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# A Possible Specific Receptor for 3-β-Androstanediol in the Human Sebaceous Gland

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Dihydrotestosterone (DHT) does not seem to be the active specific metabolite of testosterone in hypertrophic sebaceous glands of subjects affected by male pattern baldness (MPB) and several results indicate that probably 3-β-androstanediol (βDIOL) could be an active form of testosterone in those glands. Cytosol and serum from several patients affected by MPB and subjected to hair autotransplantation, was incubated with both βDIOL and 3-α-androstanediol (αDIOL). Binding patterns indicate that αDIOL binds to cytosolic proteins probably due to the contaminating sex hormone binding globulin (SHBG), whereas βDIOL exhibits an atypical binding process in cytosol in the presence of high concentrations of non radioactive βDIOL. This binding increases progressively up to 2 pmol/mg protein at the limit solubility conditions for the non radioactive steroid. This pattern is not observed in serum from the same patients, where the binding of βDIOL is typically restricted to the SHBG. These results strongly suggest the existence of a specific βDIOL-binding protein in the hypertrophic sebaceous glands and explain the lack of specific receptor for DHT in these tissues.

Key words: 3-β-Androstanediol, Male pattern baldness, Sebaceous glands, Cytosolic receptor.

Male pattern baldness (MPB) is a common form of alopecia, with a multifactorial origin, that can be induced by normal levels of androgens in genetically predisposed individuals. The altered androgenic function seems to be localized only in the affected areas of the scalp, which have an elevated production of sebum and progressive hair loss.

Previous studies demonstrated a higher uptake of testosterone (1) and sex hormone binding globulin (SHBG) (2) in biopsies from bald areas when compared with the hairy part of the scalp from the same subject. Also the conversion of testosterone into 5-α reduced metabolites has been shown to be greater in isolated hairs

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from all sites of the scalp of bald men in comparison with hairs from the corresponding sites of non-bald subjects (7). Moreover, the 5-α reductase (5-α-R) activity was maximal in the frontal region (7). It is generally accepted that dihydrotestosterone (DHT) is the active form of testosterone in most tissues and that it is formed in the target cell by means of the 5-α-R. Hence, in androgen-dependent tissues, a specific DHT receptor has been demonstrated to exist. The binding of the DHT-receptor complex to the nuclear chromatin promotes gene expression as a direct response to hormone stimuli. The extent of hormone stimulation correlates with the number of androgen receptor binding sites (6).

If DHT is the active form of testosterone in the sebaceous gland, then in bald areas a high density of DHT receptors should be expected to exist, but in a previous report (2) we demonstrated the absence of a specific DHT receptor in scalp biopsies of bald subjects. In addition, a clear presence of SHBG in seborrhoeic-alopecic areas contrasted to its absence in the hairy skin of the same subject.

DHT can be converted into α- and β-androstanediols (αDIOL, βDIOL) by means of the 3-α,β-hydroxysteroid oxoreductase (3-α,βHO) and a few studies (3, 9, 10) suggest that βDIOL can play a role in the control of sebum production. Therefore, if βDIOL is assumed to be the active form of testosterone in the sebaceous gland, then a specific receptor should be present in seborrhoeic tissues. In this study results concerning this hypothesis are presented.

## Materials and Methods

*Specimens.* — Scalp biopsies were obtained from patients affected by MPB who were subjected to hair autotransplantation. Pieces were obtained with an Orentreich punch (n = 4.25). Tissues were rinsed with saline and kept frozen at −70 °C until processed (not longer than three months). Their serum was also used in binding experiments.

*Chemicals.* — 5-α-(1α,2α(n)$^3$H)-androstane-3-β, 17β-diol (46 Ci/mmol) ($^3$HβDIOL) and 5-α-(1α,2α(n)$^3$H)-androstane-3-α, 17-β-diol (59 Ci/mmol) ($^3$HαDIOL) were purchased from the Radiochemical Centre. Unlabelled DHT and diols were obtained from Sigma. The purity of the labelled steroids was checked by thin-layer chromatography. All the other reagents were of analytical grade.

*Binding assay.* — Binding of androstanediols was estimated by saturation analysis using the method of WILSON and FRENCH (11) modified as described previously (2). Specific conditions are as follows. The cytosol obtained from skin biopsies was used undiluted immediately. In serum experiments, native serum was diluted 1:8 in the working buffer. Protein was determined by the method of Lowry and the range of protein concentrations of the samples was 1.5-2.5 mg protein/ml for cytosol and 8-10 mg protein/ml for serum. Sample volumes of 500 µl were incubated in duplicate with the labelled steroid in the presence or absence of a 100 or 1.000 fold excess of unlabelled steroid for 18 h at 4 °C. Steroids were dissolved in ethanol and the final solvent concentration in the incubation medium was not higher than 4 %. Bound steroid was recovered by adding 500 µl of a charcoal suspension and quantified in a Packard Tri-Carb 3255 spectrometer by liquid scintillation.

In some experiments βDIOL binding in cytosol was also measured by gel filtration in 10 × 0.5 cm Sephadex G-25 columns. Briefly, cytosol was incubated with $^3$HβDIOL in the presence and absence of βDIOL 2.5µM, 25 µM and DHT 25 µM, as described above. 250 µl of the incubation mixture were eluted in Tris-EDTA buffer and collected stepwise in about

70 µl fractions. Bound radioactivity was estimated as above.

## Results

The results obtained displacing the radioactive steroid by the usual 1.000 fold non radioactive androstanediol are as follows. Figure 1 shows the specific $^3$HαDIOL binding to cytosol in the bald area from 5 patients and the corresponding Scatchard plot. A linear relationship indicates the presence of only a single class of high affinity binding sites, with a $K_d$ value of $6.97 \times 10^{-9}$ M and a maximum number of binding sites n = 15 fmol/mg protein. In serum, values for $K_d$ and maximum binding sites were, respectively, $12.1 \times 10^{-9}$ M and 55 fmol/mg protein. This corresponds to 3.85 pmol/ml of serum.

In the same conditions, $^3$HβDIOL binding to cytosol in the bald area from 4 patients shows a non-saturated curve with

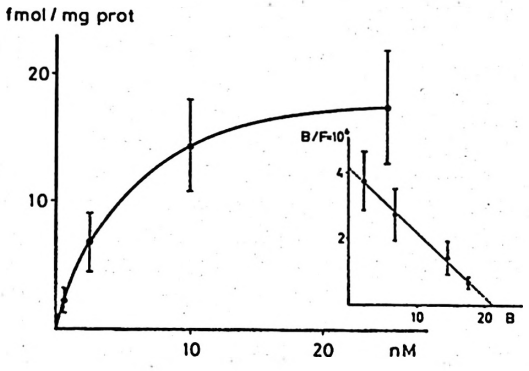


Fig. 1. *Specific $^3$H-αDIOL binding in bald scalp cytosol.*
Mean values for five subjects. Abscissa, molar concentration for the radioactive steroid; Ordinate, specifically bound steroid. Insert: Scatchard plot of the same results. Abscissa, specific binding in fmol/mg prot.; Ordinate, bound/free ratio for the radioactive steroid. Specific binding was obtained by displacement with a 1,000 fold concentration of non-radioactive steroid.

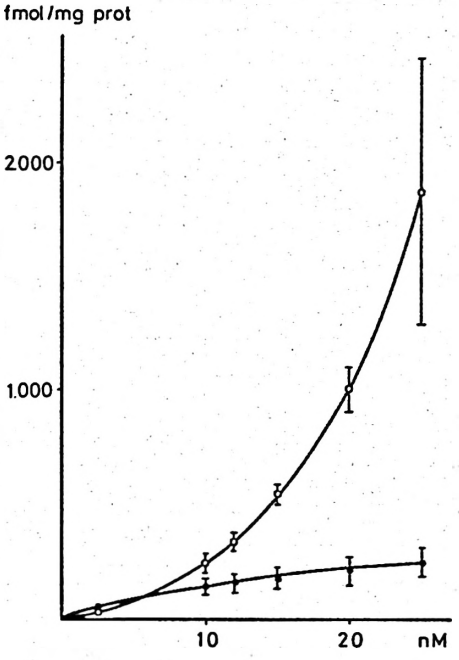


Fig. 2. *Specific $^3$H-βDIOL binding in bald scalp cytosol.*
Mean values for four subjects. Abscissa, molar concentration for the radioactive steroid; Ordinate, radioactive bound steroid. Filled circles, binding in absence of non-radioactive steroid; open circles, binding in the presence of a 1,000 fold non-radioactive steroid.

a potentiation of the binding in the presence of the unlabelled steroid (fig. 2). In serum, the kinetics is a typical saturation process, as well as in the $^3$HαDIOL experiments, with a $K_d = 3.13 \times 10^{-9}$ M and maximum binding sites n = 80 fmol/mg protein which corresponds to 5.6 pmol/ml of serum.

In contrast to these results, when $^3$HβDIOL binding to bald scalp cytosol was displaced with a 100 fold excess of unlabelled steroid, a saturation curve was obtained (fig. 3). The Scatchard analysis of the data indicates the presence of only a single class of high affinity binding sites, with a $K_d$ value of $5.5 \times 10^{-9}$ M and a

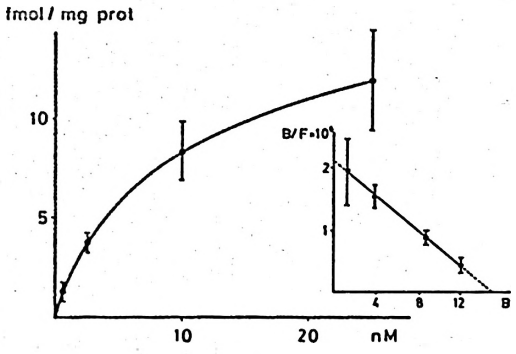


Fig. 3. *Specific $^3$H-βDIOL binding in bald scalp cytosol.*
Mean values for five subjects. Same explanations as for figure 1, except that the specific binding was attained by displacing the radioactive steroid with a 100 fold concentration of non-radioactive steroid.

maximum number of binding sites n = 21.3 fmol/mg of protein.

In cytosol, when $^3$HβDIOL was displaced with 1.000 fold DHT, the binding pattern is similar to that obtained with non radioactive βDIOL at 100 fold concentrations (fig. 4). In this case, a $K_d$ value of $5.8 \times 10^{-9}$ M and a maximum number of binding sites n = 29.44 fmol/mg protein, were calculated.

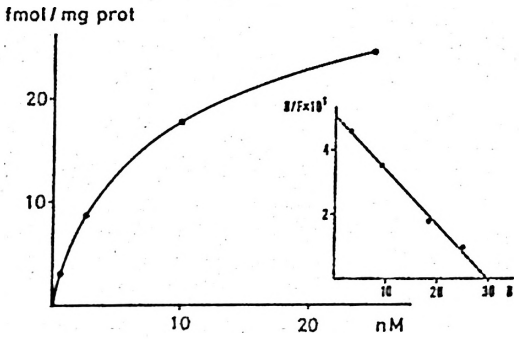


Fig. 4. *$^3$H-βDIOL binding in bald scalp cytosol.*
Same explanations as for figure 1, except that the radioactive steroid was displaced by non radioactive DHT at 1,000 fold concentrations.

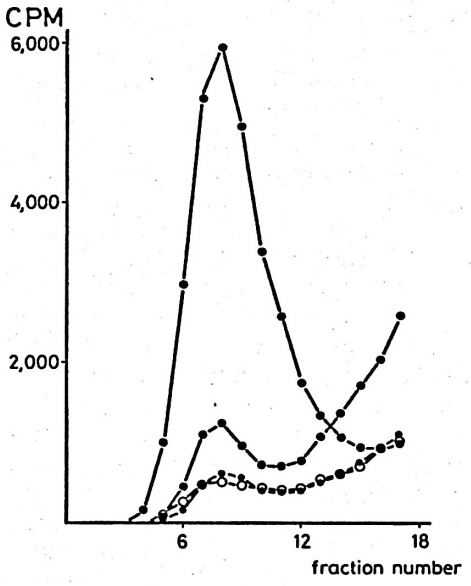


Fig. 5. *Protein-bound radioactivity in bald scalp cytosol.*
Cytosol aliquots were incubated with $^3$H-βDIOL 25 nM, alone (o) or with βDIOL 2.5 μM (*), βDIOL 25 μM (●) or DHT 25 μM (o). Protein peak at fraction number 8.

Figure 5 shows the results obtained by gel-filtration. It is clear that bound radioactivity elutes with the protein fractions. Moreover, when $^3$HβDIOL is incubated in the presence of 1,000 fold non radioactive βDIOL, the bound radioactivity eluted is greater than that obtained in the fractions incubated with $^3$HβDIOL alone. Displacement of the radioactive binding is clear and similar when samples are incubated in the presence of 100 fold βDIOL and 1,000 fold DHT. In both cases, the bound radioactivity is lower that that obtained with $^3$HβDIOL alone.

## Discussion

The etiology of MPB and other seborrhoeic states is not clear and a better

knowledge of the androgenic receptors as well as the local metabolism of testosterone can help to elucidate this kind of processes.

The results presented here are of interest because the role of DHT in the sebaceous gland is controversial. Several reports suggest that DHT is not the active form of testosterone in the sebaceous gland (8). In this sense, the absence of a specific DHT receptor in cytosolic fraction of the hypertrophyc sebaceous gland has been demonstrated (2). On the other hand, some authors have suggested that βDIOL is likely to be the active form of testosterone in the sebaceous gland (9, 10). In fact there is an increased uptake of testosterone (1) and SHBG (2) in the hypertrophic sebaceous gland, and a conversion of testosterone into DHT and finally into βDIOL. Hence, a specific role of βDIOL in the control of sebum secretion can be suspected.

The model used by us contains several types of tissues, but sebaceous glands are well known to be present to maximum extent. In fact, histological preparations from the present samples (not shown here) demonstrate that hypertrophic sebaceous glands account at least for 85 % of the tissue.

In previous results (2) plasma contamination in our samples was calculated to range up to 1 %. If a mean protein concentration of 2 mg/ml of cytosol is assumed then an αDIOL binding of about 19 fmol/mg cytosolic protein can be attained from the SHBG plasma contamination, which is roughly the value obtained in the present experiments. Therefore, the αDIOL binding in scalp biopsies probably is merely due to plasma contamination.

On the contrary, the binding pattern of βDIOL is unique among all the steroids previously described. When a 100 fold concentration of non radioactive βDIOL is used, βDIOL probably binds in the cytosolic fractions only to the contaminating SHBG. Applying the same calculations as for αDIOL, a binding of about 28 fmol/mg of cytosolic protein should be expected, which corresponds satisfactorily with the present value of 21.3. At higher concentrations of non radioactive βDIOL (1,000 fold) the binding of $^{3}$HβDIOL increases parallelly to its concentration, no saturation of the binding curve being attained with βDIOL concentrations over 25 μM when the steroid is not soluble in the medium.

The increase of $^{3}$HβDIOL binding cannot be due to the formation of steroid precipitates or to a lesser adsorption to the charcoal, since this increase is not observed in the absence of cytosol (blanks) or to the use of serum instead of cytosol. In addition, DHT gives the same results as those obtained with βDIOL at 100 fold concentrations in cytosol or when serum samples are used but DHT is not able to reproduce the increase of $^{3}$HβDIOL binding. On the other hand, the excellence of the charcoal method is demonstrated by the fact that gel-filtration gives the same results. Thus, the conclusion was reached that βDIOL at low concentrations binds in a manner that is only due to the SHBG coupling.

The binding pattern of $^{3}$HβDIOL to cytosol when a 1,000 fold βDIOL is used is more difficult to explain. Positive cooperation seems to take place, but it cannot be evaluated by the usual Hill equations as saturation is not achieved. In addition, the high binding increase seems to be too great for this interpretation. Other explanations can be considered: high amounts of βDIOL might induce a disaggregation of the βDIOL proteic ligand, thus activating the binding capacity of the system as has been described for the DHT receptor (4); furthermore at these high concentrations, βDIOL from molecular aggregations might lead to a binding pattern dissimilar to that obtained with low concentrations; in this case such pattern should be also observed in other pro-

teins like serum. When our paper was reviewed, MURPHY and HYDE (5) published their results on the enhancement of the R-1881 binding by several steroids, including βDIOL, in placenta homogenates. They speculate that some steroids can stabilize the R-1881-binding protein, thus facilitating its binding pattern.

It is concluded that in cytosol a specific protein exists which binds βDIOL in an atypical manner. The possibility that this protein could really be a specific receptor for βDIOL and that it could be related to some cellular mediators involved in the action mechanism of steroidal hormones remains to be elucidated. But, the existence of a specific receptor for βDIOL could explain previous findings carried out in the sebaceous gland, namely: the greater uptake of the testosterone-SHBG complex, the increase of the 5-α-R activity and finally the conversion of DHT into βDIOL. This opens up new possibilities in understanding the testosterone action mechanism and for the development of new antiandrogenic drugs, as well as specific inhibitors of the 3βHO system.

*Acknowledgements*

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## Resumen

Se estudia, mediante las técnicas de desplazamiento del esteroide radioactivo con esteroide no radioactivo, la fijación de 3-β-androstanodiol (βDIOL) y 3-α-androstanodiol (αDIOL) en citosol de cuero cabelludo alopécico y suero de pacientes afectos de alopecia seborreica masculina (ASM). La fijación del αDIOL en el citosol presenta un patrón típico saturante, con una sola clase de sitios de fijación, y es probablemente debida únicamente a la presencia contaminante de globulina transportadora de esteroides (SHBG). El βDIOL presenta un patrón de fijación atípico a las proteínas citosólicas, en presencia de altas concentraciones de βDIOL no radioactivo. La fijación aumenta progresivamente hasta 2 pmol/mg de proteína en las condiciones límite de solubilidad del esteroide no radioactivo. Este fenómeno no se observa en el suero del mismo sujeto, donde la fijación del βDIOL es la típica que se produce en la SHBG. Estos resultados sugieren la existencia de una proteína fijadora del βDIOL en la glándula sebácea hipertrófica que podría corresponder a un receptor específico para dicho esteroide.

Palabras clave: 3-β-androstanodiol, Alopecia seborreica masculina, Glándula sebácea, Receptor citosólico.

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