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ESTRADIOL-17β- PROGESTERONE AND 5α-DIHYDROTESTOSTERONE RECEPTORS OF UTERINE MYOMETRIUM AND MYOMA IN THE HUMAN SUBJECT

TERUHIKO TAMAYA, TOSHIHIKO MOTOYAMA, YOUSUKE OHONO,
NORIMASA IDE, TOSHIHIDE TSURUSAKI and HIROJI OKADA
Department of Obstetrics and Gynecology,
Kyoto Prefectural University of Medicine, Kyoto 602, Japan

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SUMMARY

Steroid receptors in human uterine myomas and the corresponding myometria were characterized and investigated to clarify their relation to tumor growth. The 248,800 *g* supernatant of tissue homogenates was used, in which steroid receptors were characterized and determined by Scatchard plot analysis using dextran coated charcoal at 4°C. In cytosols of both myoma and moymetrium, the estrogen receptors (ER) were sedimented at approximately the 7S, 5S and 4S regions, and the estradiol-17β(E_2)–ER complex had a dissociation constant (K_D) = 4.5×10^{-10} M. Progesterone receptors (PR) were sedimented at approximately the 7*S* and 4–5*S* regions. The K_D of the progesterone–PR complex was 1.5×10^{-9} M and the K_D of the R5020–PR complex was 5.0×10^{-10} M. Androgen receptors (AR) were sedimented at approximately the 6–7*S* and 5*S* regions and dihydrotestosterone (DHT)–AR complex had a $K_D = 4.0 \times 10^{-10}$ M. All of steroid–7*S* binding in the cytosol were easily degraded during 5–20% sucrose gradient centrifugation. Ligand specificity studies characterized the specificity of receptors.

There was no significant difference in steroid receptor levels (expressed as fmol/mg protein) between myoma and the corresponding myometrium; however if the receptor level was expressed as fmol/μDNA, the estrogen receptor level in the myometrium was higher than that in the myoma ($P < 0.05$). There was no significant difference in mitotic index between the two tissues.

INTRODUCTION

The uterine myometrium is regulated by sex steroids and sex steroids are considered to mediate their biological effects via cytoplasmic receptors. It is generally accepted that the source of uterine myoma is muscle tissue. It has been suggested that the ovarian steroids may be responsible for the growth of myomas [1].

Many investigators have described estrogen and progesterone receptors in human uterine myometrium and in uterine myoma [2–4], but androgen receptors have not yet been described.

In uterine myoma and the corresponding myometrium in the human subject, estrogen, progesterone and androgen receptors have been characterized, and the receptor levels and mitotic index investigated for cell proliferation.

MATERIALS AND METHODS

Radioactive steroids and counting of radioactivity. [1,2-^{3}H]Progesterone ([^{3}H]-P, 55.7 Ci/mmol); dimethyl-19-nor-prega-4,9-diene-3,20-dione,17,21-[17-methyl-^{3}H]([^{3}H]-R5020, 86 Ci/mmol); [6,7-^{3}H]-estradiol-17β ([^{3}H]-E_2 47.9 Ci/mmol) and [1,2-^{3}H]-dihydrotestosterone ([^{3}H]-DHT, 40 Ci/mmol) were obtained from New England Nuclear (Boston, MA). Aliquots of 0.2 ml were mixed with 3.0 ml of methanol and counted in 10 ml of toluene-based fluid (4 g of 2,5-diphenyloxazole and 0.1 g of 1,4-bis[2-(5-phenyloxazolyl)]-benzene in 1l of toluene) by a Packard 3390 scintillation spectrometer at 30% efficiency.

Steroids. Ethynyl estradiol (17α-ethynyl-1,3,5-estratriene-3,17β-diol), diethylstilbestrol [3,4-bis (p-hydroxyphenyl)-3-hexene, norethindrone (17α-ethynyl-17β-hydroxy-estra-4-en-3-one), and dihydrotestosterone (17β-hydroxy-5α-androstan-3-one, DHT) were obtained from Sigma Chemical Company (St. Louis, MO).

Preparation of cytosol. Immediately after total abdominal hysterectomy, the myometria of corpus uteri and myoma were frozen at −70°C. A part of either the endometrium, myometrium or myoma was submitted for histology. All subsequent steps were performed at 4°C. Frozen tissues were minced with a radish grater and homogenized with a Teflon–glass homogenizer (3 strokes) in 9 vol/wt of buffer A (0.01 M Tris, 1.5 mM EDTA, 10% glycerol, pH 7.4). This homogenate was centrifuged at 800 *g* for 10 min. The supernatant fraction from the 800 *g* centrifugation was further centrifuged for 1 h at 248,800 *g* in

Abbreviations: Estrogen receptor = ER, progesterone receptor = PR, androgen receptor = AR, dissociation constant = Kd, estradiol-17β = E_2, progesterone = P, dihydrotestosterone = DHT.

a Hitachi RPS 55T rotor to yield the supernatant fluid (cytosol). Cytosol protein was determined by biuret reaction [5] and a part of the 800 *g* pellet was resuspended in buffer A in order to determine the DNA content [6].

Sucrose gradient centrifugation. The cytosol or serum (0.5 ml) was incubated with labelled steroids (0.5 ng) for 2 h at 4°C, and after adsorption of free and weakly binding steroids by charcoal, the incubate (0.4 ml) was layered on 5–20% sucrose gradient (total 4.5 ml) in buffer A and centrifuged at 50,000 rev/min [248,800 *g* (max)] for 16 h at 4°C. After centrifugation, each fraction of 10 drops was collected from the bottom through a No. 21-gauge needle.

After sucrose gradient centrifugation of unlabelled cytosol (0.4 ml), each fraction was incubated with 4.9×10^{-10} M [^{3}H]-E_2, 6.2×10^{-10} M [^{3}H]-P, and 8.5×10^{-10} M [^{3}H]-DHT at 4°C for 2 h and the unbound and weakly-binding [^{3}H]-steroids in each incubate were adsorbed by charcoal.

Cow fibrinogen (7.9*S*) and bovine serum albumin (4.6*S*) were used as sedimentation coefficient markers to standardize the gradients.

Equilibrium study. The cytosol (0.5 ml) diluted with buffer A was incubated with each labelled steroid. Increasing concentrations were used: [^{3}H]-E_2 (7.4×10^{-10} M to 7.4×10^{-9} M), [^{3}H]-P (6.4×10^{-10} M to 6.4×10^{-9} M), [^{3}H]-R5020 (6.1×10^{-10} M to 6.1×10^{-9} M), and [^{3}H]-DHT (7.4×10^{-10} M to 7.4×10^{-9} M). Incubations were carried out with and without excess unlabelled steroid (200 ng, ethynylestradiol for [^{3}H]-E_2, norethindrone for [^{3}H]-P and [^{3}H]-R5020, DHT for [^{3}H]-DHT) for 2 h at 4°C. Dextran-coated charcoal (0.5 ml) (0.005% dextran and 0.5% Norite A in buffer A) was then added to the incubate. The incubate was kept for 30 min at 4°C and centrifuged at 1,200 *g* for 10 min. Methanol (3 ml) was added to 0.2 ml of the supernatant and the radioactivity of the bound form was determined. Specific binding was expressed as the difference of the binding in each sample with and without unlabelled steroid. A Scatchard plot [7] was made.

Ligand specificity study. The cytosol (0.5 ml) was incubated with [^{3}H]-steroid (1 ng) alone or together with unlabelled steroids (a 50-fold excess) at 4°C for 2 h. Bound steroid was determined by charcoal adsorption as described for the kinetic study. The % of [^{3}H]-steroid bound at the presence of unlabelled steroid was calculated.

Mitotic index. The mitotic index was expressed as the % of mitotic cells in 500 cells.

RESULTS

Sucrose gradient centrifugation

The sedimentation profiles of [^{3}H]-steroid–protein complexes are shown in Fig. 1 (serum), Fig. 2 (myometrial cytosol) and Figs 3 and 4 (cytosol of myoma). In the serum equilibrated with [^{3}H]-steroid, only 5*S* protein binding appeared with [^{3}H]-E_2 [^{3}H]-DHT and [^{3}H]-P, and even with [^{3}H]-R5020. In the cytosols of myometrium and myoma the binding with [^{3}H]-steroid appeared as peaks at approximately 4–5*S* region with or without a shoulder in the 7*S* region. The recognizable small 7*S* shoulder was found only as progestogen binding in some experiments.

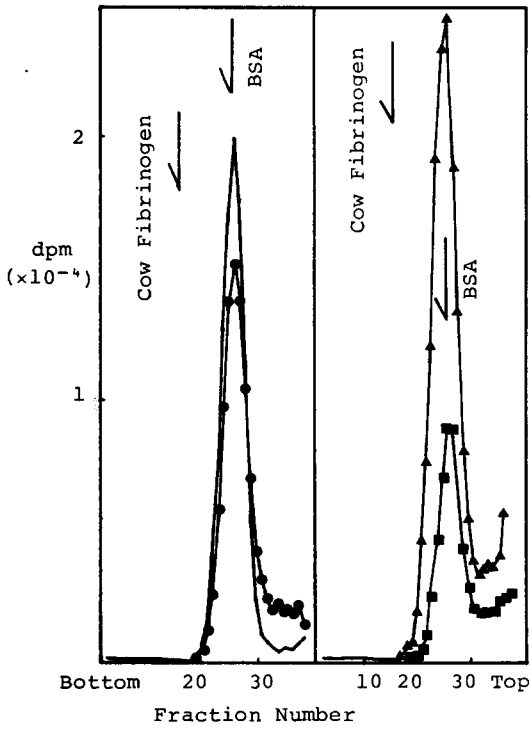

Fig. 1. Sedimentation profile of [^{3}H]-steroid binding in human serum. Aliquots of serum were incubated with 3.7×10^{-9} M [^{3}H]-E_2 (●——●), 3.4×10^{-9} M [^{3}H]-DHT (——), 3.2×10^{-9} M [^{3}H]-P (▲——▲) and 1.2×10^{-9} M [^{3}H]-R5020 (■——■). These incubates were layered on sucrose gradient. Serums were obtained from the patients having leiomyoma uteri and diluted will buffer A (protein concentration: 10.2 mg/ml except [^{3}H]-R5020 which was 8.8 mg/ml). BSA = bovine serum albumin.

In the competition by unlabelled steroids, [^{3}H]-$E_2$4–5*S* binding was reduced by ethynyl estradiol [Fig. 2(A)] and by diethylstilbestrol and DHT [Fig. 3(A)], and ^{3}H-DHT-4–5*S* binding was reduced by DHT and E_2 [Fig. 3(B)]. Sometimes diethylstilbestrol reduced [^{3}H]-E_2-4S binding but DHT did not as shown in Fig. 3(A). Cortisol decreased progesterone- or R5020-5*S* binding mainly as shown in Fig. 3 (C and D), and norethindrone reduced 7*S*-, 5*S*- and 4*S*-bindings of R5020 (mainly 7*S* and 4*S* bindings) shown in Fig. 3 (D). On the other hand, when the aliquots, fractionated after sucrose gradient centrifugation of unlabelled cytosol, were equilibrated with [^{3}H]-steroid, the following peaks appeared; 7*S*, 5*S* and 4*S* binding of [^{3}H]-E_2 [Fig. 2(A) and Fig. 4(A)], 7*S* and 4–5*S* bindings of [^{3}H]-P (Fig. 2(B) and Fig. 4(B)] and 6–7*S* and 5*S* bindings of [^{3}H]-DHT [Fig. 2(C) and Fig. 4(C)]. However [^{3}H]-P-5*S* binding was absent [Fig. 2(B) and Fig. 4 (B)].

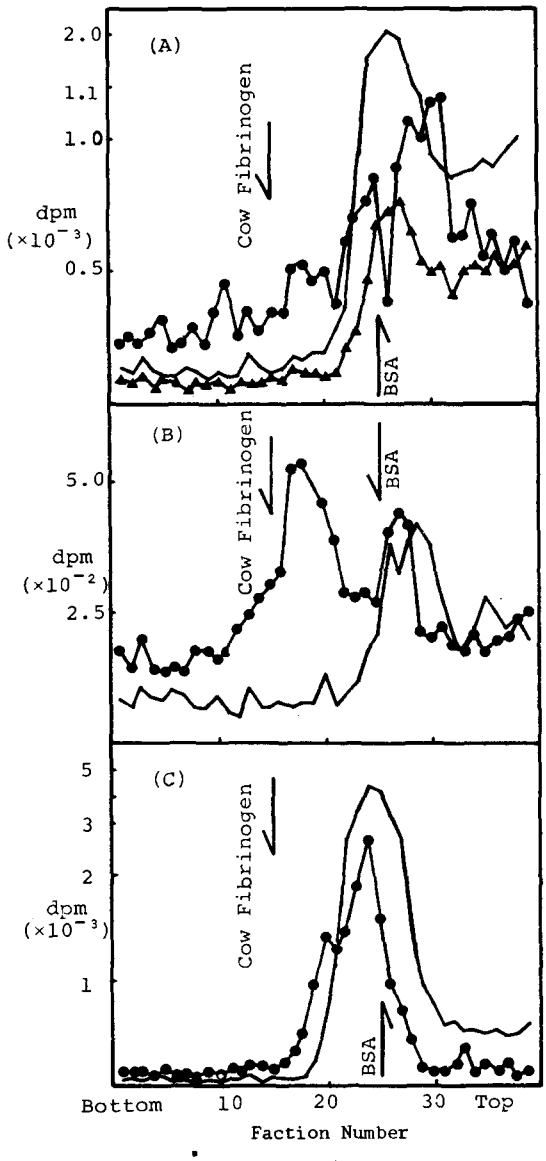

Fig. 2. Sedimentation profile of [^{3}H]-steroid binding in myometrial cytosol. The cytosol (8.4 mg protein/ml) was equilibrated with 3.7×10^{-9} M [^{3}H]-E_2 alone (——) or together with 8.4×10^{-8} M ethynyl estradiol (▲——▲) (Fig. 2A); 1.6×10^{-9} M [^{3}H]-P alone (——) (Fig. 2B), or 3.4×10^{-9} M [^{3}H]-DHT alone (——) (Fig. 2C). After free and weakly binding steroids were adsorbed by dextran coated charcoal, the incubate was centrifuged on sucrose gradient centrifugation. On the other hand, [^{3}H]-steroid was incubated with the fractionated aliquot after the cytosol (8.2 mg protein/ml), not equilibrated with [^{3}H]-steroid, was centrifuged on sucrose gradient centrifugation. The bound fraction was obtained after adsorption by dextran coated charcoal (●——●). The [^{3}H]-steroid concentration was 7.4×10^{-10} M [^{3}H]-E_2, 6.4×10^{-10} M [^{3}H]-P and 6.9×10^{-10} M [^{3}H]-DHT. This experiment was from the same individual.

These results indicate that there are 7*S*, 5*S* and 4*S* binding proteins for E_2, 7*S* and 4–5*S* proteins for progesterone and 6–7*S* and 5*S* proteins for DHT, and that each steroid-7*S* binding is easily degraded during sucrose gradient centrifugation.

Equilibrium study

When free or weakly binding steroids were adsorbed by charcoal, the dissociation constant (K_D) of [^{3}H]-steroid binding to cytosol was 4.5×10^{-10} M for E_2, 1.5×10^{-9} M for progesterone, 5.0×10^{-10} M for R5020 and 4.5×10^{-10} M for DHT, and each value of K_D in the myometrium (Fig. 5) was the same as that in the myoma (Fig. 6).

Ligand specificity study

The ligand specificity study of cytosol showed that E_2-protein binding was inhibited effectively by estrogens [Fig. 7(A) and Fig. 8(A)] and that progesterone-protein binding was decreased by progestins [Fig. 7(B) and Fig. 8(B)]. DHT-protein binding was remarkedly inhibited by DHT (>testosterone) and to a lesser extent by E_2 [Fig. 7(C) and Fig. 8(C)]. The findings in the myometrium (Fig. 7) were almost the same as those in the myoma (Fig. 8).

Steroid receptor levels and mitotic index in myometrium and corresponding myoma

The steroid receptor levels and mitotic indices in myometrium and myoma are summarized in Table 1.

The average age of patients suffering from leiomyoma uteri was 43 years. There was no significant difference of steroid receptor levels (fmol/mg protein) and the mitotic index in the myometrium and the corresponding myoma. If however the receptor level was expressed as fmol/μg DNA, the estrogen receptor level in the myometrium was higher than that in the myoma ($P < 0.05$).

DISCUSSION

Estrogen is known to have a trophic effect on some myoma as well as on the normal myometrium. Evidence has accumulated indicating that progesterone demonstrates anti-estrogen action in many species. This steroid action may be mediated partly via steroid receptor and partly perhaps without it. The growth of myoma is considered to be dependent on the interaction of these steroids, and possibly on that of ER and PR. Androgen may have some effect on the growth of such tumor. Therefore ER, PR and AR in tumors should be investigated together.

Steroid receptors should be characterized before their levels are discussed. Variable values of K_D and sedimentation coefficient are reported by different authors. ER in the myometrium is described as follows: 8*S* + 3*S* ($K_D = 10^{-9}$ M at 4°C by agarose gel filtration) [8], 5.1*S* ($K_D = 4.7 \times 10^{-10}$ M at 4°C by charcoal adsorption) [9], and 3.7*S* ($K_D = 10^{-10}$ M at 4°C by charcoal adsorption) [10]. ER in the myoma is described as 8*S* + 4*S* ($K_D = 1.3 \times 10^{-10}$ M at 4°C by charcoal adsorption), where 8*S* is specific and 4*S* is non-specific [3]. PR in the myometrium is described as follows: 7.5*S* + 3.8*S* ($K_D = 3.7 \times 10^{-10}$ M at 4°C by charcoal adsorption) [11], 4.1*S* ($K_D = 7.1 \times 10^{-10}$ M at 4°C by dialysis) [12],

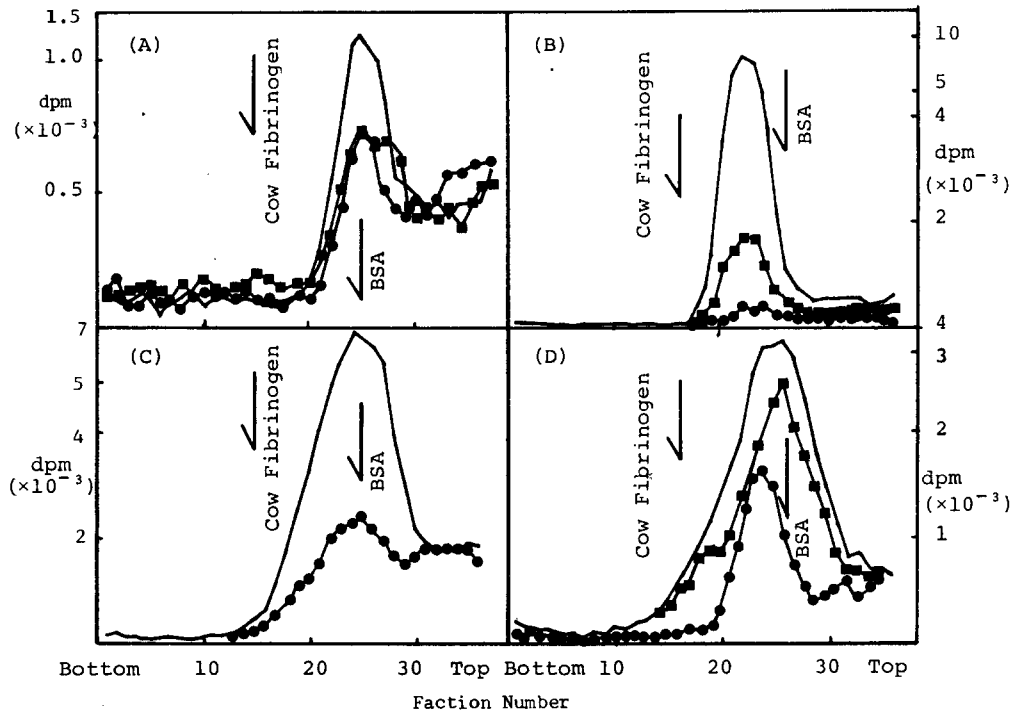

Fig. 3. Sedimentation profile of [^{3}H]-steroid binding in the cytosol of myoma. The cytosol was equilibrated with 3.7×10^{-9} M [^{3}H]-E_2 alone (——), or together with 7.5×10^{-7} M diethylstilbestrol (●——●), or 6.9×10^{-7} M DHT (■——■) (Fig. 3A); 3.4×10^{-9} M [^{3}H]-DHT alone (– – –) and together with 7.3×10^{-7} M E_2 (■——■) or 6.9×10^{-7} M DHT (●——●) (Fig. 3B); 6.4×10^{-9} M [^{3}H]-P alone (——) or together with 5.5×10^{-7} M cortisol (●——●) (Fig. 3C); 3.1×10^{-9} M [^{3}H]-R5020 alone (——) or together with 3.4×10^{-7} M norethindrone (●——●) or 2.8×10^{-7} M cortisol (■——■) (Fig. 3D). After free and weakly binding steroids were adsorbed by dextran coated charcoal, the incubate was centrifuged on sucrose gradient. Cytosol protein concentration was 8.4 mg/ml except (D) where it was 6.2 mg/ml.

7.8*S* + 4–5*S* (13), 7.5*S* + 3.8*S* [14], and 4–5*S* ($K_D = 3.7 \times 10^{-9}$ M at 4°C by charcoal adsorption) [15]. Daxenbichler *et al.* [4] report 4*S* ($K_D = 0.1$–7×10^{-9} M by charcoal adsorption) in ER and 5.5*S* + 3.5*S* ($K_D = 1.7$–3.1×10^{-9} M, using [^{3}H]-R5020 by charcoal adsorption for PR in both myometrium and myoma. Norgestrel-PR migrated to 4.3*S* with K_D of 0.96×10^{-9} M determined by sephadex chromatography when [^{3}H]-norgestrel is used for the assay [16].

The data presented here showed peaks at 7*S*, 5*S* and 4*S* for the estradiol receptor. Notides *et al.*[17] have shown that ER in the human myometrium, when isolated in buffer containing diisopropylfluorophosphate (DFP) to inhibit proteolytic activity, sediments at 8*S*, 5*S* and 4*S* proteins (3*S* protein without DFP), and undergoes a temperature dependent increase in its sedimentation coefficient from 4*S* to 5S. This indicates that the preferred form of the human ER under more physiological conditions is the 5*S* dimer. Our findings for ER indicate that 7*S* protein exists in the cytosol, but E_2-7*S* binding is easily degraded during sucrose gradient centrifugation. E_2-binding 5*S* and 4*S* proteins exist. The 5*S* is specific and testosterone binding globulin is also present. These findings are partly consistent with those of Notides *et al.*[17]. A possible explanation of the degradation of E_2-7*S* as well as other steroid-7*S* complexes may be that after E_2 binds to 7*S*, the 7*S* protein is broken or degraded to a lower molecular protein, or E_2-7*S* binding is dissociated during the long period of sucrose gradient centrifugation.

Reported K_D values for E_2 are about 10^{-10} M at 4°C and, are similar to our K_D.

The reported values of sedimentation coefficients for PR are at 7–8*S* and 4–5*S*. The data presented here demonstrate 7*S* and 4–5*S* specific protein for PR and 5S non-specific (transcortin) binding in both myometrium and myoma. Progesterone-7*S* binding was found to be easily degraded during sucrose gradient centrifugation, and this phenomenon is also reported in the human endometrium [18]. [^{3}H]-R5020 is specific for PR in other species [19,20] but binds relatively weakly to transcortin ($K_D = 1.3 \times 10^{-7}$ M at 4°C by dialysis) [21] and sediments at 5*S* as presented here. The reported K_D values and our value using [^{3}H]-P are about 10^{-10}–10^{-9} M. When [^{3}H]-R5020 is used, the K_D of PR is 1.7–3.1×10^{-9} M [4] and 5.0×10^{-10} M in both myometrium and myoma.

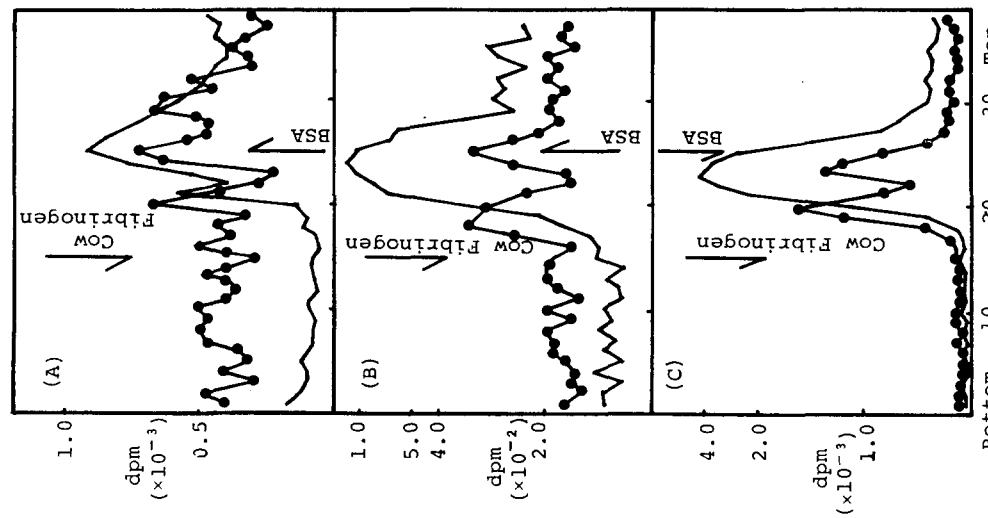

Fig. 4. Sedimentation profile of [^{3}H]-steroid binding in cytosol of myoma. The cytosol (7.2 mg protein/ml) was equilibrated with 3.7×10^{-9} M [^{3}H]-E_2 alone (——) (Fig. 4A); 1.6×10^{-9} M [^{3}H]-P alone (——) (Fig. 4B); or 3.4×10^{-9} M [^{3}H]-DHT alone (——) (Fig. 4C). After free and weakly binding steroids were adsorbed by dextran coated charcoal, the incubate was centrifuged on sucrose gradient. On the other hand, [^{3}H]-steroid was incubated with the fractionated aliquot after the cytosol (7.2 mg protein/ml), not equilibrated with [^{3}H]-steroid, was centrifuged on sucrose gradient. The bound fraction was obtained after adsorption by dextran coated charcoal (●——●), [^{3}H]-steroid concentration was 7.4×10^{-10} M [^{3}H]-E_2, 6.4×10^{-10} M [^{3}H]-P and 6.9×10^{-10} M [^{3}H]-DHT. This experiment was from the same individual.

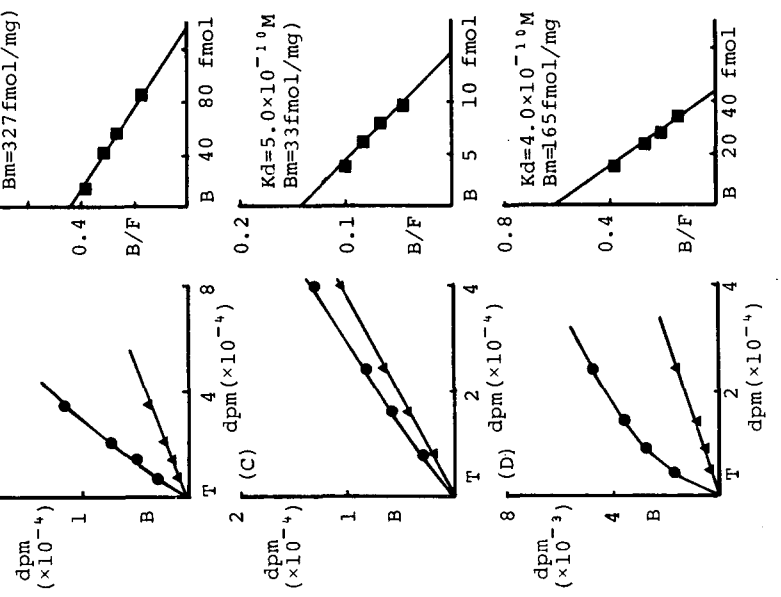

Fig. 5. Scatchard plot analysis of steroid-receptor binding in myometrial cytosol. Left panel: The cytosol was incubated with increasing concentrations of [^{3}H]-steroid alone or together with unlabelled steroid (200 ng). Unbound and weakly binding steroids were adsorbed by dextran coated charcoal. Specific binding was expressed as the difference of the bindings in each incubate with and without unlabelled steroid. Total binding (●——●) and non-specific binding (▲——▲). Right panel: Scatchard plot analysis of the data presented in left panel. Specific binding (■——■). (A): [^{3}H]-E_2 (cytosol protein: 1.8 mg/ml), (B): [^{3}H]-P (cytosol protein: 2.3 mg/ml), (C): [^{3}H]-R 5020 (cytosol protein: 2..1 mg/ml), (D): [^{3}H]-DHT (cytosol protein: 1.7 mg/ml). Each experiment was from the myometrium of a different patient having leiomyoma uteri. B = bound, T = total count/tube, K_D = dissociation constant, Bm = maximal binding sites.

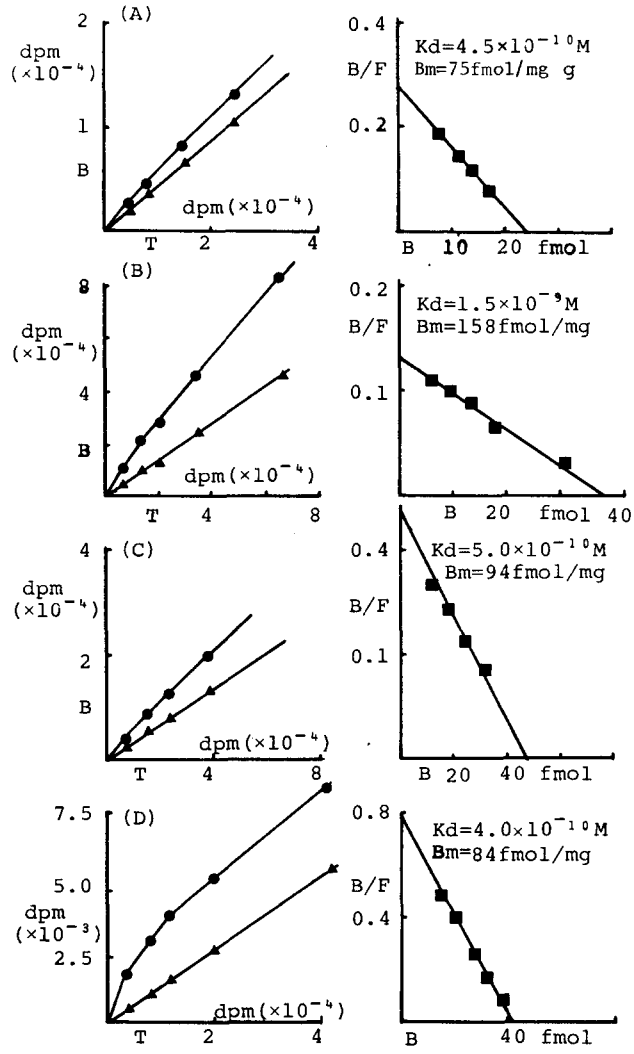

Fig. 6. Scatchard plot analysis of steroid-receptor binding in the cytosol of myoma. The additional legends were the same as those described for Fig. 7. (A): [^{3}H]-E_2 (cytosol protein: 1.6 mg/ml), (B): [^{3}H]-P (cytosol protein: 1.5 mg/ml), (C): [^{3}H]-R5020 (cytosol protein: 2.5 mg/ml), (D): [^{3}H]-DHT (cytosol protein: 2.5 mg/ml). Each experiment was from a different myoma except (C) and (D).

The 7*S* and 5*S* proteins for AR are presented for the first time in the myometrium and myoma. DHT-7*S* binding was easily degraded during sucrose gradient centrifugation. The human myometrial tissue has been reported to contain binding proteins for both E_2 and DHT, where specific DHT binding is inhibited by testosterone and DHT and moderately reduced by E_2 [22]. Our study also showed that DHT specific protein binding was reduced to a lesser extent by E_2, but E_2-specific protein binding was not decreased by either testonterone or DHT.

The cytosol ER from myoma has a ligand specificity closely resembling that of the corresponding receptor in normal human myometrium and endometrium [3]. This indicates that ER has no different biological effect in myometrium and myoma.

Myoma samples contain more ER than do the corresponding normal myometrial tissue [2, 3]. However, there is no difference of E_2 uptake between the myoma and myometrium in the same subject [23]. Our findings showed that only the ER level was higher in the myometrium than that in the corresponding myoma, if the level was expressed as a unit of DNA. Daxenbichler *et al.*[4] report that the number of binding sites was highly variable in different tissue samples and depended upon the endocrine status of the patient. The ER level expressed as fmol of sites/mg cytosol protein ranged from 10–49 for myometrium and 19–394 for leiomyomas, and the PR level was between 63–450 for myometrium and 37–302 for leiomyomas.

The action of progesterone on cell proliferation of myometrium and myoma may be complex and it is probable that progesterone alone or in combination with estrogen influence mitosis in these tissues. Mitosis indicates proliferation. Intramenstrual alter-

Table 1. Steroid receptor level and mitotic index of myoma and corresponding myometrium

	Myoma	Myometrium	Statistical analyses
Bm of ER	81.0 ± 7.8(n = 25)	79.7 ± 6.6(n = 24)	NS
fmol/mg (cytosol protein)	[range; 0(n = 2)—150]	[range; 30—172]	
fmol/μg (DNA)	1.13 ± 0.21(n = 25) [range; 0(n = 2)—5.10]	2.02 ± 0.32(n = 24) [range; 0.47—7.90]	$0 < 0.05$
Bm of PR	78.2 ± 8.9(n = 25)	61.4 ± 12.3(n = 24)	NS
fmol/mg (cytosol protein)	[range; 0(n = 1)—172]	[range; 0(n = 1)—327]	
fmol/μg (DNA)	1.26 ± 0.16(n = 25) [range; 0(n = 1)—2.83]	1.53 ± 0.28(n = 24) [range; 0(n = 1)—6.31]	NS
Bm of AR	88.2 ± 10.4(n = 25)	83.8 ± 7.4(n = 24)	NS
fmol/mg (cytosol protein)	[range; 23—263]	[range; 32—165]	
fmol/μg (DNA)	1.71 ± 0.36(n = 25) [range; 0.20—9.10]	2.52 ± 0.52(n = 24) [range; 0.61—9.76]	NS
Mitotic Index (%)	0.48 ± 0.04(n = 17) [range; 0.3—0.8]	0.43 ± 0.02(n = 16) [range; 0.2—0.6]	NS

ER = estrogen receptor, PR = progesterone receptor, AR = androgen receptor, MI = mitotic index (the value was based on the average of 3 determinations). All results were expressed as mean ± S.E. The average age of patients suffering from leiomyoma uteri was 42.7 ± 1.8 (n = 25). n = number of patients.

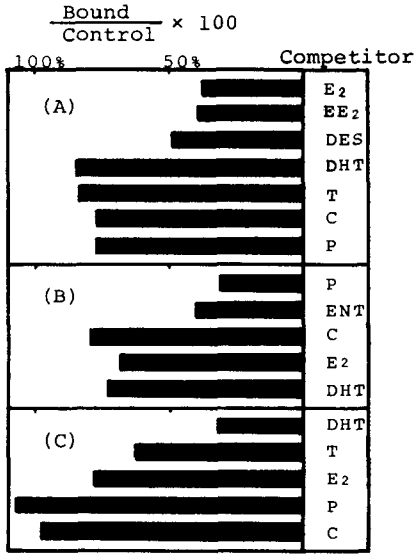

Fig. 7. Steroid specificity study in myometrium. The cytosol was equilibrated with [^{3}H]-steroid (1 ng) and unlabelled steroids (50 ng). The bound form was obtained after free and weakly binding steroids were adsorbed from the incubate by dextran coated charcoal. The value was based on the average of 2 determinations. The % of [^{3}H]-steroid bound in the presence of unlabelled steroid as a control was calculated. The cytosol protein concentration was 2.3 mg/ml. E_2 = estradiol-17β, EE_2 = ethynyl estradiol, DES = diethystilbestrol, DHT = dihydrotestosterone, T = testosterone, P = progesterone, C = cortisol, ENT = norethindrone. (A): in [^{3}H]-E_2-cytosol binding, (B): in [^{3}H]-P-cytosol binding, (C): in [^{3}H]-DHT-cytosol binding.

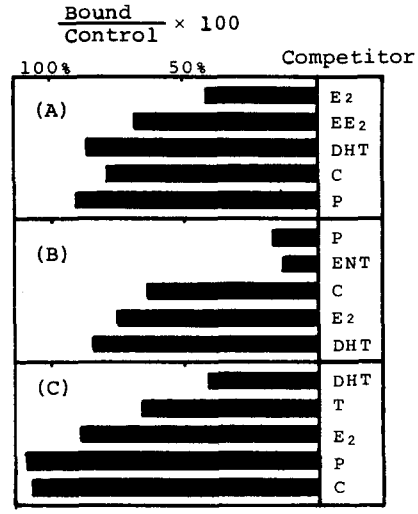

Fig. 8. Steroid specificity study in myoma. The experimental conditions were the same as those described for Fig. 7. The cytosol protein concentration was 1.0 mg/ml.

ation of mitotic activity is known to exist in the human endometrial glandular epithelium [12].

There was no difference of mitotic index between myoma and the corresponding myometrium and this indicates that there was no difference of cell proliferation between these two tissues.

Thus the steroid receptors were characterized, and the receptor levels and mitotic index were investigated in myoma and the corresponding myometrium. The steroid-dependence of the tumor growth could not be related to the steroid receptor level or the mitotic index and it is suggested that other mechanisms in the nucleus are involved.

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