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SEX STEROID BINDING PROTEIN IN THE PLASMA OF *MACACA NEMESTRINA**

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SUMMARY

A sex steroid binding protein (nSBP) has been identified in the plasma of *Macaca nemestrina*. The protein specifically binds 5α-androstane-17β-ol,3-one (K_A(male) = 1.87 ± 1.05 × 10^9 M^{-1}, K_A(female) = 2.04 ± 0.88 × 10^9 M^{-1}, K_A(pregnancy) = 2.21 ± 0.59 × 10^9 M^{-1}; 4°, 10 mM Tris, pH 7.4), testosterone (K_A = 4.10 ± 2.50 × 10^8 M^{-1}, 4°, 10 mM Tris, pH 7.4), and estradiol-17β (2.41 ± 1.34 × 10^8 M^{-1}, 4°, 10 mM Tris, pH 7.4). The steroids bind to the protein at the same site. Progesterone, cortisol, estrone, estriol, and diethylstilbestrol have relatively low affinity for the binding site. Specificity studies also suggest that 5α-androstane derivatives with a 3α-hydroxyl show little affinity compared with those having 3β-hydroxyl. Electrophoresis in 5% polyacrylamide gels yields one active molecular species with R_F 0.32. The rate constant of dissociation of the DHT–nSBP complex at 4° and pH 7.4 is 0.013 min^{-1} with a half-time of 52.8 min. The binding capacity of nSBP expressed in μg 5α-androstane-17β-ol,3-one bound/100 ml plasma for males (5 samples) and females (7 samples) is 5.62 ± 1.24 (S.D.) and 11.07 ± 1.85 (S.D.), respectively ($P < 0.001$). These results show that nSBP and human SBP are quite similar in their binding and structural properties and therefore establish *Macaca nemestrina* as a good animal model to study the physiological role of SBP.

INTRODUCTION

The existence of specific sex steroid binding proteins in the plasma of many species is becoming increasingly recognized as an entity which must play an important role in the overall mechanism of action of the sex steroid hormones. The high binding affinity, high specificity, and low concentration of these proteins in plasma taken together with the fact that they exist in most of the species of higher animals strongly attest to their biological importance. Following the discovery of a specific sex steroid binding protein, SBP[1], in the plasma of man [1, 2] much research has been published on SBP binding capacity in individuals with varying physiologic, pharmacologic, and pathologic conditions. More recently human SBP has been purified to homogeneity from fresh pregnancy serum in an attempt to understand its biochemical properties [3]. In order to answer questions on the physiological role of SBP, other animal model systems need to be developed to circumvent the inherent limitations of human studies. Very few published results demonstrate the presence of a specific sex steroid binding protein in the plasma of subhuman primates. Gauthier-Wright *et al.* [4] have shown its existence in the plasma of *Macaca speciosa*. Their results, however, suggest that the binding protein has different specificity than hSBP. The protein these authors describe binds progesterone, estrone, and androsterone, whereas hSBP has little or no affinity for these steroids. Furthermore, estradiol-17β was found to displace testosterone in competition experiments but it is not clear if both steroids compete for the same site on the binding proteins.[2] Ritzén *et al.* [5] have quantitated the presence of SBP in *Macaca mulatta*[3], and have also shown that it exists as a unique molecular species; however, no specificity studies have been described to date. Others have measured the sex steroid binding capacity of plasma from a variety of subhuman primates [6, 7, 8]. The experiments described in this paper demonstrate that SBP is present in *Macaca nemestrina* and that its steroid binding properties are similar to those of human SBP. These findings indicate that *Macaca nemestrina* is a good animal model to study the physiological role of this protein in humans.

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[1] Abbreviations: SBP, sex steroid-binding plasma protein; nSBP, *Macaca nemestrina* SBP; hSBP, human SBP; PBG, progesterone binding globulin; DHT, 5α-dihydrotestosterone.

[2] Unfortunately no data were presented in the Scatchard analyses to support the values for the equilibrium constants of association of estradiol-17β and testosterone reported in that publication.

[3] Although these authors did not report the species of monkeys in their publication, it was obtained from Dr. F. S. French through a personal communication.

MATERIAL AND METHODS

[1,2-^{3}H]-5α-dihydrotestosterone[4] (40 Ci/mmol) and Omnifluor were purchased from New England Nuclear. Radioinert steroids were obtained from Sigma Chem. Co. and Steraloids. Some were gifts from Mr. John C. Babcock of the Upjohn Company. DEAE-cellulose filter paper discs (DE-81, 2.3 cm diameter) were purchased from Reeve Angel & Company. All other chemicals were reagent grade.

Macaca nemestrina *plasma.* Venous blood was collected in heparinized glass tubes from healthy adult monkeys housed at the University of Washington Primate Center. The plasma was stored at −20°. Endogenous steroids were removed by charcoal treatment (Norit A, 50 mg/ml, 0.5 h at 25°). The suspension was centrifuged twice at 2000 *g* for 10 min and the plasma stored at 4°. Plasma was diluted freshly with 10 mM Tris–Cl, pH 7.4, at the beginning of experiments.

Determination of SBP concentration. The filter assay was carried out as originally described [9, 10]. The "efficiency" of the assay for nSBP was experimentally determined to be 80.8 ± 0.9% (S.E.). This means that 81% of the SBP–DHT complexes survives the 10 buffer washes used in the assay procedure; all values were corrected for this constant loss. The steroid was 95% bound to SBP at the highest ligand concentration used for the "efficiency" determination; no correction was applied. SBP binding capacity in plasma from males, pregnant and non-pregnant females was estimated under standard conditions in the presence of 27 nM [^{3}H]-DHT where SBP was 95–99% saturated with DHT (correction to 100% was not applied). Nonspecific binding was estimated in the presence of 27 nM [^{3}H]-DHT plus 2400 nM radioinert DHT. The concentration of nSBP was calculated from the binding capacity and expressed either as μg DHT bound/100 ml plasma or nM, assuming mol/mol stoichiometry of binding. The extent of saturation discussed above with respect to either SBP or steroid was calculated from the principle of mass action using the corresponding equilibrium constant at 4°, and SBP binding capacity obtained from extrapolated Scatchard plots.

Specificity studies. Pregnancy plasma diluted 100-fold with 10 mM Tris-buffer, pH 7.4, was used to study the specificity of nSBP. A standard curve was experimentally determined by incubating 0.5 ml of diluted plasma with 3 nM [^{3}H]-DHT and increasing concentrations of radioinert DHT (2–20 nM). Percent [^{3}H]-DHT bound to SBP was plotted versus the nM concentration of added radioinert DHT. The relative ability of radioinert steroids to compete with [^{3}H]-DHT for the steroid binding site of SBP as compared with radioinert DHT was determined with the aid of this standard curve. Two concentrations of the competitor were used. Standard solutions were prepared by dissolving the steroids in benzene–ethanol (9:1 v/v). Evaporation of aliquots was carried out at room temperature under N_2.

Scatchard analyses: The "competitive" method. The use of the standard curve described above can provide a qualitative measure of the specificity of binding for a large number of radioinert steroids [11]. When quantitative data on a particular steroid are desired, such preliminary information can be used to design an experiment which can yield the equilibrium constant of association of the steroid in question, as well as the stoichiometry of binding relative to DHT. This is done by performing two simultaneous Scatchard analyses on the same SBP solution, one in the presence of the competing steroid and the other in its absence. Relative binding affinity obtained with the original standard curve can be used to calculate the proper concentration of the competing radioinert steroid for generating a meaningful Scatchard plot. The equilibrium constant is obtained from the following equation [3]:

$$K_P = K_D(1 + K_I[I])$$

where K_P = equilibrium constant of dissociation in the presence of the competing steroid, K_D = equilibrium constant of dissociation of DHT, $[I]$ = concentration of competing steroid, K_I = equilibrium constant of association of the competing steroid. Intersection of both lines on the abscissa represents a case of competitive inhibition and indicates that the radioinert steroid and DHT bind at the same site on the protein.

Dissociation studies. The rate of dissociation of the nSBP–DHT complex was measured at 4° with the filter assay. Three ml of 100-fold diluted pregnancy plasma was incubated with 10 nM [^{3}H]-DHT at 25° for 30 min and cooled to 4°. Under these conditions 55% of the steroid was bound to SBP and 95% of SBP was saturated. At zero time the solution was made 3300 nM with radioinert DHT by adding 100 μl of 100 μM DHT (dissolved in 100% ethanol). Aliquots (100-μl) were removed at timed intervals and the amount of nSBP–[^{3}H]-DHT complex was measured. Binding was also measured after 24 h when dissociation of the trace should essentially be completed; the value was used to correct for nonspecific binding. The rate constant, k, is calculated from the slope of the line obtained by plotting the log of the remaining SBP activity as a function of time.

[4] The trivial names used are: testosterone, 17β-hydroxy-4-androsten-3-one; estradiol-17β,1,3,5(10)-estratriene-3-17β-diol; 5α-dihydrotestosterone, 5α-androstan-17β-ol-3-one; 7α-methyltestosterone, 7α-methyl-4-androsten-17β-ol-3-one; 17α-methyltestosterone, 17α-methyl-4-androsten-17β-ol-3-one; 19-nortestosterone, 17β-hydroxy-4-estren-3-one; 5β-dihydrotestosterone, 5β-androstan-17β-ol-3-one; estrone,3-hydroxy-1,3,5(10)-estratrien-17-one; dehydropiandrosterone, 3β-hydroxy-5-androstan-17-one; Epitestosterone, 4-androstan-17α-ol-3-one; progesterone, 4-pregnen-3,20-dione; 11-deoxycorticosterone, 21-hydroxy-4-pregnen-3,20-dione; estriol,1,3,5(10)-estratriene-3,16α,17β-triol; cortisol, 11β,17,21-trihydroxy-4-pregnene-3,20-dione; cortisone,17,21-dihydroxy-4-pregnene-3,11,20-trione; corticosterone, 11β,21-dihydroxy-4-pregnene-3,20-dione; 11-deoxycortisol, 17,21- dihydroxy-4-pregnene-3,20-dione.

Polyacrylamide gel electrophoresis. Analytical disc gel electrophoresis was performed according to Davis [12] as modified by Shuster [13]. Five percent gels, prepared at pH 8.9 in the presence or absence of 0.2 nM [^{3}H]-DHT, were cast into tubes (100 mm × 5 mm inside diameter) and allowed to polymerize at 25° for 2 h. The gels were cooled to 4° and pre-electrophoresed for 2 h at constant current (2 mA/gel). Samples were prepared by mixing 10 μl of plasma (pretreated with charcoal) with 190 μl of pH 8.9 gel buffer containing 10% glycerol, and incubating with 3 nM [^{3}H]-DHT for 30 min at 25°. After cooling to 4°, 4 μl of 0.05% bromophenol blue solution was added to the sample, and 50-μl aliquots were applied per gel. The samples were electrophoresed for 2.5 h at 4° until the tracking dye had migrated approximately 7 cm into the gel. Gels were sliced at the dye front, frozen on dry ice, cut transversely into 1.3 mm slices, and counted. Gels were also stained to compare the migration of albumin and other proteins with SBP.

Radioactivity measurements. Radioactivity was measured in a scintillant containing toluene and Omnifluor (4 g/l.). Radioactive steroids are completely extracted in the toluene phase. The efficiency remains constant and is independent of the vol. or salt content of the aqueous phase. Filters were counted in glass vials with 10 ml scintillant with an efficiency of 47%. Gel slices were counted in glass minivials with 4 ml scintillant. The counter was Beckman LS-100C.

RESULTS

Binding affinity of 5α-dihydrotestosterone to nSBP

The equilibrium constant of DHT association to nSBP at 4° and pH 7.4 in diluted plasma from males, pregnant and nonpregnant females were calculated from Scatchard plots as shown in Fig. 1 and 2. In the case of plasma from a male (Fig. 1A), the $K_A = 1.87 \pm 1.05 \times 10^9\ M^{-1}$ and [SBP] = 6.10 nM in the assay or 152 nM in undiluted plasma. This value corresponds well with that obtained by the standard filter assay as reported in Fig. 3 (170 nM or 4.93 μg DHT bound/100 ml plasma, second dot from the bottom). The correlation between the Scatchard plot and standard assay indicates that under the experimental conditions of the latter 99% of SBP was saturated with DHT. For plasma from a female (Fig. 1B), the $K_A = 2.04 \pm 0.88 \times 10^9\ M^{-1}$ and [SBP] = 8.68 nM in the assay or 434 nM in undiluted serum. In this case 454 nM (13.18 μg DHT bound/100 ml plasma, Fig. 3, top dot) was determined by the standard filter assay. For pregnancy plasma (Fig. 2A, top line) the $K_A = 2.21 \pm 0.59 \times 10^9\ M^{-1}$ and [SBP] = 5.68 nM in the assay or 568 nM in undiluted plasma. This corresponds to 580 nM (or 16.80 μg DHT bound/100 ml plasma; Fig. 3, highest black dot) in the standard filter assay. The differences found between all three equilibrium constants are not statistically significant.

Binding capacity of 5α-dihydrotestosterone for monkey plasma

Figure 3 describes the binding capacity of DHT in the plasma of 5 males, 7 females, and 6 pregnant females estimated by the standard filter assay. The calculated mean value for males is 5.62 ± 1.24 (S.D.) and for females 11.07 ± 1.85 (S.D.). The difference between the two means is statistically significant ($P < 0.001$). More studies are required to establish whether or not SBP concentration changes during pregnancy (work in progress).

Binding affinity of testosterone and estradiol-17β: specificity studies

The relative binding affinities of nSBP to various steroids compared with DHT were calculated according to the method of Vermeulen and Verdonck [11]. The results are shown in Table 1. nSBP binds androgens preferentially and resembles human SBP in specificity. The only significant difference is in the

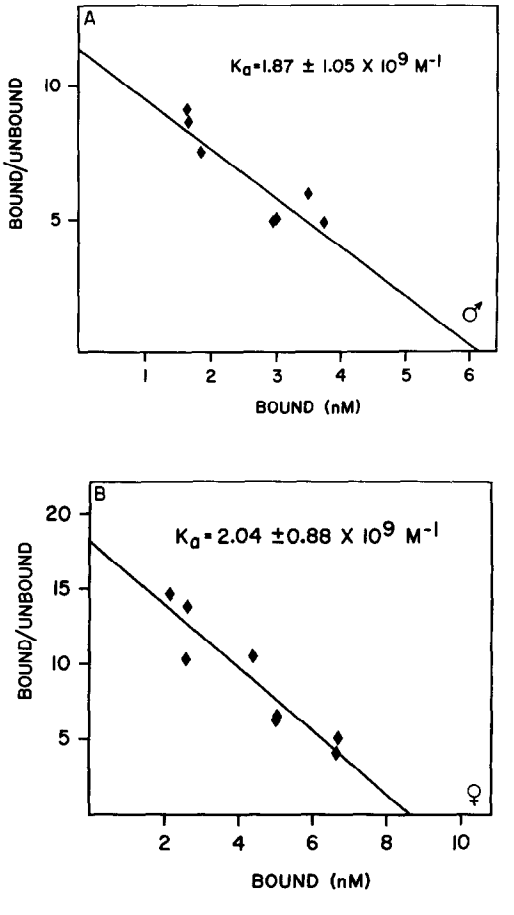

Fig. 1. Determination of equilibrium constants of association of 5α-dihydrotesterone at 4°. A: 0.5 ml samples of 25-fold diluted male plasma incubated with 1.7–4.3 nM [^{3}H]-DHT for 30 min at 25° and cooled to 4°; 100-μl aliquots were removed for counting and for the filter assay. (The filter assay "efficiency" was 80%). B: 0.5 ml samples of 50-fold diluted female plasma incubated with 2.3–8.0 nM [^{3}H]-DHT, experimental procedure similar to A.

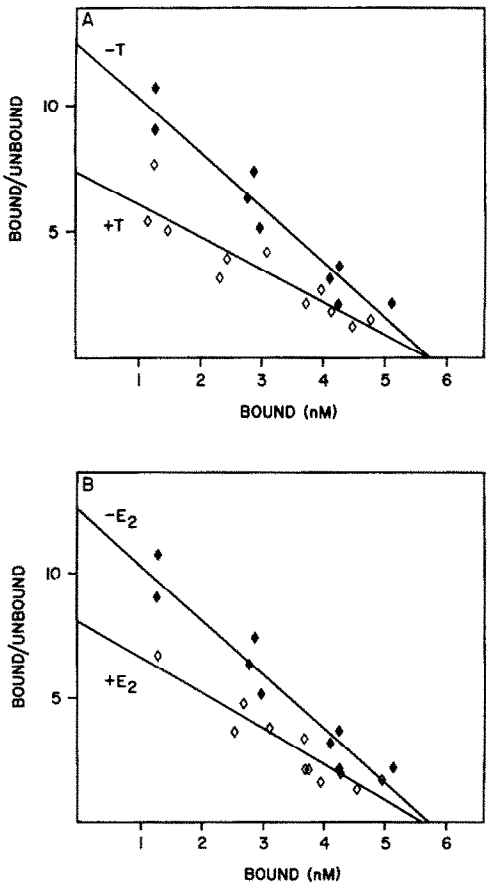

Fig. 2. Determination of equilibrium constants of association of testosterone and estradiol-17β at 4° by the "competitive" method. Experimental procedure was similar to Fig. 1. 0.5 ml samples of 100-fold diluted pregnancy plasma were incubated with 1.3–7.3 nM [^{3}H]-DHT, (◆——◆) in the absence of radioinert steroid. A, (◇——◇) in the presence of 3.53 nM radioinert testosterone in every sample. B, (◇——◇) in the presence of 4.19 nM radioinert estradiol-17β in every samples.

case of 5α-androstan-3α,17β-diol which does not compete well with [^{3}H]-DHT for the binding site of nSBP as it does for that of human SBP. This observation needs to be further examined in quantitative terms. The equilibrium constants of association of testosterone and estradiol-17β to nSBP at 4° and pH 7.4 were calculated from the "competitive" Scatchard analyses shown in Fig. 2 using the equation described in the "Methods". These are K_A (testosterone) = $4.10 \pm 2.50 \times 10^8\ M^{-1}$ and K_A (estradiol) = $2.41 \pm 1.34 \times 10^8\ M^{-1}$. Conversion of both lines of Figs. 2A and 2B at the abscissa indicates that DHT, testosterone, and estradiol-17β compete for the same binding site on nSBP. Even though the absolute values of the equilibrium constants do vary slightly from those reported for human SBP, the stereospecificity of the steroid binding site of nSBP appears quite similar to that of human SBP.

[5] Mickelson and Pétra, unpublished results.

Rate of dissociation of the nSBP–DHT complex

Figure 4 illustrates the first order rate of dissociation of the steroid–protein complex at 4° and pH 7.4. The rate constant, k, is 0.013 min^{-1}; the $t_{1/2}$ is 52.8 min. The experimental conditions at zero time (SBP = 5.2 nM, [^{3}H]-DHT = 5 nM) was chosen so that 78% of nSBP was saturated with the steroid. Extrapolation of the line of Fig. 4 to the ordinate permitted the experimental evaluation of this value (after correcting for the "efficiency" of the filter assay). This was calculated to be 67%. The value of 52.8 min is lower than that reported for human SBP, 77 minutes[6] [17]. The $t_{1/2}$ at 37° measured by the florisil assay [18] is about 2 min (data now shown) compared with 1.7 min for human SBP[6] [17]. Furthermore, preliminary experiments indicate that the dissociation of the testosterone and estradiol-17β nSBP complexes at 0° are similar to those reported for human SBP [17]. In addition, the experimental data suggest that the mechanism of dissociation agrees well with a first-order model similar to that reported for human SBP.

Polyacrylamide gel electrophoresis

The electrophoretic migration of nSBP in 5% gels is shown in Fig. 5. The existence of nSBP was demon-

Table 1. Relative binding affinities of various steroids to nSBP

Steroids	Relative binding (%) nSBP	hSBP I*	hSBP II†	hSBP III‡
5α-dihydrotestosterone	100	100	100	100
5α-androstan-3β,17β-diol	67	74	53	59
Androst-5-en-3β,17β-diol	26	38	17	—
Androst-4-en-3β,17β-diol	26	59	31	—
Testosterone	22	29	33	42
Estradiol-17β	15	18	21	12
5α-androstan-3α,17β-diol	11	59	53	41
7α-methyltestosterone	10	—	—	42
17α-methyltestosterone	9	—	13	27
19-nortestosterone	4	—	7	—
5β-dihydrotestosterone	5	—	—	—
Estrone	3	0.2	1.3	—
Dehydroepiandrosterone	3	0.7	1.5	—
5β-androstan-3α,17β-diol	1.7	0.2	8	—
Epitestosterone	0.3	0.3	1	—
Progesterone	0.2	—	0.8	—
11-deoxycorticosterone	0.2	—	2	—
Estriol	0.1	<0.1	1.1	—
Cortisol	<0.1	—	0.7	—
Cortisone	<0.1	—	1	—
Corticosterone	<0.1	—	0.7	—
11-deoxycortisol	<0.1	—	1.0	—
Diethylstilbestrol	<0.1	—	—	—

* Murphy [21]. † Vermeulen and Verdonck [11].
‡ Kato and Horton [22].
Experimental procedure: 0.5 ml samples of diluted pregnancy plasma (same as in Fig. 2, [SBP] = 5.2 nM) were incubated with 3 nM [^{3}H]-DHT plus radio-inert steroid at 2 different concentrations. Percent bound was compared to a standard DHT curve. Binding was expressed relative to DHT. The values in nSBP column represent averages of similar data from experiments carried out at two different concentrations of radioinert steroid.

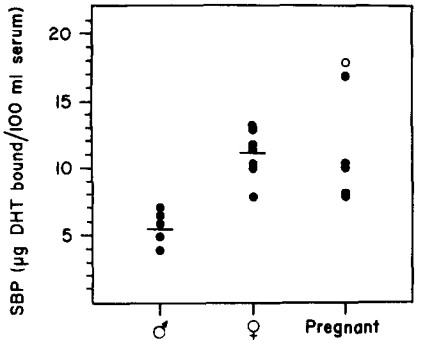

Fig. 3. 5α-dihydrotestosterone binding capacity of plasma from male, female, and pregnant monkeys. 0.5 ml samples of 50-fold diluted plasma was incubated at saturating concentrations of [^{3}H]-DHT (27 nM) in the absence or presence of 2400 nM radioinert DHT. Each point represents the mean of 4 independent determinations corrected for nonspecific binding. Same experimental procedure as in Fig. 1. Plasma was drawn at 112 days of pregnancy except for the one sample (empty circle) which was drawn at 130 days of pregnancy.

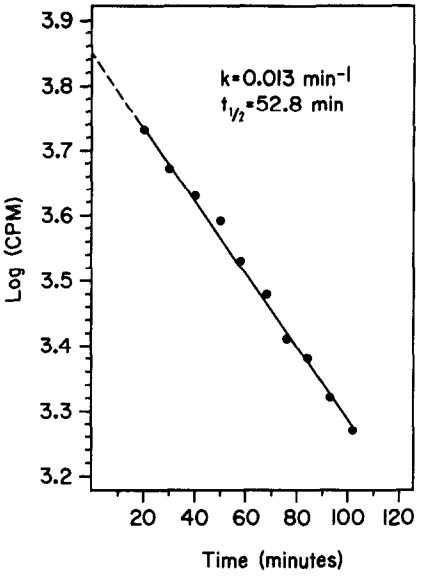

Fig. 4. Rate of dissociation of the DHT–SBP complex at 4°. Three ml of 100-fold diluted pregnancy plasma (same plasma as shown in Figs 2 and 3, [SBP] = 5.2 nM by Scatchard analysis) were incubated with 10 nM [^{3}H]-DHT for 30 min at 25° and cooled to 4°. Solution was made 3300 nM with radioinert DHT at zero time. 100-µl aliquots were assayed at indicated times. Assay after 24 h was used to correct for nonspecific binding.

strated whether or not [^{3}H]-DHT was incorporated into the gel prior to electrophoresis indicating that the nSBP–DHT complex survives during the experiment. The rate of dissociation reported in Fig. 4 as well as the high efficiency of the filter assay agrees with the electrophoretic migration data. Specificity of binding is demonstrated by the disappearance of the radioactive peak when 100-fold molar excess of radioinert DHT is added in the incubation prior to electrophoresis. The same results were obtained with plasma from males and females strongly suggesting that nSBP exists as one molecular entity. The calculated R_F of 0.32 is similar to 0.30 R_F for hSBP when electrophoresed under the same conditions[6].

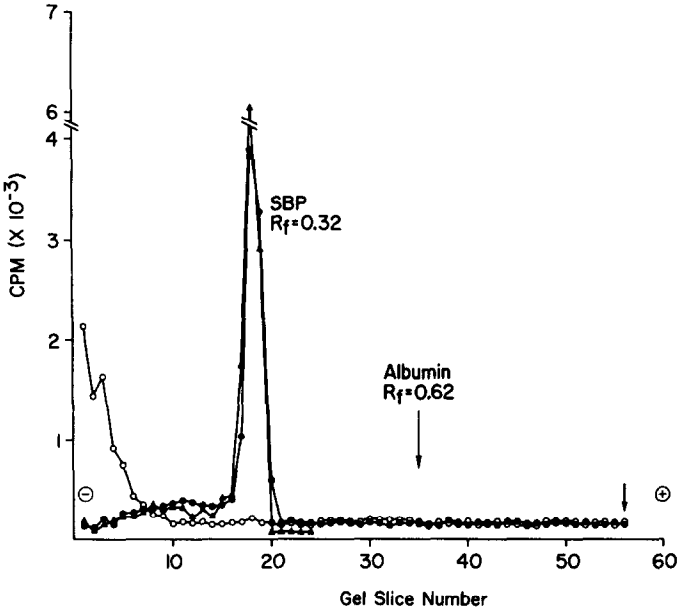

Fig. 5. Analytical polyacrylamide gel electrophoresis of pregnancy plasma 10-µl of pregnancy plasma (pretreated with charcoal) was mixed with 190-µl of pH 8.9 gel buffer containing 10% glycerol and incubated with 3 nM [^{3}H]-DHT for 30 min at 25° and cooled to 4°. 4-µl of 0.05% Bromophenol blue solution was added, and 50-µl aliquots were applied to pre-electrophoresed gels. After 2.5 h (2 mA/tube), gels were sliced and counted, or stained. (●——●) electrophoresed in the presence of 0.2 nM [^{3}H]-DT in the gel. (▲——▲) electrophoresed in the absence of [^{3}H]-DHT in the gel. (○——○) samples incubated with 3 nM [^{3}H]-DHT plus 300 nM radioinert DHT prior to electrophoresis. Dye front is indicated by the arrow. The same results in nSBP mobility were obtained for plasma from males and females.

Table 2. Equilibrium constants of association at 4°

Steroid	hSBP $K_A \times 10^{-9}$ (M^{-1})	nSBP* $K_A \times 10^{-9}$ (M^{-1})
5α-dihydrotestosterone	2.76 ± 0.61†	1.87 ± 1.05** (Male)
	2.8‡	2.04 ± 0.88** (Female)
		2.21 ± 0.59** (Pregnancy)
Testosterone	0.93‡	0.41 ± 0.25** (Pregnancy)
	1.7§	
	1.2‖	
	4.97 – 6.34¶	
17β-estradiol	0.46‖	0.24 ± 0.13** (Pregnancy)
	0.6§	
	3.15 – 3.37¶	

* Data in this paper. † Mickelson and Pétra [9]. ‡ Shanbhag *et al.* [14]. § Mercier-Bodard and Baulieu [23]. ‖ Lebeau *et al.* [24]. ¶ O'Connor *et al.* [17]. ** ± values represent the upper and lower limits of the slope from a single Scatchard plot at the 95% confidence level.

Statistical analysis of the data

The equilibrium constants were calculated using both the Newton–Raphson non-linear treatment of the observation equation, and the linear regression analysis of Scatchard plots. The Scatchard plots were linear in the 20–70% range of the saturation, and consequently the assumption of the model based on only one set of binding sites was valid for the fitting. Both methods yielded almost identical values for the equilibrium constants. In Table 2, as well as throughout the text, we report the constants calculated by linear regression analyses of Scatchard plots since this method gave the maximum estimation of the error.

DISCUSSION

The results obtained in this report not only establish the presence of SBP in *Macaca nemestrina* but also provide evidence for a good experimental model system to study the physiological role of SBP. Concentrations of nSBP in females are significantly higher than in males. Similar SBP concentrations are found in the Macaca mulatta[6] and the Macaca speciosa [4]. In humans a similar sex difference exists but the concentrations are 3–4 times lower. The similarities between nSBP and hSBP demonstrated here indicate that the Macaca nemestrina should serve as a useful model for man. This reasoning is further substantiated by a recent observation in *Macaca mulatta* suggesting an inverse relationship between metabolic clearance rate of testosterone and SBP concentrations [19], similar to that shown in man some years ago [20]. The results obtained here support the idea that nSBP and hSBP appear similar in their binding and structural properties. There are, however, differences which require discussion. First, whereas the DHT binding affinity of nSBP correlate well with that of hSBP, the K_A's for testosterone and estradiol-17β are somewhat lower (Table 2). The differences, however, are probably not significant due to the extensive scatter of the published data on hSBP. Second, it appears that the steroid binding site of nSBP may accommodate 5α-androstan-3β-17β-diol better than 5α-androstan-3α,17β-diol (Table 1). Such a structural requirement does not exist in hSBP. A quantitative binding analysis will be needed to evaluate further this preliminary observation (in progress). Third, the rate constant of dissociation at 4° of the DHT–nSBP complex, $K = 0.013\ min^{-1}$, is higher than that of hSBP, $K = 0.009\ min^{-1}$ [17]. Since the filter assay used in these studies [9, 10] and the ammonium sulfate precipitation method [17] yield similar results for the determination of rate constants[6], we conclude that the rates of dissociation are probably different. This may explain the slight differences in specificity reflected by changes in dissociation rates as shown by Stroupe and Westphal in the case of PBG–steroid complexes [25]. The mechanism of the dissociation process is similar for both complexes since first-order kinetics is maintained. Further work will be required to explain these variations in terms of possible structural differences in the steroid binding sites of the two proteins. The most convincing results which bear out the similarity between the two proteins are the electrophoretic mobility and the specificity of binding. Progesterone and estrone have no measurable affinity for nSBP and hSBP, in contrast to the sex steroid binding protein of *Macaca speciosa* [14]. "Competitive" Scatchard analyses conclusively show that 5α-dihydrotestosterone, testosterone, and estradiol-17β all compete for the same binding site on the protein. The same procedure carried out on hSBP yields identical results.[5] Although the absolute values of the K_A's for testosterone and estradiol-17β for nSBP are lower than those reported for hSBP (Table 2), the trend in the binding affinity is the same for both proteins, i.e., DHT > testosterone > estradiol-17β SPB. The rates of dissociation of steroid–

[6] Burry, Tabei, Pétra, Schiller, Resko and Heinrichs, unpublished results.

nSBP and steroid–hSBP complexes also follow the same but reverse trend, i.e., DHT·SBP < testosterone·SBP < estradiol-17β-SBP. It is therefore likely that the structural differences in the binding sites which reflect the specificity differences will be similar for both proteins. We believe that such structural differences in the binding site are probably the result of minor genetic variations in the primary structure of these proteins. While these species differences exist, the similarities between hSBP and nSBP are more striking and support our contention that nSBP should serve as a useful human model. Furthermore, if these conclusions are correct, antibodies prepared with pure hSBP should cross-react with nSBP (work in progress). Availability of antibodies will permit the exploration of the biological role of SBP without the necessity of purifying nSBP.

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