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Guanylate Cyclase Activity in Male Pattern Baldness. Stimulating Effect of 3-β-Androstanediol

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Guanylate cyclase (GC) activity was measured in the cytosolic fraction of sebaceous gland-enriched skin samples obtained from alopecic and non-alopecic areas of subjects affected by male pattern baldness. GC activity was significantly higher in alopecic scalp (7.13 ± 2.7 pmol/min/mg prot; n = 22) than in non-alopecic samples (3.91 ± 0,48 pmol/min/mg prot; n = 8). 3-β-androstanediol was able to increase GC activity up to 15 % when added to the incubation medium (1×10^{-5} M). The regional differences observed should be due to the larger size of the sebaceous glands in alopecic areas. The higher production of dihydrotestosterone and 3-β-androstanediol in alopecic areas may also contribute to determine the level of GC in scalp skin. The role of second messenger systems in sebaceous glands to understand certain aspects of the action mechanism of androgens is discussed.

Key words: Male pattern baldness, Guanylate cyclase, 3-β-Androstanediol.

It is widely accepted that androgens exert their action in target organs by coupling with a soluble specific protein which carries the steroid to the cell nucleus to initiate the specific hormonal response (2).

The mechanisms by which testosterone reaches the cytoplasm and its biotransformation in target cells are of the utmost importance; it is well known that most androgenic actions are in fact carried out by the specific metabolite dihydrotestosterone (DHT) but not by testosterone (12). In periferal tissues oxo-reductases convert testosterone to androstenedione (AEDIONE) (17-β-hydroxysteroid oxoreductase) and DHT in 3-α (β)-androstane-

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diol (ADIOL) (3-α (β)-hydroxysteroid oxoreductase) or androstanedione (AADIONE) (17-β-hydroxysteroid oxoreductase). A complete knowledge of the role of these metabolites has not been reached but, at any rate, they are involved in the complex steroidal biotransformation pathway and contribute to regulating the cellular levels of some androgens (4, 10).

Sebaceous gland activity is recognized to be, at least in part, under androgenic control and many studies have been published concerning the androgen metabolism and the mechanism of androgen activity in this tissue (8). That human alopecic tissue presents higher activity of 3-α (β)-hydroxysteroid oxoreductase than the hairy tissue of the same subject and that the opposite is true for 17-β-hydroxysteroid oxoreductase, has been previously reported (10). In addition, ADIOL binds to the cytosolic fraction of sebaceous gland-enriched alopecic tissue in an unusual manner and it was concluded that this metabolite may represent an active form of testosterone in this gland (3).

Cyclic nucleotides play an important role as second messengers in many tissues and some reports indicate that sexual steroids regulate the activity of adenylate and guanylate cyclases (1, 11). This relationship has not been previously described in human scalp sebaceous glands and it seems worthwhile to study the possible involvement of guanylate cyclase (GC) in sebaceous hypertrophy. In the present paper we describe the preliminary results on the activity of GC in alopecic and non-alopecic tissues and the stimulating effect of ADIOL on this enzyme.

Materials and Methods

Scalp biopsies of the alopecic frontoparietal region were obtained with the aid of an Orentreich trocar of 4.5 mm diameter from individuals submitted to hair auto-transplantation. In some subjects, pieces from the hairy occipital donor area were also obtained.

Samples were rinsed with saline and dissected to obtain the segment corresponding to the sebaceous glands. Tissues were minced with scissors and homogenated in an Omni-Mixer blender (Sorvall) using potassium phosphate buffer 0.1×10^{-3} M, pH 6.6 (1/10; W/V). During the homogenization process, the temperature was kept cool with crushed ice. Good performances were obtained with 4 homogenization cycles (30 s each, and with a 30 s pause between cycles) at the maximal speed of the system. Homomogenates were filtered through a gauze to eliminate gross particles and centrifuged 60 min at 105,000 x *g* to obtain the cytosol. The floating lipid layer was carefully removed and discarded.

Guanylate cyclase assay: The method of PALMER *et al.* has been used (9). Briefly, cytosol aliquots (40 µl) were incubated in tubes containing 120 µl of the mixture: phosphocreatine (3×10^{-3} M), creatinphosphokinase (15 µg), bovine serum albumin (15 µg), GTP (0.5×10^{-3} M), β-mercaptoethanol (2×10^{-3} M), caffeine (25×10^{-3} M), magnesium chloride (3×10^{-3} M) and calcium chloride (1×10^{-3} M). The tubes were placed in a thermostated shaking incubator at 37 °C for 10 min and the reaction was stopped by adding 10 µl of 0.2×10^{-3} M EDTA and heating the tubes in boiling water for 3 min. The tubes were rapidly cooled with crushed ice and centrifuged at 5,000 x *g* for 15 min. Cyclic GMP (cGMP) was measured in 100 µl aliquots of the supernatant with a commercial kit (Amersham, U.K.) using ^{3}H-cGMP as standard. Radioactivity measurements were carried out in a Packard Tri-Carb 3255 spectrometer, using Unisolve (Koch Light, U.K.) as scintillation liquid.

To study the influence of ADIOL on the GC activity, this steroid was added to the incubation medium at several concentrations up to 10×10^{-6} M.

Protein was calculated by the method of Lowry.

Results

Kinetic studies: In scalp homogenates coming from the alopecic areas, since the amount of tissue was large enough, complete kinetic analyses were performed. However it was very difficult (for evident ethical reasons) to obtain samples from the occipital donor areas; thus only comparative studies were performed with those specimens.

Guanylate cyclase activity has been proved to be linear during the first 30 min of the reaction, so that a 10 min incubation period was used for routine experiments. The amount of cGMP formed also correlated well with the amount of protein up to 100 μg in the total volume of the reaction. Kinetic parameters were calculated by varying the GTP concentrations from 0.05 to 1.5 x 10^{-6} M. Equilibrium occurs around 0.5 x 10^{-6} M of GTP. Complete kinetic studies were developed in ten patients in the alopecic frontal area; mean values obtained were: Vmax = 14 ± 4.2 pmol/min/mg protein and Km = 59 ± 11 x 10^{-5} M.

Table I. *Guanilate cyclase activity in scalp skin.* Skin biopsies from alopecic and non-alopecic areas were studied. Cytosol was incubated with GTP 0.5 × 10^{-6} M for 10 min.

	pmol/min/mg prot.	pmol/min/g tissue
Bald scalp		
all cases (n = 22)	7.14±2.70*	93.7±35.5**
paired samples (n = 8)	7.81±3.36*	87.8±37.8***
Hairy scalp		
paired samples (n = 8)	3.91±0.48	59.5±7.2

* $p < 0.005$; ** $p < 0.01$; *** $p < 0.05$, comparing the values obtained in bald scalp with those of non-bald scalp.

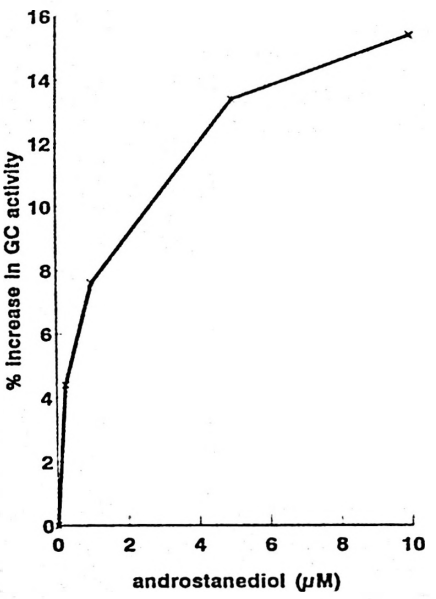

Fig. 1. *Effect of 3-β-androstanediol on the guanylate cyclase (GC) activity.* Bald scalp cytosol was incubated with different amounts of steroid. The increase of GC activity is expressed as a percentage of the basal activity in absence of androstanediol.

Comparative regional studies: GTP at 0.5 x 10^{-6} M and 10 min incubation time were used in these experiments. Specimens were obtained from both bald and non-bald areas in eight patients. Fourteen more alopecic specimens from different patients were also included. GC activity per mg of total cytosolic protein was higher in bald scalp samples. The differences were highly significant both by comparing the results of the 22 alopecic samples with the eight non-alopecic specimens, and by comparing the eight paired samples (table I).

If the GC activity is calculated per gram of tissue, similar results were obtained.

The addition of ADIOL to the incubation medium increases GC activity. In

five experiments carried out with alopecic samples the steroid at 10 x 10^{-6} M increased the enzyme activity by 14 ± 2 %. Figure 1 depicts one of these experiments.

Discussion

The important role of adenylate and guanylate cyclases in many tissues is well known. Thus, it is well accepted that the balance between both enzymes is of the outmost importance to maintain a normal turnover in epidermal cells. An increase in GC activity would determine an excess of epidermal layers with abnormal exfoliative processes as happens in psoriasis (5). Data about GC activity in skin appendages (7), such as the sebaceous gland, are scarce and a comparative study of this activity in bald and non-bald scalp has not been reported. Our results demonstrate a higher GC activity in samples coming from bald skin than in those coming from hairy skin. Histologic control of the specimens demonstrated that in samples coming from alopecic areas, the sebaceous glands accounted for more than 80 % of the whole biopsy, whereas in the non-bald tissues the size of the sebaceous glands was much smaller and accounted for about 15-20 % of the tissue. Consequently the higher GC activity in alopecic skin correlates well with the greater size of the sebaceous glands in these areas and points out the possible role of cGMP in the sebaceous activity.

These regional differences agree with previous findings on testosterone metabolism. Testosterone is converted into DHT and androstanediols predominantly in the bald scalp, whereas conversion of testosterone into AEDIONE and DHT into AADIONE is higher in the hairy scalp (10). GC activity seems to be partially regulated by androgens and other steroidal compounds, since these substances stimulate the enzyme in several tissues (1, 11).

The results presented here indicate that ADIOL is able to stimulate GC activity but to a lesser extent than does DHT in other tissues (11). Since ADIOL and DHT predominate in the alopecic tissue, this could justify the higher activity of GC and the larger size of the sebaceous glands in alopecic areas. Whether the sebaceous hypertrophy is the consequence or the cause of a specific androgenic pattern and of the increased GC activity remains to be elucidated.

Sebaceous glands hypertrophy is associated with several skin disorders: seborrhoea, male pattern baldness, acne, etc. (8). Ethiopathogenic studies on such disorders have focused on several factors, i.e. androgen metabolism, but the involvement of the GC system has not been considered. Second messenger systems may represent an important factor in understanding not only the normal pattern of the androgenic response but also in explaining certain disorders in androgen target tissues. Some of the cellular events derived from the androgen administration cannot be satisfactorily explained by the formation of the androgen-protein complex above mentioned. In addition, the administration of specific androgen-receptor blockers are unable to antagonize all the cellular androgen effects. Therefore it must be assumed that androgens can exert some of their biological effects by mechanisms other than the activation of specific cytosolic proteins (6). One of these mechanisms would be the activation of specific nucleotide cyclases, increasing the amounts of cAMP and cGMP. Thus, the effects of androgens can be elicited by at least two mechanisms: a direct nuclear action on the nucleus by the androgen-receptor complex and an indirect mediated effect by the second messenger mechanisms (cGMP and cAMP).

If the excess of GC activity contributes to the ethiopathogeny of male pattern baldness as well as other androgenic disturbances, then a novel approach for the

therapeutic management of such states can be considered.

Resumen

Se valora la actividad guanilato ciclasa (GC) en fracción soluble de glándula sebácea de muestras cutáneas procedentes de zonas alopécicas y no alopécicas de pacientes afectos de alopecia seborreica masculina. Dicha actividad es superior en la zona alopécica (7,13 ± 2,7 pmol/min/mg proteína; n = 22) que en la no alopécica (3,91 ± 0,48 pmol/min/mg proteína; n = 8). La adición de 3-β-androstanodiol (1 x 10^{-5}) al medio de incubación incrementa la actividad GC en un 15 %. Las diferencias observadas en las dos zonas cutáneas pueden ser debidas al mayor tamaño de las glándulas sebáceas en las zonas alopécicas. La mayor producción de dihidrotestosterona y androstanodiol en el tejido alopécico puede contribuir a determinar el nivel de GC en la hipertrofia sebácea. Se discute la importancia de este segundo mensajero en la secreción sebácea y en el mecanismo de acción de los andrógenos.

Palabras clave: Alopecia androgenética, Guanilato-ciclasa, 3-β-androstanodiol

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