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PROGESTERONE-BINDING PROPERTIES OF MICROSOMES FROM PREGNANT RAT UTERUS

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SUMMARY

The progesterone binding characteristics of microsomes from pregnant and post partum rat myometrium were investigated using a competitive protein binding method.

The myometrial microsomal preparations showed progesterone binding of high affinity ($K_a \sim 6 \times 10^7\ M^{-1}$) and limited capacity in addition to low affinity binding of high capacity. The microsomal progesterone binding components of high affinity were characterized by rapid dissociation and exchange of bound steroid at +6°C and 0°C.

The progesterone binding activity of the rat myometrial microsomes was resistant to freezing. The microsomal progesterone binding components were shown to be specific for progesterone. Of 18 different steroids tested only testosterone, 5α-dihydrotestosterone (17β-hydroxy-5α-androstan-3-one) and 5α-pregnane-3,20-dione exhibited moderate competition with [^{3}H]-progesterone for the binding sites.

Microsomal preparations from rat striated muscle and heart displayed only low concentrations of non-specific progesterone binding whereas rat liver microsomes exhibited progesterone binding resembling that of the myometrial microsomes.

The myometrial microsomal progesterone binding capacity was lower post partum than one day before parturition.

The microsomal progesterone binding components described in the present study are distinctly different from the cytosolic high affinity progesterone binding proteins found in rat myometrium.

INTRODUCTION

Cytosolic progesterone receptors have been found in the human uterus [1–3] and the uteri of several mammals [4–7]. Rat myometrial cytosol contains a progesterone-specific receptor [6, 7] and a non-specific progesterone binding protein of high affinity which closely resembles corticosteroid binding globulin (CBG) [8]. The concentration of this CBG-like progesterone binder falls to undetectable levels just before parturition [3, 9].

The existence of microsomal estrogen binding proteins in pig uterus has been reported [10–12]. These microsomal estrogen binders are assumed to be precursors of soluble cytoplasmic receptors [11]. It is also known that injected [^{14}C]-progesterone is retained in the microsome fraction (sedimented at 20000 *g*) of pregnant rat myometrium in higher amounts per mole N_2 than in the other subcellular fractions [13]. The capacity of this subcellular fraction to retain [^{14}C]-progesterone was found to decrease steeply just prior to, during or after parturition [14]. Whereas rat liver microsomes contain high affinity binding components for oestradiol and testosterone [15, 16], the possible existence of progesterone binding proteins in the microsomal fraction of pregnant rat uterus has not been reported.

In the present study the microsomal progesterone binding components of pregnant rat myometrium have been characterized. These progesterone binders differ from the cytosolic progesterone receptor of rat myometrium. In addition microsomal progesterone binding capacity seems to decrease at parturition.

MATERIALS AND METHODS

Chemicals

The chemicals used were as described previously [3]. Preparation of tissue samples and progesterone binding studies were carried out in a medium containing 25 mM Tris–HCl buffer, 1·3 mM EDTA and 250 mM sucrose, pH 7·4 (TES buffer).

Steroids

[1,2,6,7-^{3}H]-Progesterone (S.A. 81·1 Ci/mmol and 96·0 Ci/mmol) was obtained from the New England Nuclear Corporation, U.S.A. The purity of this material was checked every 4th month by t.l.c. (chloroform–acetone 9:1 v/v) and was found to be 94–96% during the study.

17β-Hydroxy-5α-androstan-3-one was obtained from Steraloids, Inc., Pawling, New York, U.S.A. 3α-Hydroxy-5α-androstan-17-one, 4-androstene-3,17-dione, 5α-androstane-3,17-dione and 5β-androstane-3,17-dione were obtained from Ikapharm, Ramat-Gan, Israel. The other steroids used were the same as described previously [3, 17]. The purity of the non-radioactive steroids was checked by g.l.c. In all cases these compounds were chromatographically pure and were used without purification. The trivial and systematic names of the steroid standards used are given in Table 1.

Table 1. The steroid specificity of the microsomal progesterone binding components of rat myometrium. The values given represent the percentage of [^{3}H]-progesterone (2 nM) bound in the presence of 167 nM non-radioactive steroid. The amount of tritiated progesterone bound in the absence of other steroids is given as 100%. All the steroids tested were assayed in duplicate and using microsomes isolated from two different rats on two separate days. The values given represent the mean of the 2 determinations. The values given for progesterone is based on 12 different determinations (12 rats)

Trivial name	Systematic name	[^{3}H]-progesterone bound, %
progesterone	4-pregnene-3,20-dione	42 ± 6
testosterone	17β-hydroxy-4-androsten-3-one	55
5α-dihydrotestosterone	17β-hydroxy-5α-androstan-3-one	56
5α-pregnanedione	5α-pregnane-3,20-dione	65
5β-pregnanedione	5β-pregnane-3,20-dione	77
5α-androstanedione	5α-androstane-3,17-dione	79
R-2323	13-ethyl-17α-ethynyl-17β-hydroxy-4,9,11-gonatrien-3-one	84
17-hydroxyprogesterone	17α-hydroxy-4-pregnene-3,20-dione	90
medroxyprogesterone acetate	6α-methyl-3,20-dioxo-4-pregnen-17α-yl-acetate	91
20α-dihydroprogesterone	20α-hydroxy-4-pregnen-3-one	97
norethisterone	17α-ethynyl-17β-hydroxy-4-estren-3-one	97
norethandrolone	17α-ethyl-17β-hydroxy-4-estren-3-one	98
androsterone	3α-hydroxy-5α-androstan-17-one	98
androstenedione	4-androstene-3,17-dione	99
5β-androstanedione	5β-androstane-3,17-dione	99
megestrol acetate	6-methyl-3,20-dioxo-4,6-pregnadien-17α-yl-acetate	100
oestradiol	1,3,5(10)-oestratriene-3,17β-diol	100
cortisol	11β,17α,21-trihydroxy-4-pregnene-3,20-dione	100
cholesterol	5-cholesten-3β-ol	100

Animals

Non-pregnant (15), pregnant (56) and post partum (13) Sprague Dawley rats were used. The day of vaginal sperm positivity was designated day 0 of pregnancy and the next day as day 1. The rats thus delivered on the 22nd day of pregnancy. The duration of pregnancy, near term, was also checked by determining the mean foetal weight from which the exact duration of pregnancy can be determined [18].

Microsomes

The rats were killed by decapitation. The foetuses of rats 18–22 days pregnant were weighed and the uterus prepared as already described [3, 18] except for the following modifications: the myometrial homogenate was not filtered but was directly centrifuged at 10,000 *g* for 15 min, at +2°C. The supernatant thus obtained was then centrifuged at 105,000 *g* for 60 min at +2°C. The 105,000 *g* supernatant was decanted, the tube and the surface of the 105,000 *g* pellet were washed with TES buffer. The pellet was then gently suspended in TES buffer using 10 strokes of a homogenizer with a teflon pestle (1·0 ml TES buffer per 1·0 g of myometrial wet weight). This microsomal suspension was either stored at −20°C overnight, or was immediately used in the progesterone binding studies. In the case of non-pregnant rats or when the uterine weight of pregnant rats was <1·0 g microsomal suspensions were prepared from 2–4 pooled uteri. Microsomal suspensions of the liver, striated muscle and heart were similarly prepared.

Protein assay

Protein was determined according to the method of Lowry *et al.*[19].

Enzyme treatment

Microsomes prepared from the myometrium of a 21 days pregnant rat were suspended in TES buffer and then divided into 4 aliquots of 0·5 ml. Protease (from Streptomyces griseus with starch extender, no. P-5005, Sigma Chemical Co., St. Louis, Mo., U.S.A.) to a final concentration of 5·7 mg/ml was added to one of the tubes and RNase (Ribonuclease-A, type 1-A, from bovine pancreas, no. R-4875, Sigma) to a final concentration of 0·9 mg/ml to another and 25 mM Tris buffer pH 7·4 to the two control tubes. The tubes containing the enzymes and one control tube were incubated for 30 min at +22°C. The second control tube was kept at 0°C. After incubation the tubes were cooled and the progesterone binding capacity of the samples was measured by the CPB method [3]. Similarly treated enzyme control samples containing TES buffer and the same volume of enzyme solution as the microsomal samples were run in parallel with the analytical samples.

Charcoal pretreatment

Charcoal pretreatment of the microsomal suspensions was performed by dialyzing the microsomes suspended in TES buffer against 0·5% (w/v) activated charcoal (Norit A), overnight at +6°C. Charcoal suspension (10 ml) was used in the outer phase per 2·5 ml of microsomal suspension in the inner phase. The charcoal-pretreated microsomes were then assayed for their progesterone binding capacity.

Assay for progesterone binding capacity

The progesterone binding capacity of the myometrial microsomes was determined using a competitive protein binding (CPB) method which has been described in detail elsewhere [3]. In this method Dextran-coated charcoal is used for the separation of bound and free steroids. The microsomes were incubated for 1 h at +6°C with 2 nM [^{3}H]-progesterone and non-radioactive progesterone (0–1667 nM). The reaction time with Dextran-coated charcoal (0·5% Norit A, 0·005% Dextran T 70) was usually 5 min. Most microsomal suspensions were only assayed at a [^{3}H]-progesterone concentration of 2 nM and a CPB curve could only be constructed for 21 of the samples as the microsomal pellet derived from a single uterus was very scanty. All incubations were carried out in duplicate. The Dextran-coated charcoal was sedimented by centrifugation at 12,000 *g* for 2 min, conditions under which the microsomes remained in the supernatant. From protein determinations it was obvious that the microsomes were not absorbed on the charcoal during the assay. Steroid specificity was tested by comparing the ability of different steroids and progesterone (167 nM) to reduce the amount of [^{3}H]-progesterone (2 nM) bound by the microsomes.

Calculations

The Dextran-coated charcoal absorbed 98–99% of the unbound steroid. In every assay 4 buffer controls were included to determine the amount of radioactivity left in the supernatant after charcoal adsorption. This background radioactivity (usually 900–1500 d.p.m.) representing the amount of unbound steroid not adsorbed by the charcoal was always subtracted from the supernatant radioactivity content in the samples analysed (3000–6000 d.p.m.) before the final results were calculated. The results are expressed as fmol of [^{3}H]-progesterone bound per mg microsomal protein at a [^{3}H]-progesterone concentration of 2 nM. To estimate the approximate number of total high affinity binding sites and the association constant (K_a) reduced Scatchard plots were constructed [20, 21].

Thin-layer chromatographic (t.l.c.) analysis of microsome-bound [^{3}H]-labelled steroid

To investigate whether progesterone was metabolized when bound to the myometrial microsomes a microsomal suspension prepared from the uterus of a 21 days pregnant rat was incubated with 2 nM [^{3}H]-progesterone for 1 h at +6°C and the unbound steroid was then adsorbed with Dextran-coated charcoal in the usual way. The supernatant containing the bound steroid was extracted twice with 5 vol. of diethyl-ether–ethyl acetate (2:1 v/v) and evaporated to dryness in a stream of nitrogen. The dry residue was then dissolved in 100 µl of ethyl acetate and applied to a t.l. plate (precoated silica gel plates 20 × 20 cm., Eastman Kodak Co., Rochester, New York, 14650, U.S.A., prewashed with methanol). The plate was developed in chloroform–acetone (9:1 v/v). Non-radioactive progesterone (located with a U.V.-light) and [^{3}H]-progesterone were used as standards and were added, prior to chromatography to the same amount of biological extract as was present in the unknowns. After development the areas containing the radioactive steroids were cut into 1·0–1·5 cm. segments, and the radioactivity quantitated in liquid scintillation counting vials containing the gel and 7·0 ml of Insta-Gel.

Dissocation of microsome-bound [^{3}H]-progesterone in the presence of excess non-labelled progesterone

To determine the dissociation rate of bound progesterone microsomal suspensions were prepared from combined pregnant rat (19–20 days) uteri and incubated with [^{3}H]-progesterone (final concentration 2 nM) for 1 h at +6°C. Then an excess (1667 nM) of non-labelled progesterone was added, the tube was mixed on a vortex mixer and incubated at +6°C or 0°C. Duplicate samples were taken from the tube at fixed time intervals and adsorbed with Dextran-coated charcoal for 5 min before centrifugation. The amount of [^{3}H]-progesterone bound was then calculated. The results were plotted as fmol of tritiated progesterone bound against incubation time with the non-radioactive progesterone.

Measurement of radioactivity

Radioactivity was measured in a LKB Wallac liquid scintillation counter 81000 (LKB Wallac Inc., Turku, Finland) using Insta-Gel (Packard Instrument Co., Ill., U.S.A.) as the scintillation solution. No correction for quenching was made as standards were counted simultaneously with all analytical samples and in identical media.

Radioimmunoassay (RIA)

The concentration of progesterone in the microsomal suspensions was determined by radioimmunoassay using a method described in detail elsewhere [18].

RESULTS

Binding of progesterone by myometrial microsomes

Progesterone was bound by the myometrial microsomes of non-pregnant, pregnant and post partum rats. Figure 1 shows a CPB-curve and a Scatchard

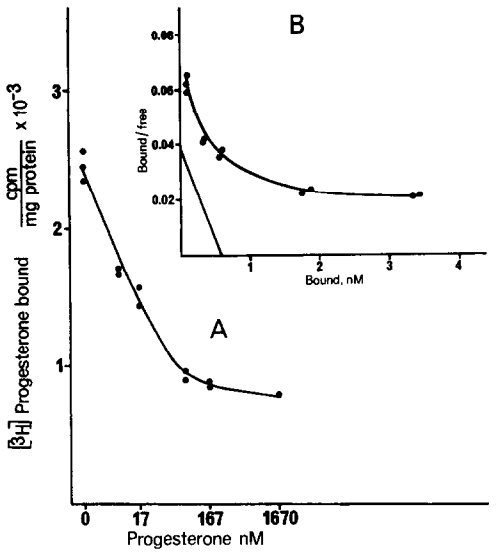

Fig. 1. A competitive protein binding curve (A) and the Scatchard plot (B) obtained with microsomes prepared from the uterus of a pregnant rat. The duration of pregnancy was 21 days. The concentration of [^{3}H]-progesterone was 2 nM and the final protein concentration 0·77 mg/ml. The incubation time was 1 h at +6°C and the reaction time with Dextran-coated charcoal 5 min. The straight line in Fig. 1 B is the reduced Scatchard plot obtained as described in the references nos. 20 and 21 and gives an association constant of $7 \times 10^7\ M^{-1}$ for progesterone in this case.

plot obtained with myometrial microsomes. As seen from the non-linear Scatchard plot, at least two kinds of progesterone binding exist, to high affinity progesterone binding components of limited capacity and to low affinity binding components of high capacity. The [^{3}H]-progesterone bound by the microsomes could not be displaced totally by a 1000-fold concentration of non-radioactive progesterone which demonstrates the existence of high capacity binding sites. The high affinity binding components were saturable with progesterone. The association constant of the high affinity binding sites was $\sim 6 \times 10^7\ M^{-1}$ for progesterone and the concentration of binding sites was ~1·0 pmol/mg microsomal protein. These mean values were determined using microsomal preparations from 2 post partum and 2 late pregnancy rats known to contain low concentrations of endogenous progesterone [18].

Association of [^{3}H]*-progesterone and microsomes*

The association of [^{3}H]-progesterone and myometrial microsomes was rapid at +6°C as seen in Fig. 2. The 1 h incubation time used in the routine binding studies was sufficient as shown.

Dissociation of microsome-bound [^{3}H]*-progesterone*

The dissociation and rate of exchange of progesterone bound to microsomes was measured at +6°C and at 0°C (Fig. 3). The exchange was initially rapid and the amount of 2 nM [^{3}H]-progesterone bound by the microsomes was reduced by ~50% in the pres-

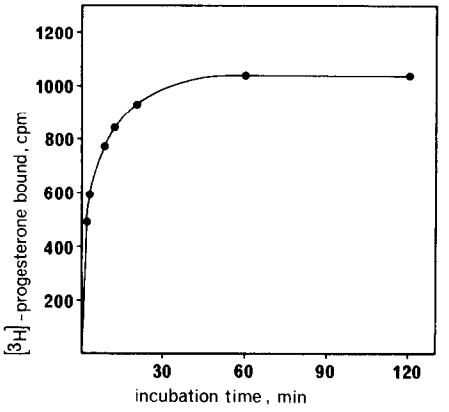

Fig. 2. Association of [^{3}H]-progesterone and pooled myometrial microsomes at +6°C. The microsomes were obtained from 3 rats, 20–21 days pregnant. The concentration of [^{3}H]-progesterone was 2 nM and the final protein concentration 0·93 mg/ml. The reaction time with Dextran-coated charcoal was 5 min. The results are plotted as amounts of [^{3}H]-progesterone bound c.p.m. v. incubation time.

ence of 1667 nM non-radioactive progesterone within 10 min at +6°C. After this rapid initial exchange the amount of [^{3}H]-progesterone bound remained constant showing the existence of high capacity binding sites which were unsaturable by the added progesterone. The half-life of the binding sites receptive to progesterone was ~6 min at +6°C and ~10 min at 0°C. The rapid dissociation of microsome-bound pro-

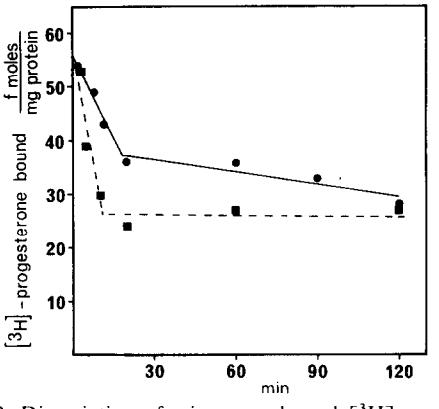

Fig. 3. Dissociation of microsome-bound [^{3}H]-progesterone (2 nM) in the presence of 1667 nM non-radioactive progesterone. The microsomes and [^{3}H]-progesterone was first incubated for 1 h at +6°C after which the excess of non-radioactive progesterone was added and incubation was continued at +6°C or 0°C. Duplicate samples were taken at fixed time intervals and adsorbed with Dextran-coated charcoal for 5 min before centrifugation. The microsome suspensions used were prepared from different pregnant rats. ● ●: 0°C, 19th day of pregnancy, ■ ■: +6°C, 20th day of pregnancy. The results are plotted as fmoles of [^{3}H]-progesterone bound/mg microsomal protein v. incubation time with excess progesterone. The time in min given indicates the interval between the addition of non-radioactive progesterone and Dextran-coated charcoal. The half-life of bound [^{3}H]-progesterone replaceable by progesterone (shown by the steep parts of the curves) was ~10 min at 0°C and ~6 min at +6°C and indicates the rapid exchange rate of microsome-bound progesterone.

gesterone was also observed when the reaction time with Dextran-coated charcoal at 0°C was varied: the amount of microsome-bound progesterone decreased linearly when the reaction time with the charcoal suspension was varied from 2 to 15 min, with only 45% of the amount bound at 2 min remaining bound after 15 min.

Steroid specificity

The microsomal progesterone binding components of high affinity were shown to be specific for progesterone. None of the other steroids tested behaved like progesterone in competing with [^{3}H]-progesterone for the binding sites (Table 1). The progesterone metabolites 17-hydroxyprogesterone and 20α-hydroxy-4-pregnen-3-one had a minimal effect on [^{3}H]-progesterone binding to the microsomes whereas 5α-pregnane-3,20-dione showed moderate competing efficiency. 5β-Pregnane-3,20-dione was less effective than the 5α-isomer. Synthetic progestins (5) were tested and only R-2323 (13-ethyl-17α-ethynyl-17β-hydroxy-4,9,11-gonatrien-3-one) had a moderate effect on [^{3}H]-progesterone binding whereas megestrol acetate, norethisterone, norethandrolone and medroxyprogesterone acetate were not bound to any significant extent by the microsomes. Of the 6 androgens tested testosterone and 5α-dihydrotestosterone were found to compete considerably with [^{3}H]-progesterone for the binding sites, 5α-androstane-3,17-dione had a moderate effect and 4-androstenedione 3β → 3α-hydroxy- 5α-androstan-17-one and 5β-androstane-3,17-dione were practically without effect. Oestradiol, cortisol and cholesterol had no effect on [^{3}H]-progesterone binding.

Target organ specificity

Microsomes from heart and striated muscle samples of pregnant rats exhibited constant and low [^{3}H]-progesterone binding which was not abolished by excess non-radioactive progesterone. Rat liver microsomes possessed progesterone binding components resembling those of the myometrium (Haukkamaa, unpublished data).

Effect of charcoal pretreatment

The microsomal progesterone binding capacity (measured at the 2 nM [^{3}H]-progesterone concentration) increased by 24% on the average (range 14–49% $n = 5$) from the basal levels after pretreatment of the microsomes from pregnant rat myometrium with charcoal. The concentration of endogenous progesterone in a pool of microsomes from 15 and 16 days pregnant rats decreased from 11·4 to 0·9 pmol/mg microsomal protein after charcoal pretreatment.

Effect of enzyme treatment

Treatment with Protease for 30 min at +22°C decreased the microsomal [^{3}H]-progesterone binding whereas RNase had no apparent effect on the binding of tritiated progesterone. However, the non-specific binding of progesterone shown by the enzyme preparations lead to difficulties in the quantitative interpretation of the results.

Effect of storage and temperature on myometrial microsomal progesterone binding

The progesterone binding activity of the microsomes resisted freezing and storage for short periods when suspended in TES buffer: 15% of the original binding capacity was lost during overnight storage at +6°C or −20°C and 40% of the binding capacity was lost during storage for 3 nights at these temperatures. Further it was observed that the specific progesterone binding activity of the myometrial microsomes was irreversibly lost when they were exposed at +37°C for 20 min.

T.l.c. of microsome-bound [^{3}H]-labelled steroid

The microsome-bound progesterone was not metabolized under the conditions used in the binding studies as 90% of the total radioactivity representing the microsome-bound [^{3}H]-labelled steroid was located in the area of the progesterone and [^{3}H]-progesterone standards on the t.l. plates. The purity of the standard [^{3}H]-progesterone was 94–96% when assessed in the same chromatographic system. Progesterone is well separated from 5α-pregnane-3,20-dione and 3α-hydroxy-5α-pregnan-20-one, the main progesterone metabolites found in rat myometrium [13, 24] under these chromatographic conditions [13].

Myometrial microsomal progesterone binding during pregnancy and parturition

The microsomal progesterone binding capacity was measured at a fixed [^{3}H]-progesterone concentration of 2 nM during the last third of pregnancy and until 3 days post partum (Table 2). The final protein concentration varied between 0·41–1·11 mg/ml in the

Table 2. The progesterone binding capacity of myometrial microsomes during the last third of pregnancy and 0·5–3 days post partum. n indicates the number of rats used in the determination of the values given. The results are expressed as fmol of [^{3}H]-progesterone bound/mg microsomal protein at a fixed [^{3}H]-progesterone concentration of 2 nM. The final protein concentration varied between 0·41 and 1·11 mg/ml

Duration of pregnancy days	n	[^{3}H]-progesterone bound fmoles/mg protein
14-16	6	72 ± 17
19	3	59 ± 13
20	10	59 ± 11
21	9	77 ± 25*
22	5	51 ± 27
Days post partum		
0.5-3	11	47 ± 18*

* $P < 0{\cdot}01$.

assay tubes. The results have not been compensated for the binding of endogenous progesterone present in the microsomal suspensions from pregnant rats [18]. The microsomal progesterone binding capacity did not differ significantly from day to day during the last third of pregnancy. The progesterone binding capacity in post partum rats was lower than on the 21st day of pregnancy ($P < 0.01$). The progesterone binding capacity was low on the day of delivery (22nd day of pregnancy) but only 5 rats were studied and the range of results was great. It was concluded that the difference between the binding capacities on the 21st day of pregnancy and 0·5–3 days post partum was significant since the concentration of endogenous progesterone is the same on these days [18].

DISCUSSION

A microsomal estrogen receptor in pig uterus has been described recently [10–12]. In the present study microsomal progesterone binding components of rat myometrium are reported. The microsomal receptor in pig uterus showed high affinity for oestradiol and was proposed to be a precursor of the cytosolic oestradiol receptor [11]. However, the affinity of this receptor for oestradiol ($K_a \sim 1 \times 10^9\ M^{-1}$) was higher than the affinity of the progesterone binding components described above for progesterone indicating that the functional roles of these microsomal steroid binding components probably differ from each other.

The microsomal progesterone binding components found in this study are characterized by high affinity, limited capacity and steroid specificity, all of which are characteristics of steroid receptors. The association constant ($K_a \sim 6 \times 10^7\ M^{-1}$) of the microsomal high affinity progesterone binders is lower than the K_a of the cytoplasmic progesterone receptor ($K_a \sim 10 \times 10^7\ M^{-1}$) of the pregnant rat [3]. The difference in these values, however, would not rule out the possibility that the microsomal binder is a precursor of the cytoplasmic receptor. However, the steroid specificity of these two binders is distinctly different [3].

The progesterone binding components of rat liver microsomes have a K_a of the same order as those of the myometrial microsomes (Haukkamaa, unpublished results). The fact that rat liver microsomes possess progesterone binding components resembling the myometrial microsomal binders, whereas microsomes from rat heart and striated muscle do not, raises the question of their being characteristic components of target organs only.

The ligand specificity of the microsomal progesterone binders demonstrates that both C-19 and C-21 steroids are accepted by the same binding sites. A 4-en-3-one structure is not essential for ligand binding as ring-A saturated steroids, 5α-pregnane-3,20-dione and 17β-hydroxy-5α-androstan-3-one, are bound but with decreased affinity. With regard to the binding of C-21 steroids, reduction of the C-20-keto group of progesterone or introduction of a 17α-hydroxyl-group reduces binding to a minimum. The microsomal progesterone binding components of rat myometrium do not bind synthetic progestins while the cytosolic progesterone receptors do [3, 17]. It is interesting to note that testosterone and 5α-dihydrotestosterone, the specific steroid ligand bound by androgen receptors [22, 23], are effective competitors of progesterone binding to the microsomal sites.

During the present study we strongly suspected that the microsomal progesterone binding protein was an enzyme. Metabolic conversion of progesterone during the binding studies was ruled out as no metabolites of progesterone were observed. The myometrial microsomes of pregnant rats do contain 4-ene-5α-reductase activity as was shown in this laboratory [13] and 5α-pregnane-3,20-dione, the principle progesterone metabolite found in rat myometrium [13, 24], binds to the microsomal progesterone binding component to some extent (Table 1). However, if the progesterone binding was due to substrate–enzyme complex formation, it is surprising that it did not tolerate exposure at +37°C for 20 min. The microsomal progesterone binding protein of high affinity is very selective in regard to steroid specificity as only certain 4-ene-3-keto and 5α-reduced steroids are accepted (Table 1). It seems that the substituents in or near ring D of the steroid nucleus are very critical in the determination of binding affinity as is the configuration of ring A, a property not usually characteristic of steroid metabolizing enzymes.

The values presented in Table 2 give the progesterone binding capacity of myometrial microsomes at a fixed [^{3}H]-progesterone concentration of 2 nM during the last third of pregnancy. The concentration of endogenous progesterone in the microsomes remains high until the 21st day of pregnancy [18]. Thus the progesterone binding capacity can be reliably determined and compared using this low concentration of [^{3}H]-progesterone during and after the 21st day of pregnancy only. Charcoal pretreatment of the myometrial microsomes (obtained from rats on the 14–20th day of pregnancy) increased their progesterone binding capacity and decreased the amount of endogenous progesterone to hardly detectable levels. From these observations it is concluded that before the 21st day of pregnancy (days 14–20 in Table 2) the microsomal progesterone binding capacity is potentially higher than the results indicate. This finding is supported by the results of a previous study with [^{14}C]-progesterone [14], which showed that injected radioactive progesterone was retained in the microsomal fraction in greater amounts than in other subcellular fractions before parturition but not post partum. The question of the progesterone binding capacity of myometrial microsomes at parturition may be of great physiological importance and requires further investigation.

Considering these and earlier findings [3, 25] we

conclude that the cytosolic progesterone-specific receptor of rat myometrium and the microsomal progesterone binder are different entities. This conclusion is based on the following: the association constants and ligand specificities of the cytoplasmic and microsomal progesterone binders are different and moreover, the regulation of binder concentration differs since the concentration of the cytoplasmic progesterone receptor is low throughout the last third of pregnancy and does not change at parturition [3, 25].

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