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Rapid Communication

Binding of Sex Steroid Binding Protein to Plasma Membranes of Human Testis

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The existence of a specific binding site for sex steroid binding protein (SBP or SHBG) was detected on plasma membranes prepared from the testis of a patient affected by a variant form of testicular feminization. A binding technique using [^{125}I]SBP as a tracer allowed us to identify a single set of binding sites, characterized by a K_d of 1.917×10^{-11} M. The maximum number of binding sites was 5.2 fmol/mg membrane protein. Membranes were also prepared from a sample of genital skin from the same patient, but no binding for [^{125}I]SBP was detectable. The evidence of the SBP membrane receptor in the testis of a patient affected by Morris syndrome extends our knowledge about the tissue distribution of the SBP receptor and suggests the possible implication of SBP and its recognition system in a disorder related to peripheral androgen insensitivity.

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INTRODUCTION

Specific membrane binding sites for sex steroid binding protein (SBP, SHBG) were identified in several human steroid-sensitive tissues, prostate [1], decidual endometrium [2], premenopausal endometrium [3], endometrial adenocarcinoma [4], as well as in cultured cells [5–7].

Although the exact role of SBP membrane receptor still awaits full clarification, it is suggested that it may be linked to the action of steroids at the target site [8]. Recently, the SBP–membrane receptor interaction in MCF-7 cells (human breast cancer cultured cells) has been shown to reduce significantly the estradiol-induced cell proliferation [6]. In addition, a significant increase in intracellular cAMP following the androgen–SBP-receptor interaction in LnCaP cells [5], and the estradiol–SBP-receptor interaction in MCF-7 cells [9] has been reported. The relationships between the mechanism of action of steroid hormones and the second messenger cAMP is not fully understood, but several authors have recently suggested that cAMP and steroid receptors could be linked to additional and more sophisticated control of steroid hormones action [10, 11].

A receptor-mediated endocytosis of human SBP has been observed to occur in the epididymal epithelial cells of the rat [12]. An enriched fraction of membranes extracted from epididymal epithelial cells of immature and adult rats has been reported to have specific binding sites for both rat androgen binding protein (rABP) and human SBP [13].

The present study demonstrates the existence of a specific membrane binding site for SBP in the testis of a patient suffering from a variant form of testicular feminization.

MATERIALS AND METHODS

Patient

Testis and genital skin samples were obtained from a 51-year-old patient (phenotypically female, karyotype 46, XY) who underwent surgery for correction of a right inguinal hernia and bilateral orchiectomy (both testes were located in the inguinal canal). The patient

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presented: primary amenorrhea, severe hirsutism, female external genitalia with a blind vaginal pouch and testes located in the inguinal canal bilaterally; elevated plasma LH, FSH, testosterone (18 ng/ml), dihydrotestosterone (70 ng/ml), androstenedione (3 ng/ml), a normal male cortisol and 17-hydroxyprogesterone response to a standard i.v. ACTH stimulation test, absent estrogen and progesterone receptors and extremely low levels (4 fmol/mg protein) of androgen receptor in genital skin. The disease was identified as a variant form of complete androgen resistance (testicular feminization or Morris Syndrome).

Preparation of plasma membranes

Samples of testis (4.0 g) and of genital skin (3.0 g) were placed in ice-cold saline immediately after surgical removal, extensively washed to remove any blood contaminant, blotted dry and frozen at −80°C until processing. The testis sample was used without any further attempts to fractionate different cell types. Plasma membranes were obtained by differential centrifugation, as described previously [3]. In brief, samples were homogenized in 25 mM Tris–HCl and 0.3 M sucrose, pH 7.4, using a Polytron homogenizer (PT 1035, Kinematica, GmBH, Lucerne, Switzerland). The homogenate was serially centrifuged at 15,000 and 125,000*g*. The final 125,000*g* pellet, considered the source of plasma membranes, was assayed both for protein concentration with the Bradford technique [14] and for 5′-nucleotidase activity, a specific marker for plasma membrane [15].

SBP binding assay

Human purified [^{125}I]SBP (10 mCi/mg) was obtained from Eurodiagnostica (Malmö, Sweden) and, before use, checked for its purity by sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS–PAGE) under denaturing conditions [16]. Radioinert highly purified human SBP was a gift from Dr PH Petra (Department of Biochemistry, University of Washington, Seattle, WA, U.S.A.). Both the labeled and the radioinert protein were tested for their capacity to bind dihydrotestosterone as described previously [17].

Binding of [^{125}I]SBP to plasma membranes took place in τ-counter plastic tubes (11 × 75 mm) pretreated with 50 mM Tris–HCl, 30 mM $CaCl_2$, and 0.2% bovine serum albumin, pH 7.4 (binding buffer), at 37°C for 1 h to decrease test tube blanks.

Aliquots of membrane suspensions (0.1–0.2 mg protein/tube) were incubated with [^{125}I]SBP (0.1 nM, approx. 200,000 dpm/tube, in the absence and in the presence of 0.1 μM radioinert SBP for single point determination; [^{125}I]SBP ranging from 0.05 to 0.1 nM, in the absence and in the presence of 21 nM of radioinert SBP for saturation/Scatchard analysis) [18] in binding buffer (final volume 1.0 ml). Incubation was accomplished at 4°C with continuous rotation for 24 h. At completion, tubes were centrifuged to pellet membranes at 6000*g*, 4°C, 30 min. Supernatants were discarded and pellets counted for radioactivity in a Packard τ-counter at 72% efficiency.

Data obtained with the saturation study were processed with the PC program LIGAND [19] to calculate both K_d and R (maximum number of binding sites) values.

RESULTS

Preparation of plasma membranes

Membrane suspensions were successfully prepared from both tissues. The protein concentration, measured in both preparations, were: testis, 1.9 mg/ml; genital skin 0.34 mg/ml, respectively. In both preparations, 5′-nucleotidase activity was increased more than twice with respect to the initial homogenate, showing the good quality of preparation.

SBP binding assay

Both sample membranes (testis and skin) were assayed for SBP-receptor with a single point assay. Testis membranes were shown to bind 4.89 fmol SBP/mg protein, while no binding was observed on skin membranes (0 fmol SBP/mg protein).

The binding of SBP to testis membranes was further characterized. It was shown to reach saturation at a dose of 15–20 pM of added SBP (Fig. 1). The Scatchard analysis of the binding demonstrated the existence of a single set of binding sites, characterized by a K_d of 1.917×10^{-11} M. The maximum number of binding sites was 5.2 fmol SBP/mg protein (Fig. 2).

DISCUSSION

The membrane receptor for SBP has been described on several tissues, either androgen or estrogen-dependent [1–7, 20–22]. Its biological role is, unfortunately, still unclear, as well as its involvement in human pathophysiology. We have previously shown that the SBP receptor is present on membranes of neoplastic endometrium [4], suggesting a possible role for SBP on estrogen-responsive neoplasms. More recently, our studies on MCF-7 cells [6, 9] strongly suggest that SBP and its membrane receptor could be involved in the modulation of estradiol action.

The present data demonstrate the presence of a specific binding site for SBP with high affinity on membranes of a pathologic human testis. Felden *et al.* [13] have recently shown that epididymal cells obtained from rat testis bind both ABP and SBP at a receptor site. Interestingly, these authors reported that SBP binds to a single binding site with a K_a of 2.6 nM^{-1}. In the present study we observed that SBP recognizes a receptor on human testis membranes and interaction occurs at a single binding site. The K_d we calculated is approximately 10-fold lower than the one reported by Felden *et al.* for rat testis. The difference could be due

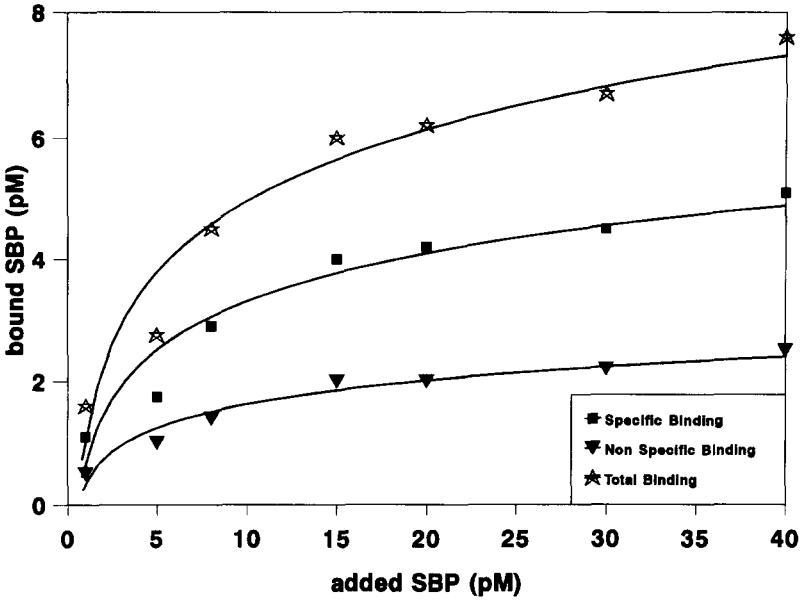

Fig. 1. Saturation curve of SBP specific binding to testis membranes. Aliquots of membrane proteins were incubated with [^{125}I]SBP ± a molar excess of radioinert SBP for 24 h at 4°C. Specific binding was calculated by subtracting NSB values (obtained in the presence of radioinert SBP) from TB values (obtained in the absence of radioinert SBP). Experiment was performed in duplicate and repeated twice. The figure shows the best of the two obtained curves.

to the different species used (human vs rat), the different kind of SBP (radioiodine labeled vs photoaffinity labeled), or the different incubation conditions (4°C for 24 h vs 4°C for 1 h). In any case, the most impressive aspect of both reports is the single binding site recognized by SBP in male gonads. The experimental conditions used in the present study failed to demonstrate another binding site; the range of [^{125}I]SBP concentrations used is, however, wide enough to cover the possible existence of other binding sites. All previous reports about other human tissues and cultured cells described a double-site interaction, characterized by two different affinities for SBP [1–6]. Felden *et al.* [13] suggested that the male rat gonad, which is the site of synthesis of ABP, presents a membrane receptor undergoing a high level of receptor recycling. This high level of recycling is due to the high tissue concentration of ABP, and, in turn, is responsible for the single affinity site for ABP/SBP. We have recently observed that liver cells [7] which produce SBP express a specific receptor for the protein; in this case, two binding sites at different affinities were observed, but the low

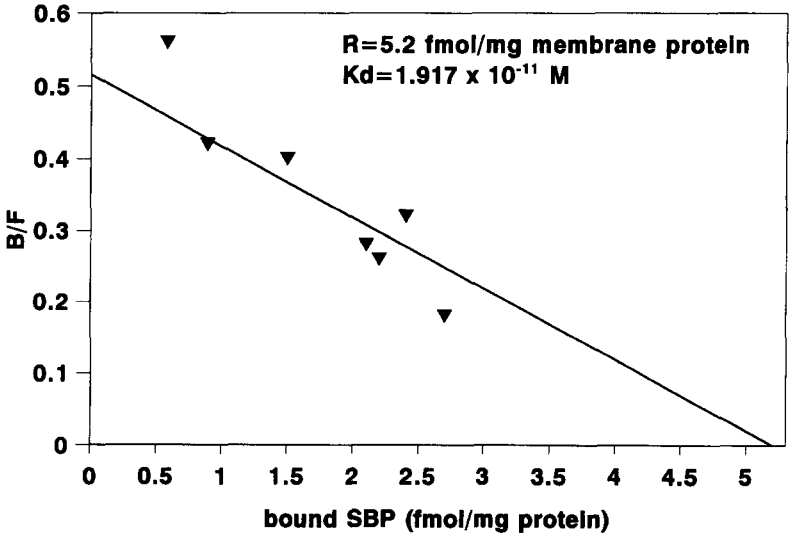

Fig. 2. Scatchard plot of the SBP binding to testis membranes. Experimental data of Fig. 1 were elaborated with the PC program LIGAND. Binding parameters obtained from Scatchard analysis are the following: $K_d = 1.917 \times 10^{-11}$ M; R (maximum number of binding sites) = 5.2 fmol/mg membrane protein.

affinity, high capacity sites were represented much more than in other human tissues. The present data are definitely not enough to draw any conclusion but the suggestion that cells producing SBP/ABP present a membrane receptor slightly different from the receptor of other tissues deserves further study.

We could not observe any significant binding of SBP to membrane obtained from genital skin, but the low protein concentration of the membrane sample is likely to be responsible for the negative result.

In conclusion, our data show that plasma membranes from a human testis present a specific binding site for SBP, characterized by a single site of binding. Unfortunately it was not possible to study the SBP binding to membranes from non-pathological testis, since suitable samples have not been available until now. Thus, the present data cannot be used for any comparison between physiological and pathological tissue. However, our observation is of some interest, since it extends our knowledge about the tissue distribution of the SBP membrane receptor.

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