France, who also provided the corresponding non-radioactive steroid. The structures of the labeled progestins investigated are shown in Fig. 1.

Buffers

In most cases, TEND-10G buffer (10 mM Tris-HCl, pH 7.5 at 23°C, 1.5 mM Na_2EDTA, 3 mM NaN_3, 2 mM dithiothreitol and 10% (v/v) glycerol) was used. Occasionally, in order to improve the stability of the receptor, the glycerol concentration was elevated up to 25% and the buffer termed accordingly TEND-25G.

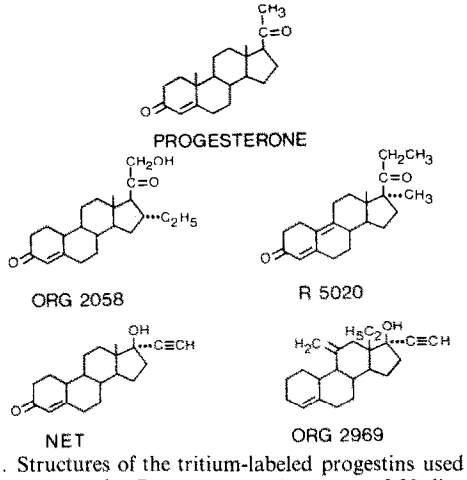

Fig. 1. Structures of the tritium-labeled progestins used in the present work: Progesterone (4-pregnene-3,20-dione); ORG 2058 (16α-ethyl-21-hydroxy-19-nor-4-pregnene-3,20-dione); R 5020 (17,21-dimethyl-19-nor-4,9-pregnadiene-3,20-dione); NET (norethisterone, 17α-ethinyl-17β-hydroxy-4-estren-3-one); and ORG 2969 (13-ethyl-11-methylene-18,19-dinor-17α-4-pregnen-20-yn-17-ol).

Preparation of partially purified cytosol progestin receptor from the myometrium

A detailed description of the methods used in isolation of progestin receptors from human myometrium has been reported previously [3, 9]. In short, the following technique was employed: After homogenization of the tissue in TEND-10G or TEND-25G buffer (4 ml buffer/g tissue), the homogenate was centrifuged at 105,000 ***g*** to yield a soluble supernatant fraction (cytosol). Cytosol progestin receptor was then precipitated (without prior charging with a radioactive ligand) with $(NH_4)_2SO_4$ by adding 19.7 g $(NH_4)_2SO_4$/100 ml cytosol (35% fractional saturation). The precipitate formed was harvested by centrifugation at 20,000 ***g***. The resulting pellet contained the bulk of high-affinity progesterone-binding sites [4, 9]. Following centrifugation, the walls of the tubes were carefully wiped with paper to remove all the supernatant. The pellets were then frozen at −70°C until used.

Just before each experiment, a frozen pellet was taken up in a small vol. of TEND-10G buffer. The redissolved receptor fraction was first cleared by centrifugation (10,000 ***g***) and then passed through a Sephadex G-25 column equilibrated with TEND-10G, in order to remove excess of salt. All binding kinetic studies were performed with preparations purified to this extent.

Breast cancer cytosol

Breast cancer samples were stored at −70°C until the preparation of the cytosol. Cytosol fraction from the tumor was prepared in TEND-10G buffer as previously described [10], and was used immediately for assays of estrogen and progestin receptors.

Measurement of steroid receptor concentrations

Progestin receptor level was measured from the human myometrial cytosol using [^{3}H]-progesterone as the labeled ligand, essentially as previously outlined [3, 4, 9]. We have, however, recently switched to the use of [^{3}H]-ORG 2058 or [^{3}H]-R 5020 in these assays (see below). Breast cancer estrogen receptor content was estimated as described by Jänne *et al.* [10].

Progestin receptor level was measured from breast cancer cytosol as follows: Portions (0.1 ml) of the cytosol fraction were incubated in duplicate with 10, 25, 50, 100, 250, 500, 1000 and 2500 fmoles of the synthetic progestin [^{3}H]-ORG 2058 in 0.1 ml TEND-10G at +4°C overnight (occasionally, identical incubations were conducted with [^{3}H]-R 5020). Parallel sets of tubes in duplicate contained a 100-fold excess of the synthetic progestin in non-radioactive

form, in order to allow correction for the non-specific binding of the steroid. Following incubation, bound and unbound steroids were separated by a dextran-coated charcoal technique [11] and the bound fraction counted for radioactivity. The method of Scatchard [12] was utilized to calculate receptor levels from the binding data. Receptor values are expressed relative to cytosol protein concentration which was measured by the method of Lowry [13] with bovine serum albumin (BSA) as standard.

Density gradient centrifugation was conducted as previously described [3, 4], using 5–20% sucrose gradients made up in TEND-10G buffer.

Estimation of the biological activity of progestins was performed by measuring histological changes in rabbit endometrium after systemic administration of the hormone, employing the criteria of McPhail [14]. The biological activity of a given steroid was estimated relative to progesterone, as described by Kontula *et al.* [3].

Determination of binding kinetics

The rate of association was measured utilizing the methods described in detail by Best-Belpomme *et al.* [15] and Schrader and O'Malley [16], as previously outlined [4]. The initial rate of association was plotted and calculated as described by Best-Belpomme [15]. The first-order dissociation rate constant was determined, as described in detail in refs. [4, 15, 16]. All series of experiments contained appropriate controls for checking of the stability of the receptor at different time points [4].

Dissociation and association rate constants were measured in the absence of any additional salt for all the labeled progestins. In addition, dissociation rate studies in the presence of 0.15 M KCl and 0.3 M KCl were performed utilizing progesterone, NET and ORG 2058 as labeled ligands.

Determination of free steroids

Both serum and myometrial cytosol estradiol-17β and progesterone concentrations were measured using radioimmunological techniques [17, 18].

RESULTS AND DISCUSSION

Steroidal regulation of progestin receptor level in human myometrium

Animal experiments have clearly shown that the progestin receptor level in mammalian uterus is under dual steroidal control: Exogenous estrogens are positive effectors promoting the synthesis of new progestin receptors, whereas exogenous progesterone (and progestins?) seems to degrade its own receptor [19–21]. A situation consistent with the results from studies with exogenous hormones apparently prevails during the estrous cycle of the animals [22], when endogenous steroids regulate the cytosol progestin receptor level in the uterus. Exogenous estrogens also control progestin receptor synthesis in human myometrium by increasing its concentration in uteri of postmenopausal women (Fig. 2, estrogen-treated women vs. non-treated controls). Moreover, exogenous estrogen administration almost invariably led to the appearance of a 7S progestin receptor component in the myometrial cytosol of the patients treated [3, 4]. This was in marked contrast to the situation during the normal menstrual cycle of fertile women, when only 4S receptor could be found in the myometrial cytosol. During the normal menstrual cycle, the level of progestin receptor in the myometrial cytosol is lower during the latter half of the cycle (Fig. 2) favoring the contention that progesterone elicits a negative control over its own receptor. Whether the decline is due to nuclear translocation of the cytosol receptor or to its degradation, is not known at present. When cytosol estradiol-17β and progesterone concentrations were related to cytosol progestin receptor levels, two separate correlations were found: There was a direct correlation between cytosol estradiol-17β concentration and progestin receptor level, whereas cytosol progesterone content and progestin receptor concentration correlated in an inverse manner [18]. Thus, steroidal regulation of the progestin receptor synthesis in and disappearance from the human myometrium seems to compare well with the initial findings which emerged in animal experiments.

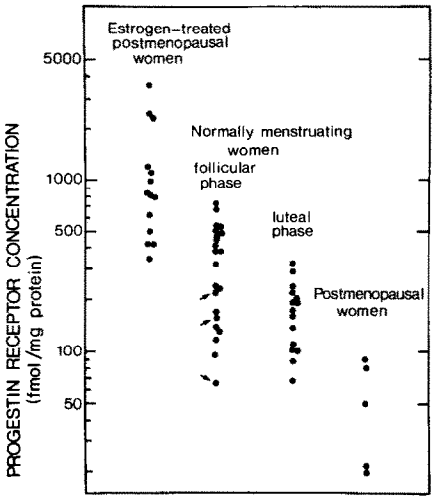

Fig. 2. Progestin receptor levels in cytosol samples prepared from human myometrial tissue. Tissue specimens were obtained from fertile women at various stages of the menstrual cycle, from postmenopausal women after 1 week's estrogen treatment and from non-treated postmenopausal women. Arrows denote cases, who underwent hysterectomy at an early follicular phase (prior to the 7th day of the cycle).

Biological potency of progestins in relation to their receptor binding activity

We have previously shown that estrogen-induced cytosol progestin receptors in the myometrium and endometrium of rabbit and sheep have very similar, if not identical, steroid binding properties with both

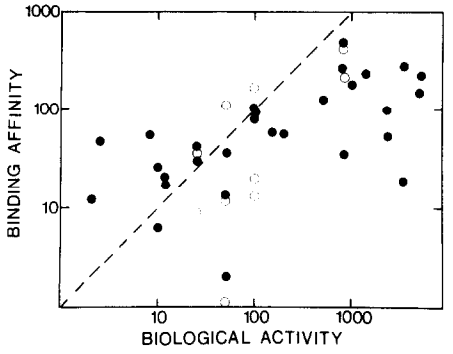

Fig. 3. Comparison of the *in vitro* receptor binding of various progestins with the biological activity of the same steroids, using rabbit as the experimental animal. In each case, the value obtained for progesterone was set as 100 and the figures for other compounds then expressed relative to progesterone. Data is obtained from refs. [3, 4]. ● = subcutaneous, ○ = oral administration of the steroid during the test of its biological activity (see Experimental). Dotted line shows the situation when y is equal to x.

endometrial and myometrial progestin receptors in the human uterus [3, 4]. These findings thus justify comparison of *in vivo* endometrial response to *in vitro* binding to myometrial receptor and also, application of biological data from suitable animal models to man. The biological activity of a number of steroidal compounds was extensively evaluated employing rabbit as the experimental animal and glandular endometrium as the target tissue in the estimation of the *in vivo* potency of a steroid [3, 4]. A modest correlation was present between the receptor binding activity and biological potency of the various steroids investigated as illustrated in Fig. 3. Certain modifications in the steroid structure brought about relatively greater increment in the biological potency of the steroid than what was found in its receptor binding (Fig. 3). This greater increase in the biological activity may have resulted from changes in various factors involved in the metabolism and excretion of the steroid in question, since some bulky groups linked to the steroid are likely to change these parameters. On the other hand, longer residence of the steroid within the target cell due to other factors, such as tighter binding of the progestin to its receptor and altered interaction of the complex with the nuclear acceptor sites, might have contributed to enhanced biological activity of the modified progestin. Mechanisms consistent with the latter proposals have been recently suggested as explanations for the differential biological activity of various estrogens, too [6, 7]. The *in vivo* biological potency of one synthetic progestin, medroxyprogesterone acetate, in guinea pig uterus seems to derive in part from its longer nuclear retention [23]. We have been interested in studying whether kinetics of interaction between various potent progestins and progestin receptor from the human uterus would be different from that between the same receptor and its physiological ligand, progesterone.

Receptor binding kinetics of some tritium-labeled progestins

The results from the kinetic studies are summarized in Table 1 and Figs. 4 and 5. Four out of the five labeled progestins associated to the receptor with rates fairly similar to each other, but one of them, ORG 2969 lacking the 3-keto group, associated with the myometrial cytosol receptor at a much slower rate (Fig. 4 and Table 1). This may have been due to hydrophobic interactions between different regions of the molecule (e.g. methylene group at C-11) and the receptor than in the case of other steroids, in order to overcome the absence of the 3-keto group, previously shown by us to be of major importance in the binding of progesterone to its cytosol receptor [3, 4]. The rate constants for association (k_a's) given in Table 1 are greater than that given by us previously for progesterone [4] which was, however, measured in the presence of 20% glycerol instead of 10% used in this work.

The rate constants for dissociation of the 5 progestins from the partially purified cytosol receptor differed remarkably from each other (Fig. 5 and Table 1). The fastest k_d was estimated for progesterone itself ($13.0 \times 10^{-5}\ s^{-1}$; half-time of dissociation 90 min), followed by NET and ORG 2969. It was of interest to notice that the 3-deoxy steroid, ORG 2969, which associated slowly to the receptor did not dissociate at the fastest rate from the complex, as could have been predicted on the basis of the absence of the 3-keto group. The two potent progestins carrying hydrophobic substituents, ORG 2058 and R 5020, dissociated very slowly from the protein-steroid complex. Half-times for their dissociation were approximately 10-times longer than that of progesterone, being 1000 and 620 min, respectively. Each of the latter progestins have a high *in vivo* potency in relation to progesterone: ORG 2058 is 36-fold [3] and R 5020 approx. 100-fold [24] more active than progesterone.

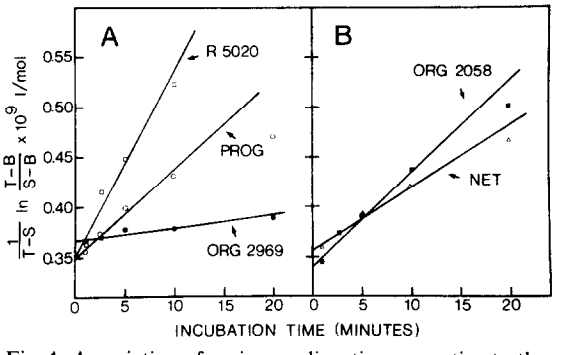

Fig. 4. Association of various radioactive progestins to the human myometrial progestin receptor partially purified from the cytosol fraction, as described in the text. The second-order association rate plots are constructed according to Best-Belpomme *et al.* [15]. S = concentration of binding sites (2.74 nM in each case); T = total hormone added (PROG, 3.1 nM; NET, 3.0 nM; ORG 2058, 3.3 nM; R 5020, 3.0 nM; and ORG 2969, 2.8 nM); B = amount of bound progestin at a given time.

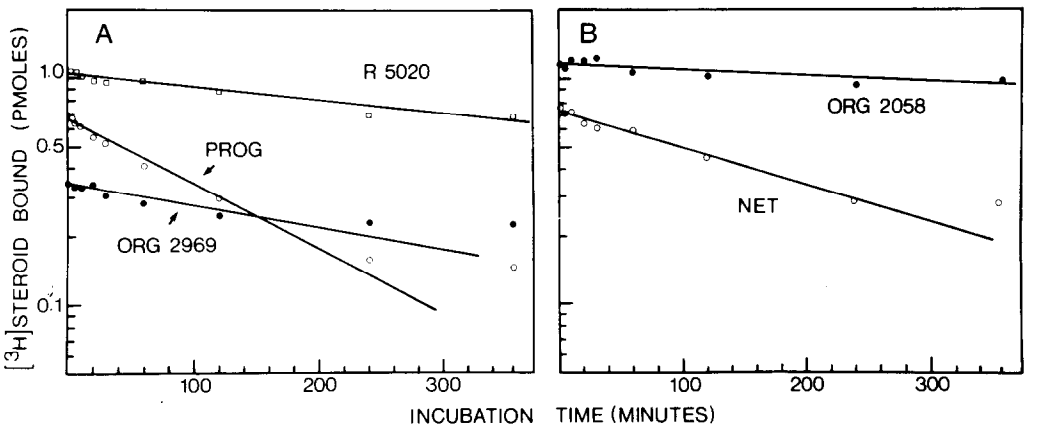

Fig. 5. Analyses of dissociation rates for the myometrial receptor–progestin complexes. After the initial labeling of the receptor with the steroid and removal of excess tritiated progestin by pellets of Dextran-charcoal, a 1000-fold excess of unlabeled progestin was added to the respective set of tubes to start the experiment. The amount of labeled receptor–progestin complex was measured at indicated time intervals by the dextran-charcoal assay.

It is tempting to suggest that these figures would be partially due to slower dissociation and thus better stability of the receptor–progestin complexes also *in vivo*. This might subsequently lead to more stable steroid receptor–chromatin complexes and thereby, to a longer duration of the biological action of the progestin.

When the apparent intrinsic dissociation constants (K_D's) were calculated from the kinetic data, the values obtained for different progestins were 5-15-fold lower (Table 1) than those derived from the Scatchard analyses shown in Fig. 6. Similar results were found for ORG 2969 (not shown in Fig. 6): In this case kinetic studies yielded K_D's which were 4–5 times lower than those derived from Scatchard analyses. The reason for this discrepancy between kinetic and equilibrium data is not known at present. It seems to be, however, rather uniform finding for most steroid–receptor interactions thus far studied with the two different approaches [2].

Inclusion of either 0.15 M KCl or 0.3 M KCl in the TEND-10G buffer during the dissociation reaction enhanced the rate of exchange of the labeled steroid to a respective non-labeled compound. The higher KCl concentration (0.3 M) decreased the half-lives of receptors complexed with progesterone, NET and ORG 2058 to a similar degree (to 50–60% of the control), whereas complexes with a lower rate of dissociation seemed to tolerate 0.15 M KCl somewhat better than the fast-dissociating progesterone (cf. Table 2). In this respect, human myometrial progestin receptor differs from the corresponding cytosol receptor in guinea pig uterus, the half-time of dissociation of which is longer in the presence of 0.4 M KCl than in a low-salt medium [25]. In accordance with our previous findings, 0.15 M or 0.3 M KCl did not decrease significantly the number of receptor binding sites for progesterone over the period of 18-h incubation [26], neither was a loss of binding of NET or ORG 2058 noticed in these control incubations (results not shown).

Sedimentation profile of the human myometrial progestin receptor complexed with various progestins

In our previous studies, we have shown that all potent synthetic progestins are capable of displacing [^{3}H]-progesterone from both 7S and 4S binding components of the partially purified cytosol progestin receptor isolated from myometra of estrogen-treated postmenopausal women [3, 4]. In order to gain more information about the specificity of the binding characteristics of the two components, we repeated

Table 1. Association and dissociation rate constants for the interaction between various labeled progestins and human uterine progestin receptor[1]

Steroid	Association rate constant (k_a) ($l \times mol^{-1} \times s^{-1}$)	Dissociation rate constant (k_d) (s^{-1})	k_d/k_a ($mol \times l^{-1}$)
Progesterone	2.3×10^5	13.0×10^{-5}	5.65×10^{-10}
NET	1.9×10^5	5.7×10^{-5}	3.0×10^{-10}
ORG 2058	3.0×10^5	1.2×10^{-5}	0.4×10^{-10}
R 5020	4.6×10^5	1.9×10^{-5}	0.41×10^{-10}
ORG 2969	0.2×10^5	4.9×10^{-5}	24.5×10^{-10}

[1] The rate constants were measured as described in the text at +4°C employing $(NH_4)_2SO_4$ purified and desalted cytosol receptor fraction from the human myometrium.

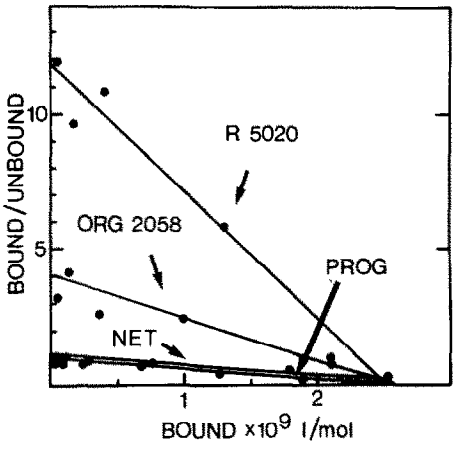

Fig. 6. Scatchard analysis of the binding of different progestins to the human myometrial receptor. Cytosol receptor fraction was prepared by ammonium sulfate precipitation and Sephadex G-25 filtration from the uterus of an estrogen-treated woman, as described in the Experimental-section. Portions of the same receptor preparation were allowed to interact with various amounts of the tritium-labeled progestins (18 h at +4°C), after which bound and free radioactivities were separated by the dextran-charcoal technique.

the sedimentation studies employing this time various [^{3}H]-labeled synthetic progestins. All the labeled steroids were bound to each of the two receptor entities, as shown in Fig. 7. More radioactivity was bound to both 7S and 4S peaks, when the labeling was performed with progestins exhibiting a low rate of dissociation (ORG 2058 and R 5020) than with other compounds, although equimolar steroid concentrations were used to charge identical portions of an ammonium sulfate purified and desalted receptor preparation. This is obviously eminating from the lower rate of dissociation during centrifugation of the receptors complexed with [^{3}H]-ORG 2058 or [^{3}H]-R 5020 than those associated with other progestins. It was of interest to notice that the relative proportions of radioactivity in the 7S and 4S peaks were

Table 2. Effect of salt on the dissociation of the labeled progestin-cytosol receptor complex[1]

Steroid	Reaction allowed to proceed in the presence of No KCl	0.15 M KCl	0.3 M KCl
Progesterone	155 min[2]	105 min	80 min
NET	276 min	203 min	155 min
ORG 2058	1110 min	1000 min	690 min

[1] Progestin receptor was partially purified by $(NH_4)_2SO_4$ precipitation from the myometrial cytosol from the uterus of an estrogen-treated patient, and desalted prior to charging with the labeled progestin. First-order rate of dissociation was determined as described in the text.

[2] Half-time of dissociation. The time required to exchange half of the bound labeled progestin to a respective unlabeled steroid, added in excess to start the dissociation reaction.

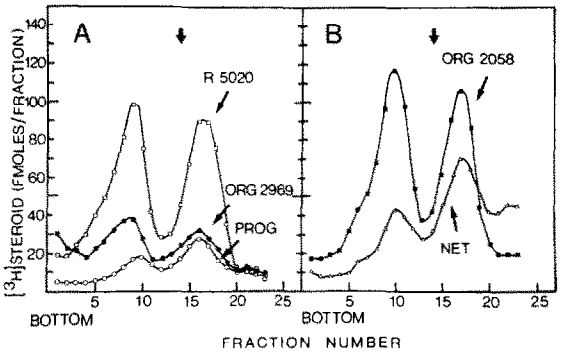

Fig. 7. Sucrose density gradient centrifugations of the myometrial progestin receptor from the uterus of an estrogen-treated postmenopausal woman. Myometrial receptor was partially purified by $(NH_4)_2SO_4$ precipitation and desalted with Sephadex G-25 filtration. Identical portions of the preparation were charged with equimolar amounts of the various [^{3}H]-progestins for 3 h at +4°C, after which excess of label was removed by Dextran-charcoal pellets. Centrifugation was conducted at 49,000 rev./min for 18 h utilizing 5–20% sucrose gradients made up in TEND-10G. The arrow points to the sedimentation position of BSA.

not similar for each labeled progestin: High binding affinity of the steroid seemed to be accompanied with an increase in the size of the 7S peak, when compared to the sedimentation profile of progesterone (Fig. 7). We do not know at present, whether this phenomenon is simply derived from a better stability of the 7S receptor–progestin complex during rather lengthy centrifugation or whether a potent progestin preferentially promotes an *in vitro* formation of the 7S complex. Partially purified myometrial progestin receptors did not show any major non-receptor binding of the progestins studied: apparently the same binding sites were measured with each of them in the Scatchard analyses (cf. Fig. 6) and, furthermore, both excess of non-labeled progesterone and the re-

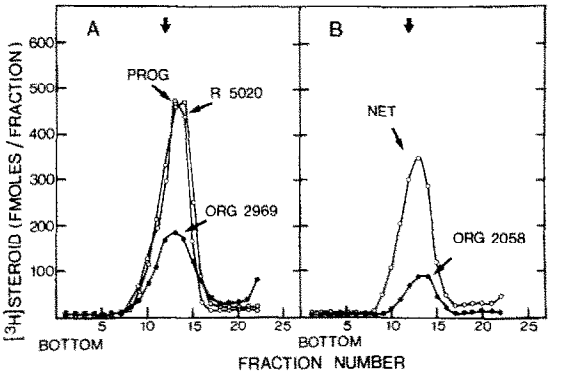

Fig. 8. Sucrose density gradient centrifugations of human pregnancy serum in the presence of various [^{3}H]-labeled progestins. Pregnancy serum was first depleted of endogenous steroids by treatment with Dextran-charcoal pellets. Aliquots of serum (0.6 ml) were then labeled with equimolar amounts of [^{3}H]-progestins for 3 h at +4°C, and the excess of label removed by a second treatment with Dextran-charcoal. Portions from the diluted serum sample were then run on density gradients, as explained in the text and the legend to Fig. 7.

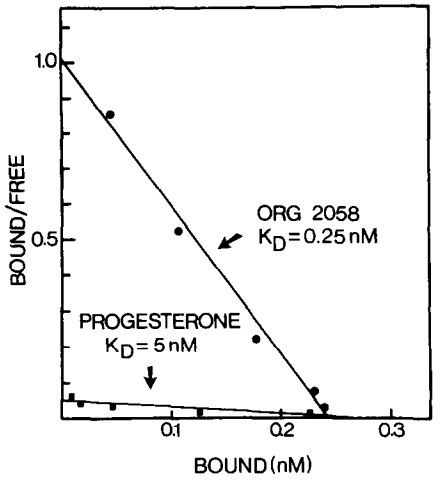

Fig. 9. Analysis of the binding of [^{3}H]-progesterone and [^{3}H]-ORG 2058 to the progestin receptor in human breast cancer cytosol by a Scatchard-type plot. The cytosol sample contained progestin receptor 90 fmol/mg protein. The binding of labeled progesterone was measured in the presence of a 100-fold excess of non-radioactive cortisol (see ref. [18]).

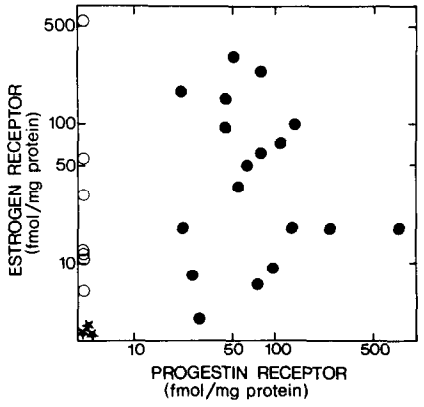

Fig. 10. Estrogen and progestin receptor levels in breast cancer cytosol samples. The receptor levels were measured utilizing [^{3}H]-estradiol-17β and [^{3}H]-ORG 2058, respectively, as the ligands in the receptor assays (see text). ● = both estrogen and progestin receptors detected; ○ = progestin receptor level below 5 fmol/mg cytosol protein; * = no measurable estrogen or progestin receptor.

spective progestin were capable of completely abolishing the formation of 7S and 4S macromolecule complexes during the density gradient centrifugation. Our current work is aimed at elucidating whether these two receptor forms have different kinetic properties and whether chromatin binding of progestin receptor prefers one of these forms.

Even though R 5020 and ORG 2058 are two promising progestins with a high potential use in progestin receptor measurements, they still exhibit binding to non-receptor components as well. This is clearly indicated in Fig. 8, where binding to female serum of the various progestins is illustrated utilizing density gradient centrifugation technique. Of the slowly-dissociating, high-affinity progestins, R 5020 seems to be bound to a much greater extent to non-specific sites in female serum than ORG 2058. The binding to these components apparently does not involve corticosteroid-binding globulin [27], and is high-capacity, low-affinity type of binding in nature (not shown). In spite of the low affinity, the binding of R 5020 (and ORG 2058 to a lesser extent) to the 4S-like material on density gradient centrifugation should be taken into account, when quantitative results from analyses of crude cytosol samples with this technique are interpreted.

Table 3. Estrogen (ER) and progestin (PR) receptors among Finnish breast cancer patients

	PR + [1]	
ER + [2]	18/25	(72%)
ER −	0/3	(0%)

[1] PR + denotes cases where progestin receptor was measured using [^{3}H]-ORG 2058 as the ligand. PR range: 0–781 fmol/mg cytosol protein.

[2] ER + = estrogen receptor level measurable; ER − = no ER detected. ER range: 0–507 fmol/mg cytosol protein.

Measurement of progestin receptors from breast cancer cytosol with [^{3}H]-*labeled ORG* 2058

In accordance with the results derived from studies of uterine progestin receptor, ORG 2058 was bound with a much higher affinity to the cytosol breast cancer progestin receptor than progesterone itself (Fig. 9). Progesterone (in the presence of excess cortisol, see [18]) and ORG 2058 also seemed to involve the same binding sites on the breast cancer binding protein (Fig. 9). The binding of ORG 2058 was inhibited with excesss of unlabeled progesterone and ORG 2058, but not with estradiol-17β, testosterone, 5α-dihydrotestosterone or cortisol. At present, we have found in 18 out of 25 (72%) Finnish estrogen receptor positive breast cancer samples also measurable progestin receptor level (Table 3). None of the 3 estrogen receptor negative tumors contained progestin receptor level which was demonstrable with [^{3}H]-ORG 2058. The results are in harmony with those obtained by McGuire *et al.* [28] and Raynaud *et al.* [29] in extensive studies using labeled R 5020 as the tracer during progestin receptor measurements.

In our initial and limited material, the levels of estrogen and progestin receptors within the same tumor sample do not have any significant correlation with each other (Fig. 10), although the receptor concentrations have approximately the same quantitative range: Progestin receptor 0–781 fmol and estrogen receptor 0–507 fmol/mg cytosol protein.

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DISCUSSION

Bell. Regarding your comments that the equilibrium constants were found to be lower than the ratio of rate constants, one only needs a very low rate of receptor inactivation to cause radical differences in apparent equilibrium values. A very low rate of degradation, possibly comparable with some of your dissociation rates, will result in true equilibrium never being reached and will lower the values measured by means of equilibrium binding studies. If we plot the time course of binding on the basis of rate constants, we often find that we would expect to reach equilibrium at a time much later than in practice we measure. Have you attempted to estimate the diffusion-limited rate of association in your system, because these association rates seem low for a diffusion-limited process?

Janne. We have not done anything like that.

Jungblut. I second Dr. Bell's remark. In order to differentiate between dissociation and destruction it is necessary to assess the ability for reassociation.