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Experimental Study on the Male Pattern Alopecia

I. Differences between the Testosterone Transport Protein in Bald and Hairy Areas

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Summary. A cytosol testosterone binding protein is found in the bald and hairy zones of male pattern alopecia. Simple kinetic differences between both regions towards testosterone binding indicate structural differences and greater affinity of the binding protein in bald regions towards testosterone and its steroid competitors. These differences may be linked to the pathogenic cause of baldness.

Zusammenfassung. Ein testosteronbindendes Protein wurde in den haarlosen und behaarten Hautarealen der männlichen Glatze gefunden. Die unterschiedliche Kinetik der Testosteronbindung in beiden Hautarealen weist sowohl auf strukturelle Unterschiede, als auch auf eine größere Affinität des testosteronbindenden Proteins zum Testosteron und seinen Steroidanalogen in haarlosen Bezirken hin. Diese unterschiedliche Bindungsaktivität spielt möglicherweise bei der Glatzenbildung eine pathogenetische Rolle.

Earlier clinical observations indicate the genetical control of baldness in male pattern alopecia [14, 15], as well as its association to the effect of testosterone [8]. However no major differences in the testosterone plasma levels on bald and hairy people are found [4, 5, 10, 16].

The testosterone action on target tissues seems to be mediated through binding to specific proteins [11]. A cytosol [16] protein with several components [4] have been identified in the prostate [6]. Additionally, a nuclear binder appears to play an important role on the testosterone action [12]. Similar situations are found in sebaceous glands [1].

The purpose of this paper is to show evidence of the differences existing between the cytosol binding protein from regions normally resistant to baldness (temporal and occipital areas) as compared with the bald regions in the same patient. These differences may suggest the association of baldness with the intracellular metabolism of testosterone as related to hairy and bald areas.

Material and Methods

Material from Transplants

Hairy and bald skin pieces were taken with an Orentreich No. 4,25 trocar from 10 male patients with male pattern alopecia as the only apparent disease.

Reagents

Testosterone (androsta-4-ene, 17 β-ol, 3-one).
Androsterone (5-α-androstan, 3-α-ol, 17-one).
Androstandiol (5-α-androstan, 3-α,17-β-diol).
Epiandrosterone (5-α-androstan, 3-β-ol, 17-one).
Progesterone (pregnan-4-ene, 3,20-dione).
Estrone (estra-1,3,5, (10) triene, 3-ol, 17-one).
Estradiol (estra 1,3,5, (10) triene, 3,17-β-diol).
Estriol (estra-1,3,5, (10) triene 3,16-α-, 17-α-triol) were bought from the Merck & Co. (Darmstadt, West-Germany); Testosterone H^3 (1α, 2α) (n) with an specific activity of 56 mCi/mM, was purchased to the Amersham Radiochemical Centre (England).

Dextran type D was supplied by Schwarz Mann, Orangeburg N. Y. (U.S.A.).

Charcoal was obtained from the Merck & Co. PPO (2,5-diphenyloxazol) and POPOP (2,4-bis 2,5-phenyloxazolyl) benzene were purchased to the Amersham Radiochemical Centre, England.

Tripsyn was bought to the Worthington Laboratories, U.S.A.

The rest of the reagents were the highest purity grade commercially available.

Sample Preparation

All the operations were performed at 4° C unless otherwise indicated. Dermo-epidermal pieces were fast frozen in presence of dry ice and kept at $-20°$ C until assayed.

Before testing, the fat portion of the dermo-epidermal pieces were cut out and then suspended on the double volume of 0,9 % sodium chloride and 1 mM phosphate buffer (pH 7.4). The suspension was homogenized and centrifuged at $2000 \times g$ for 10 min. Tests are run within 1 h of homogenizing.

Assay of the Binding Protein

On 5 ml polystyrene tubes, 1 ml final volume of the incubation mixture contained: 3.3 picomoles of unlabelled testosterone, 0.3 picomoles of H^3 testosterone (35—40000 c.pm.), 0.5 mg protein from the supernatant, 10 μmoles of Tris-HCl buffer at pH 7.5 or 9.0, depending upon the tested sample. After mixing the tubes were incubated at 4° C for 24 h.

Separation of the Free and Bound Testosterone

Separation was carried out by using a suspension of 5 % activated charcoal and 0.25 % Dextran in 1 mM Tris-HCl buffer at pH 7.5 or 9.0 depending upon the zone being tested. On each tube 1 ml of this suspension was added. After mixing, tubes were allowed to stand for 15 min and then centrifuged at $3000 \times g$ for 20 min. Supernatant is twice extracted with 5 ml of 0.5 % PPO, and 0.05 % POPOP in toluene solution and counted in a Nuclear Chicago Scintillation Counter set to 10 min, or until 10000 c. were obtained.

Equilibrium Constants

Assays were performed as above mentionned but increasing the testosterone concentration up to 10^{-6} M.

Location of the Binding Protein

The association of the testosterone binding protein to the cell soluble portion was shown as follows: aliquots of 0.2 ml from each of the patients were pooled

together, and then centrifuged at 2000 × g for 10 min. The supernatant was again centrifuged on a Spinco-L5 ultracentrifuge by using a 40 rotor set to 105000 × g for 2 h. On each precipitate and supernatant, assays were run as previously described.

Optimum pH

Aliquots of 0.1 ml from each sample patient were pooled together. Two different types of pools were formed depending upon the origin of the material (bald and hairy portions). Assays for bald and hairy pools were performed as above described but changing the pHs from 5.5 to 9.0. In addition to the assays of the pools, the optimum pH was independently tested on each sample from bald and hairy portions of each one of the patients.

Competition Studies

Testing of the various testosterone binding inhibitors were performed under two conditions: 1) by using the testosterone binding protein coming from the hairy and bald portions of each patient. 2) Performing the studies with binder coming from bald and hairy separated pools. On either cases incubation mixtures contained in 1 ml final volume: 10 μmoles of Tris-HCl buffer at pH 7.5 or 9.0 depending upon the origin of the binder (bald or hairy); 3.3 picomoles of unlabelled testosterone; 0.3 picomoles of H^3 testosterone, and 3.3 picomoles of the binding inhibitor being studied, named: androstandiol, androsterone, epiandrosterone, estrone, estradiol, estriol and progesterone. Incubation, separation (free and bound portions) and counting were carried out as described above.

Miscelaneous Determinations

Protein was determined by the Lowry *et al.* method [9]. Spectrophotometric readings were performed on a U.V.-Vis 139 Hitachi Perkin Elmer Spectrophotometer. The protein nature of the binder was shown by the loss of activity after the crude extract was incubated with trypsin [13].

Results

Cell Location of the Testosterone Binding Protein

The presence of most of the testosterone binding activity on the 105000 × g supernatant, indicates the association of the binder with the cell soluble portion (Table 1).

Table 1. Aliquots of 0.1 ml from each crude extract were pooled together and then centrifuged at 2000 × g for 10 min. Supernatant was again centrifuged at 105000 × g for 2 h on a Spinco-L_5 ultracentrifuge
Precipitates and supernatants were tested for the testosterone binding activity as described in "Methods"

Centrifuged portion	H^3-c. p. m. bound per mg of protein
Crude extract (pool)	9846
Supernatant 2000 × g	11695
Precipitate 105000 × g	611
Supernatant	17069

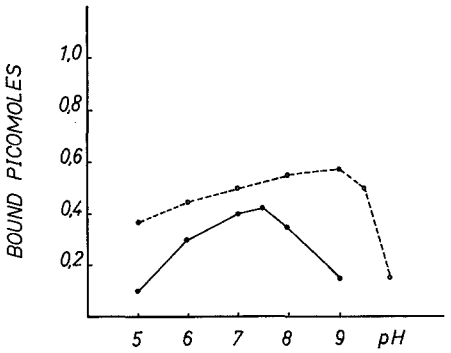

Fig. 1. Comparison of the pH Optimum. Assays at different pHs as described in Methods were performed with pooled samples from hairy (o) and bald (•) regions

Optimum pH

The optimum pH at which the testosterone binding takes place is 9.0 for the hairy and 7.5 for the bald regions. Both results were demonstrated on the different pools (bald and hairy) (Fig. 1) and on the separated samples from each patient.

Equilibrium Constants

Assays performed as described in methods, shown that the equilibrium constants of the hairy and bald portions were 8.8×10^{-10} M and 5.2×10^{-12} M.

Competition Studies

1. Androstandiol Effect. The androstandiol inhibitory effect is meaning among the different head zones and patients. It varies between a 33 % inhibition and 3 % stimulation. Though no significant differences between the bald and hairy portions are found, however, the mean values indicate a higher inhibitory effect on the hairy portions as compared to the bald zones (Table 3). Similar results were obtained when binding studies were carried out on bald and hairy pools (Table 2).

2. Androsterone and Epiandrosterone Effects. The inhibitory effects of both steroids towards the testosterone binding activity on bald and hairy portions were similar to the androstandiol effect. The relative comparison of androstandiol, androsterone and epiandrosterone inhibitory effects show a higher inhibition on the case of epiandrosterone (Table 2 and 3).

3. Estrone, Estradiol and Estriol Effects. From higher to lower, the inhibitory effects were: estrone, estradiol and estriol. The inhibitory effect was higher on the bald region than on the hairy portion. It is

Table 2. Competitive Studies
Assays contained in 1 ml final volume, 3.6 picomoles of testosterone, 0.3 of them tritiated, buffer at the appropiate pH (7.5 and 9.0 respectively for the bald and hairy regions), 0.5 mg of protein from a pool mixture of crude extracts (bald and hairy) from the different patients, and 3.3 picomoles of the steroid competitor under study
Incubation and separation of free and bound testosterone were carried out as described in "Methods"
The amount of testosterone bound by the bald region is stated as 100

	Bald region	Hairy region
Testosterone	100	82
Androstandiol	81	74
Androsterone	78	65
Epiandrosterone	69	62
Estrone	54	62
Estradiol	74	81
Estriol	98	97.8
Progesterone	58	61

worth mentioning the low or nule inhibitory effect shown by the estriol. Significant differences were shown between the androstandiol, androsterone, epiandrosterone group and the estrone, estradiol and estriol as far as the relative inhibitory effect existing on the bald and hairy zones.

4. Progesterone. The inhibitory effect was very important on both tested zones. The progesterone absolute inhibitory power was similar to, or slightly lower, than those of the estrone (Tables 4 and 5).

Discussion

Results described herein seem to indicate the presence of a cytosol testosterone binding protein in the hairy and bald head regions. Location on the supernatant after high speed ultracentrifugation and the activity loss following trypsin digestion, appears to support this view.

The different equilibrium constants, existing on the bald and hairy regions indicate a higher affinity of the binding protein in the bald areas as compared to the hairy zones.

The distinct optimum pH at which the testosterone binding takes place in the bald (7.5) and hairy (9.0) regions, suggest the existence of functional and therefore structural differences between the testosterone binding protein from both regions.

Competition studies also shown significant differences between both regions. There appears to be a greater quantitative inhibitory testosterone binding effect on the bald regions in relation to the steroids being tested. It is worth mentioning that the competitive inhibitory action of the steroids seems to be related to the presence of a ketonic

Table 3. Competitive effect of the Androstandiol, Androsterone and Epiandrosterone group
The assays were carried out as described in Table 2, but the protein binders were tested for each patient

Origin binder		Patient No.									
		1	2	3	4	5	6	7	8	9	10
Testosterone	Bald	100	100	100	100	100	100	100	100	100	100
Testosterone	Hairy	91	82	96	88	93	94	89	82	79	93
Androstandiol	Bald	80	76	69	91	72	75	67	88	74	103
Androstandiol	Hairy	62	70	62	81	84	92	74	87	65	64
Androsterone	Bald	74	61	46	55	42	62	88	41	59	61
Androsterone	Hairy	70	72	39	42	81	42	59	32	49	56
Epiandrosterone	Bald	65	74	81	88	32	36	40	29	52	89
Epiandrosterone	Hairy	52	29	39	86	42	21	15	32	41	64

Table 4. Competitive effect of the Estrone, Estradiol and Estriol group

Origin Binder		Patient No.									
		1	2	3	4	5	6	7	8	9	10
Testosterone	Bald	100	100	100	100	100	100	100	100	100	100
Testosterone	Hairy	91	82	96	88	93	94	89	82	79	93
Estrone	Bald	42	55	61	42	58	31	46	66	74	29
Estrone	Hairy	56	71	44	62	76	49	51	68	69	71
Estradiol	Bald	80	72	76	69	73	39	82	59	69	78
Estradiol	Hairy	84	82	74	81	76	69	84	77	75	80
Estriol	Bald	—	—	—	—	101	93	88	87	74	90
Estriol	Hairy	—	—	—	—	99	96	81	79	76	91

Table 5. Competitive effect of the progesterone

Origin Binder		Patient No.									
		1	2	3	4	5	6	7	8	9	10
Testosterone	Bald	100	100	100	100	100	100	100	100	100	100
Testosterone	Hairy	91	82	96	88	93	94	89	82	79	93
Progesterone	Bald	41	58	69	40	54	59	69	88	67	52
Progesterone	Hairy	61	42	71	59	64	72	78	80	71	42

group in the C_3 position. Furthermore, the addition of hydroxyl groups to the steroid molecule leads to a relative, loss of the competitive activity. Therefore it may be stated that this activity in some way depends on the number of OH groups present in the steroid molecule. In the case of the androgenic metabolites, the epiandrosterone containing a single OH group acts as a more potent competitor than androstandiol which possesses two OH groups. Similarly in the estrogen group, the estrone containing a single OH group is a more active competitor than the estradiol, which has two OH groups. Estriol is even less active.

These differences between the behaviour of the cytosol testosterone binding protein from bald and hairy regions may be explained by a higher binding affinity of that protein for the bald area. A higher affinity may indicate that a higher amount of testosterone per gram of tissue and time unit is being transported inside the cell and therefore a higher activity of the testosterone action may be implied. This higher activity could be produced through a faster rate of testosterone intracellular metabolism or by increased levels of intracellular testosterone [2]. Similar conclusions can be drawn from the testosterone uptake experiments in which the bald skin pieces appear to take up a greater amount of testosterone as compared to the hairy ones [7]. The higher functional activity of the testosterone and its most active metabolite, dehydrotestosterone, on the hair bulb, would lead to bulb exhaustion and atrophy with the expected consequences [3].

Attempts to locate the cytosol binding protein within a specific cell of the pilosebaceous gland are being pursued.

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