Increased Androgen Binding Capacity in Sebaceous Glands in Scalp of Male-pattern Baldness

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Sebaceous glands were isolated by manual dissection under a microscope from surgical specimens of scalp skin with male pattern baldness and skin specimens of hairy and bald scalp obtained at autopsy. The 800 × g pellet (nuclear fraction) and the 164,000 × g supernatant fraction (cytosol) of homogenates of the sebaceous glands were used for measurements of androgen binding characteristics, using dextran-coated charcoal and sucrose gradient methods. Scatchard plots showed high affinity binding for [^{3}H]dihydrotestosterone (DHT) and [^{3}H]methyltrienolone (R1881). Nuclei prepared from bald scalp contained greater total androgen binding capacity than nuclei of hairy scalp, although Kd values of type I binding were similar (0.68 vs 0.56 nM, respectively). On sucrose gradient, the binding protein from cytosol was found in the 7 to 8S density range. Androgen binding by cytosol of sebaceous glands of hairy scalp had Kd of 1.89 ± .79 and 2.05 ± .56 nM for DHT and R1881, respectively, and Bmax of 18.7 ± 4.4 and 20.0 ± 4.6 fmol/mg protein for DHT and R1881, respectively. Cytosol from sebaceous glands of bald scalp had Kd values approximately half those of hairy scalp, and Bmax values 50%–100% higher. The bound ^{3}H labeled DHT and R1881 could be partially displaced by testosterone (40–50%), moxestrol (28–32%), promegestone (19–26%), and Δ^4-androstenedione (6–12%), but not by dehydroepiandrosterone. These data demonstrate the presence of specific androgen binding protein in sebaceous glands, and that sebaceous glands of bald scalp have greater binding affinity and capacity for androgens than those in hairy scalp. This difference may explain the greater androgenic response in androgenic alopecia. *J Invest Dermatol 92:91–95, 1988*

The development of secondary sex characteristics in the skin is regulated by sex hormones [1–3] and the androgens especially have profound influences on cellular activities of the skin [2,3]. Action of steroids in skin is assumed to be mediated as in other target organs, by intracellular receptors, which bind the specific steroid, and after a temperature-dependent translocation of the receptor-steroid complex into the nucleus of the cell, the steroid binds to a specific DNA segment to affect genetic expression. Recent studies indicate that steroid receptors may be intranuclear [4,5] and nuclear binding of steroids especially deserves attention.

Androgen action has been studied in cultured human fibroblasts [6,7] and whole human skin of various body sites [8,9]. It has been observed that the androgen binding capacity of skin varies with anatomical sites and other conditions, but detailed information on the molecular events of androgen action in the skin is lacking. Because the sebaceous gland is especially sensitive to androgenic stimulation, a detailed study of androgen action in the sebaceous gland is desirable. In this paper we report a study of androgen binding properties of human sebaceous glands isolated by a microdissection procedure. We found greater androgen binding capacity in the cytosol and nuclei of sebaceous glands isolated from bald scalp of patients with androgenic alopecia than glands isolated from hairy scalp.

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Abbreviations:
Bp: bald scalp specimen obtained at autopsy
Bs: bald scalp specimen obtained at surgery
Δ5-3 β-hydroxysteroid dehydrogenase: 3βHSD
DHA: dehydroepiandrosterone
E2: estradiol
H: hairy scalp
R1881: 17α-methyltrienolone
R2858: moxestrol
R5020: promegestone

MATERIALS AND METHODS

Chemicals [1,2-^{3}H]5$^\alpha$-dihydrotestosterone ([^{3}H]DHT, 55 Ci/mmol), [17α-methyl-^{3}H]methyltrienolone, ([^{3}H]R1881, 86 Ci/mmol), [1,2-^{3}H]-testosterone (60 Ci/mmol), [1,2-^{3}H]dehydroepiandrosterone ([^{3}H]DHA, 55 Ci/mmol), [^{3}H]promegestone ([^{3}H]R5020, 87 Ci/mmol), moxestrol ([^{3}H]R2858, 87 Ci/mmol), and nonradioactive R1881, R5020 and R2858 were purchased from Dupont New England Nuclear (Boston, MA). Nonradioactive DHT, testosterone, androstenedione, and DHA were purchased from Sigma Chemical Co. (St. Louis, MO). Both radiolabeled and unlabeled steroids were purified by thin layer chromatography before use.

Scalp Specimens Specimens of bald scalp were collected from men with male pattern baldness (age 25–39 years) undergoing hair transplant or scalp reduction surgery. Specimens were also collected from hairy and bald scalp of adult male trauma victims (30–47 years) at autopsy within 3 h post-mortem. The specimens were put on ice and transported to the laboratory. Subcutaneous fat and the lower portions of dermis were trimmed off. The remaining skin specimens were cut into 3-mm pieces and soaked in Hank's buffered salt solution (HBSS) containing 25 mM HEPES, pH 7.4, at 4°C for 20–60 min. Dissection was performed under a stereomicroscope, using Dumont stainless steel tweezers (Nos. 5 and 55) and dispos-

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able scalpels. Scalp plugs excised from the recipient sites during hair transplant were treated in the same manner. About 1 g of skin or 13–15 scalp plugs were used in each experiment and the dissection required 2 to 3 h for completion.

Preparation of Cytosol The isolated sebaceous glands were homogenized in 10 mM Tris-HCl, pH 7.4 buffer, containing 1 mM EDTA, 1 mM dithiothreitol (TED buffer), and 10 mM sodium molybdate, with a Ten Broeck (glass to glass) tissue homogenizer. Cytosol was prepared by ultracentrifugation of the homogenate at 164,000 × g for 1 h at 2°C in a Beckman L5-65B Ultracentrifuge, with SW 50.1 swinging bucket rotor in 5 ml centrifuge tubes (Beckman Instruments, Inc., Spinco Div., Palo Alto, CA). Supernatant was removed with a Pasteur pipette, and the floating lipid layer was carefully avoided.

This cytosol preparation was diluted to 2 mg protein per ml, as determined by the Lowry method [10]. Aliquots (200 μl) of the diluted cytosol were incubated at 0°C for 30 min with 50 μl of radiolabeled steroid in the presence or absence of 1.6 μM nonradioactive steroid (200 times greater than the concentration of the radioactive ligand). Labeled ligand in final concentrations ranging from 0.25 to 8 nM, was added to make up 300 μl. The incubation was carried out for 16–20 h at 2–4°C. The incubation mixture was then treated with dextran coated charcoal suspension [11] (DCC; 0.25% Norit A, 0.0025% dextran Grade C, in 0.01 M Tris, pH 8.0), and centrifuged at 1600 × g for 10 min at 2°C to sediment the charcoal with adsorbed unbound steroid, according to Mowszowicz and Wright [12]. Radioactivity was assayed in a Packard Tricarb liquid scintillation counter with tritium counting efficiency of 35%. Data were analyzed according to Scatchard [13], after subtraction of the nonspecific binding in the samples containing 200-fold excess of nonradioactive steroid.

Preparation of Nuclear Fraction For the preparation of nuclei, the dissected sebaceous glands were homogenized in five volumes of 10 mM Tris-HCl with 1 mM EDTA, pH 7.4, in a Ten Broeck homogenizer. The homogenate was filtered through 3 layers of cheesecloth and centrifuged for 20 min at 800 × g to obtain the nuclear pellet which was washed 3 times with buffer and resedimented at 800 × g for 15 min, to break up fine aggregates and reduce non-specific binding. The washed nuclei were suspended in the same buffer, and the DNA content of the suspension was determined according to the method of Burton [14]. Examination under an inverted phase contrast microscope revealed intact nuclei free of particulate contaminations. Tests for the nuclear marker enzyme, 5' nucleotidase were positive.

Sucrose Density Gradient Analysis Sucrose density gradients were prepared by layering five sucrose solutions (5, 10, 15, 20, and 25%, w/v) in TED buffer in cellulose nitrate tubes and allowing diffusion for 2 h at 4°C, before application of cytosol samples to the top [15].

Cytosol samples were pre-incubated with 20 nM ^{3}H-labeled ligand with or without 200-fold excess (4 μM) nonradioactive ligand for 4 h at 0°C, and unbound ligand was removed by mixing 240 μl of the incubation mixture with a pellet of DCC (obtained from centrifugation of 1 cc of DCC suspension). The mixture was centrifuged at 1600 × g for 10 min at 2°C to sediment the charcoal-adsorbed free ^{3}H-ligand. Then 200 μl of incubation mixture was carefully pipetted and layered on top of the 5 ml (5–25%) sucrose gradient and centrifuged in a Beckman SW 50.1 rotor for 18 h at 190,000 × g at 0°C. Parallel gradients contained catalase (11.2S) and human hemoglobin (4.5S) as colored external marker proteins. The gradients were fractionated by puncturing the bottom of the tube, and collected in 0.2 ml fractions. Radioactivity in each fraction was plotted versus fraction number and the amount of specific bound steroid was expressed in fmol/mg protein.

Assay for Nuclear Androgen Binding Aliquots of 250 μl of the nuclear suspension were incubated with ^{3}H-methyltrienolone (0.4–12 nM) for 30 min at 37°C for androgen binding. Saturation analysis for total nuclear type I receptor sites was performed as previously described [16,17] by resuspending the nuclear pellet with buffer (10 mM Tris-HCl, 1 mM EDTA, 1 mM DTT, 10 mM Na Molybdate, pH 7.4). Parallel incubation tubes contained 200-fold excess unlabeled ligand to determine non-specific binding.

Quantitation of nuclear type II receptor sites was examined by incubating the nuclei with the radioactive ligand at concentrations of 10–30 nM, and with parallel tubes of ≥300 times unlabeled hormone for 30 min (37°C) in buffer of 10 mM Tris-HCl, with 1 mM EDTA (pH 7.4).

The incubation was terminated by the addition of 1 ml of ice cold buffer, and the mixture was then centrifuged at 800 × g for 10 min. The supernatant was decanted, and the nuclear pellets washed twice in buffer before addition of 1 ml of ethanol for extraction of radioactivity. The tubes were then placed in a water bath at 30°C for 30 min, vortexed, then centrifuged at 800 × g for 10 min. The total ethanol extract was radioassayed. Results were expressed as fmoles of bound ligand/mg DNA.

RESULTS

Steroid Binding by Cytosols Binding of [^{3}H]DHT and the synthetic ligand [^{3}H]methyltrienolone (R1881) was demonstrated by incubation with cytosols of sebaceous glands obtained from specimens of hairy scalp (H), or bald scalp obtained at autopsy (Bp) or surgery (Bs). Figure 1 shows a Scatchard plot of data obtained from [^{3}H] R1881. The results show typical specific binding of the steroid. An analysis of data from a study with [^{3}H]DHT gave similar linear graphs (data not shown). The dissociation constant (Kd) and binding capacity (Bmax) for the two androgens estimated from these plots are shown in Table I. For cytosol of the sebaceous glands the Kd values were 1.89 and 2.05 nM for DHT and R1881, respectively. For Bs and Bp glands, the values were approximately half. Bmax values were 20.0 and 18.7 fmol/mg protein for DHT and R1881, respectively, for cytosol of H glands. Bmax values for Bs and Bp glands were 35%–70% greater. Thus, cytosol of Bs and Bp glands had stronger affinity and greater binding capacity for the androgens than that of H glands.

Kd and Bmax values for [^{3}H]promegestone (R5020) and [3H]moxestrol (R2858) are shown in Table II. When compared with data in Table I, these Kd values were several-fold greater, and Bmax values were less than half of the values for the androgens.

Specificity of the Binding Complex Specificity of binding was evaluated by displacement of [^{3}H]methyltrienolone and [^{3}H]DHT from the androgen binding sites by 200-fold excess of non-radioac-

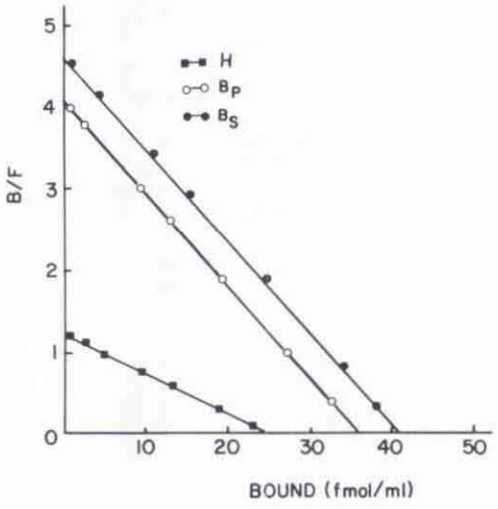

Figure 1. Binding of [^{3}H]methyltrienolone by cytosol of human sebaceous glands analyzed by Scatchard plots. *Closed squares* represent sebaceous glands isolated from specimens of hairy scalp. *Open circles* represent specimens of bald scalp obtained at autopsy. *Closed circles* represent surgical specimens of bald scalp.

Table I. Comparison of Androgen Binding Properties of Sebaceous Gland Cytosols[a,b]

Source of Sebaceous Gland	[3H]DHT Kd, nM	[3H]DHT Bmax fmol/mg protein	[3H]methyltrienolone (R1880) Kd, nM	[3H]methyltrienolone (R1880) Bmax fmol/mg protein
H	1.89 ±.79	20.0 ±4.6	2.05 ±.56	18.7 ±4.4
B_s	0.79 ±.04	34.1 ±4.1	0.90 ±.08	30.1 ±4.3
B_p	0.95 ±.09	27.0 ±3.1	0.90 ±.30	26.8 ±3.0

[a] Cytosols of sebaceous gland isolated from specimens of hairy scalp (H), and bald scalp obtained at surgery (B_s) or at autopsy (B_p) were incubated with [3H]DHT or [3H]DHT or [3H]methyltrienolone. Values and Kd and Bmax were estimated by the Scatchard method. The values are mean ± SD of 6 samples.

[b] Values of B_s and B_p glands are significantly differed from values of H gland ($p < 0.001$).

tive steroids. Nonspecific binding, measured as that portion of 3H not displaced after the addition of excess nonradioactive methyltrienolone, was subtracted from the measurements. The result (Table III) shows that DHT displaces approximately 80%, while methyltrienolone displaces 80%–85% of the bound 3H. Testosterone displaces between 39%–47% of bound [3H]DHT and 47%–52% of bound [3H]methyltrienolone. Androstenedione displaces approximately 10% of bound [3H]DHT, while no displacement occurred when DHA was tested. The synthetic ligands promegestone and moxestrol displaced approximately 20% and 30% of bound [3H]DHT, respectively.

Sucrose Density Gradient Analysis The androgen receptor binding complexes were further characterized by sucrose density gradient centrifugation. Cytosol samples were incubated with 20 nM [3H]methyltrienolone at 2°C for 4 h and subjected to sucrose density gradient centrifugation. A peak of radioactivity was found sedimenting at 7.5S (Fig 2). 3H in this peak was displaceable by 200-fold excess of non-radioactive methyltrienolone. The estimated Kd and Bmax values were in agreement with data in Table I.

Nuclear Androgen Receptor Content Table IV shows the results of nuclear androgen receptor content in H and B sebaceous glands from three separate preparations. The B glands had 20% higher type I receptor content than the H glands. The type II binding data reveal nearly three times greater receptor content in B (1786 fmol/mg DNA) than H glands (665 fmol/mg DNA). The Kd's for B and H were similar, 0.68 and 0.55 nM, for type I binding, and 8.0 and 8.5 nM for type II, respectively.

DISCUSSION

Elaborate procedures have been used in previous investigations to obtain sebaceous glands from human skin, requiring lengthy enzyme digestion [18], chemical treatment [19], or freeze-drying of tissue preparations [20]. The procedure used in the present study

Table II. Binding Properties of Sebaceous Gland Cytosols[a]

Source of Sebaceous Gland	[3H]Promegestone (R5020) Kd, nM	[3H]Promegestone (R5020) Bmax fmol/mg protein	[3H]Moxestrol (R2858) Kd, nM	[3H]Moxestrol (R2858) Bmax fmol/mg protein
H	5.25 ±0.41	7.03 ±1.71	7.30 ±1.50	9.20 ±2.50
B_s	4.85 ±0.71	7.00 ±1.10	7.27 ±1.20	9.60 ±2.30
B_p	6.56 ±0.72	5.27 ±0.60	8.43 ±0.68	8.06 ±1.19

[a] Each value is the mean ± SD of estimates from three independent Scatchard plots. There was no significant difference between values of glands from hairy and bald scalp.

Table III. Displacement of 3H Labeled Ligands by Steroids[a]

Steroid	% Displacement [3H]DHT Source of Sebaceous Gland H	B_s	B_p	% Displacement [3H]R1881 Source of Sebaceous Gland H	B_s	B_p
dihydrotestosterone (DHT)	74	69	75	79	78	76
17α-methyltrienolone (R1881)	77	78	69	84	86	79
Testosterone	39	46	47	47	52	49
4-androstenedione	6	10	9	6	11	12
dehydroepiandrosterone	0	0	0	0	0	0
promegestone	22	22	19	25	26	22
moxestrol	28	28	32	33	31	35

[a] Each value is the average of three independent binding measurements.

was milder, and required soaking the scalp specimens in HBSS for approximately 1 h at 4°C prior to dissection without use of digestive enzymes. The procedure yielded intact sebaceous glands, and permitted the study of androgen receptor proteins, which are known to be thermolabile [12,21], and are likely to be denatured if subjected to long periods of incubation at 37°C during the isolation procedure.

The data in Table I demonstrated highly specific binding in cytosol of human sebaceous glands, although the number of binding sites was low for both DHT and methyltrienolone. DHT and methyltrienolone apparently bind the same sites of the receptor, as they displace each other with equal efficiency (Table III). Testosterone displaced approximately 45% of methyltrienolone and DHT, reflecting weaker affinity of T to the receptor.

The data in Table I show that H sebaceous glands had cytosol receptor with significantly less affinity and binding capacity for DHT and methyltrienolone. These differences may reflect a state of hyperandrogenization in bald scalp and play a role in the pathogenesis of the balding process. It is not clear whether any allosteric regulators or inhibitory proteins are present to influence the receptor activity, as found with estrogen receptors [22]. Similar studies of melanoma, benign nevi [23], and lentigo maligna [24] have also shown variations in steroid receptors when compared to normal tissues. The present data revealed no significant difference between sebaceous glands isolated from surgical and postmortem bald scalp specimens.

In the evaluation of androgen binding, DHT is used as a ligand to ensure specificity [12], while methyltrienolone has the advantage of not binding to the circulating sex binding proteins [25]. The relative affinities of the sebaceous gland cytosol receptor for the steroids

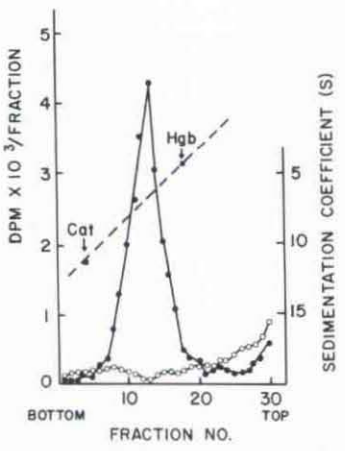

Figure 2. Distribution of protein-bound 3H in a sucrose gradient after incubation of [3H]methyltrienolone with cytosol of sebaceous glands. Details of the procedure are given in the text. Human hemoglobin (*Hgb*) and catalase (*Cat*) are used as markers. The peak of 3H corresponds to 7.5S.

Table IV. Nuclear Androgen Receptors[a]

Source of Sebaceous Gland	Type I		Type II	
	Kd (nM)	Bmax fmol/ mg DNA	Kd (nM)	Bmax fmol/ mg DNA
B	0.68	311	8.0	1786
H	0.55	239	8.5	665

[a] Nuclei of sebaceous glands were isolated from specimens of hairy scalp (H) and bald scalp (B) as described in *Materials and Methods*.

tested in this study were methyltrienolone = DHT > T > E2 = P > A, similar to that reported by other authors [10,21]. On sucrose density gradient, the receptor sedimented as 7–8S. The data clearly demonstrate the presence of a soluble receptor protein that specifically binds androgens, but binds estrogen and progesterone analogs with much less affinity (Table II). The data in Table III show specificity of the cytosol receptor protein for binding DHT, R1881, and testosterone, and relatively weak binding for moxestrol and progesterone. Biologic effects of progestins and estrogens in the sebaceous gland have been studied previously [27,28]. Circulating levels of estrogen and progesterone are low in man [29] and neither steroid is considered a trophic hormone for the male sex accessory tissue. However, it is known that progestational agents can modify androgen action by potentiation or inhibition. These mechanisms are not well understood, but binding of these hormones to the androgen receptor (as revealed by data in Tables II and III) can influence the extent of androgen binding and, therefore, the tissue response to androgens.

Table IV shows that the nuclei of sebaceous glands isolated from bald scalp had 20% greater type I binding of hairy scalp. Kd's for type I binding were similar between B and H glands, and were smaller than Kd's for type II binding. Our data on type I and type II nuclear binding for androgen are in the same range as those for estrogen receptors reported by Markaverich et al [16,17]. Those authors interpreted that type I nuclear binding sites represent the classical intracellular receptor which binds the hormone with high affinity (Kd ~ 1 nM), and translocates to the nucleus. Nuclear type II sites are inducible, more abundant, and have lower binding affinity. The exact role of type II binding sites is still unclear; however, estrogen binding capacity, especially type II binding in uterine growth, is elevated under long-term chronic elevated hormone stimulation [16,17]. The increased nuclear androgen content of B glands may be interpreted as reflecting the overandrogenic state.

The present study of quantitative analysis of steroid receptors in isolated human sebaceous glands will be followed by a report of our study on receptor in human hair follicles. The scalp has more sebaceous glands than any other part of the body and these glands are androgen target organs. The dissociation constants and binding capacities of cytosol receptors we found are in the same range as reported for genital skin and other androgen-sensitive target tissues [25,30].

Androgenic response of a tissue depends on the presence of specific androgen binding protein. In addition, enzymes of steroid metabolism alter chemical structure of the steroids and also their hormonal potency. In a recent study we demonstrated the conversion of DHA into androstenedione, testosterone, and DHT in human sebaceous gland, and the greater enzymic activity of Δ^5-3β-hydroxysteroid dehydrogenase (3βHSD) activity in sebaceous gland of bald scalp than in hairy scalp [31]. Taken together, greater 3βHSD activity and increased androgen binding capacity may provide a biochemical explanation for the disease mechanism of androgenic alopecia. Further such studies may provide new insight into the mechanism of skin diseases caused by hyperandrogenization, such as acne, hirsutism, and seborrhea, besides male pattern baldness.

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