ADENYL CYCLASE IN HUMAN HAIR FOLLICLES: ITS INHIBITION BY DIHYDROTESTOSTERONE

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Received October 6, 1970

SUMMARY: Particulate fractions obtained from human scalp hair follicles contained adenyl cyclase activity which was activated by estrone and inhibited by dihydrotestosterone (5α-androstan-17β-ol-3-one).* The marked inhibition of this enzyme activity by dihydrotestosterone but not by testosterone suggests that the former is the tissue-active androgen. We then speculate that the initial step in the development of pattern baldness is controlled by the intracellular level of dihydrotestosterone rather than testosterone as mediated by adenyl cyclase (and cyclic AMP).

It has been well established that adenosine-3', 5'-phosphate (cyclic AMP) is the common mediator for various hormones acting on their respective target tissues (1). The intracellular level of cyclic AMP appears to be controlled by adenyl cyclase (the synthetic enzyme) rather than by the specific phosphodiesterase (degradative enzyme) since, in most of these cases, the hormones show no effect on the latter.

The adenyl cyclase system has been found to occur in nearly all mammalian tissues, however, its occurrence in mammalian skin and

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*The following trivial names are used in this text: dihydrotestosterone = 5α-androstan-17β-ol-3-one; androstenedione = 4-androstene-3, 17-dione; DHA = dehydroepiandrosterone = 5 - androsten-3β-ol-17-one; estrone = 1, 3, 5 (10)-estratrien-3-ol-17-one; estradiol - 17β = 1, 3, 5 (10)-estratrien-3, 17β-diol; progesterone = 4 - pregnen-3, 20-dione; and pregnenolone = 5-pregnen-3β-ol-20-one.

appendages has not yet been reported. Clinical evidence indicates that the skin appendages are sensitive target tissues of sex hormones (2). Terminal body hairs appear at puberty (their distribution represents a secondary sex characteristic) and the common (pattern) baldness occurs only in adolescence. We investigated in our present study the role of adenyl cyclase in human scalp hair follicles as a common mediator of sex hormones.

The scalp hair follicles were obtained fresh from 3 adult Japanese males by plucking scalp hairs. The growing and resting hair follicles were easily identified by gross observation (3). One hundred twenty growing hair follicles were pooled and homogenized in 3 ml of the isolating medium consisting of 0.25 M sucrose, 0.2 M tris-HCl buffer, 1 mM EDTA and 1 mM $MgCl_2$. The homogenate was centrifuged at 8,000 g for 10 min at 4° C and the precipitate washed twice with the isolating medium without EDTA and Mg^{++}. At the final washing step, samples of 100 µl of pellet suspension were centrifuged in microcentrifuge tubes of 3 mm inner diameter so that each tube contained a final pellet equivalent of 4 original growing hair follicles. The adenyl cyclase activity of the pellet was assayed by the addition of 10 µl of assay reagent mixture which consisted of 4 mM adenosine triphosphate (ATP), 40 µc/ml reagent mixture ATP-8-^{14}C (New England Nuclear), 20 mM theophylline, 10 mM NaF, 5 mM caffeine, 5 mM 2-mercaptoethanol, 5 mM $MgCl_2$, 0.05% bovine plasma albumin and 40 mM tris-HCl buffer at pH 7.4. The mixture was generally incubated for 1 hour at 37° C and the reaction stopped by boiling for 3 min immediately after the addition of carriers consisting of 45 µg cyclic AMP, 12.5 µg each of adenine and adenosine, and 25 µg each of ATP and AMP in a total volume

of 10 μl. The cyclic AMP-^{14}C was isolated on a cellulose layer (Brinkmann Instruments, Inc., Polygram) with 4 different solvent systems as described by Tao and Lipmann (4). The solvent system, 1 M NH_4OAc:95% ethanol (30:75, V/V), yielded consistent and reproducible results and was routinely used. The other 3 systems were used for identification (4). The spot corresponding to cyclic AMP was cut out and immersed in 10 ml of counting medium (Omnifluor 4 grams in 1 liter toluene) for radioactivity determination in a liquid scintillation spectrometer. An enzyme blank (with the boiled pellet) and a reagent blank (without the addition of enzyme) were always run simultaneously. Protein was determined by the method of Lowry et al. (5). The spot corresponding to cyclic AMP was further subjected to degradation by cyclic AMP phosphodiesterase prepared from rabbit brain by the procedure of Drummond and Perrot-Yee (6). The degradation product was identified as 5'-AMP by thin layer chromatography (4).

Cyclic AMP phosphodiesterase activity was assayed with a system essentially similar to that for adenyl cyclase assay except for the substitution of cyclic AMP-^{3}H for ATP-^{14}C. The inhibitors such as theophylline, NaF and caffeine were also omitted when base values of the phosphodiesterase activity were determined. After incubation, the reaction was stopped by boiling for 3 min and the mixture subjected to the same thin layer chromatography procedure as for the routine adenyl cyclase activity. The spots corresponding to cyclic AMP, AMP, adenosine and adenine were cut out and eluted with 1 ml of 10 mM phosphate buffer, pH 7.4. The radioactivity of the eluate (250 μl) was counted in 10 ml of Omnifluor-toluene counting medium to which 0.5 ml of Biosolve (Beckman) was added.

Adenyl cyclase activity of the hair follicle was proportional to both

Table I

Effects of activators on adenyl cyclase activity of hair follicles

	Complete assay system	100%
Minus:	Magnesium chloride	56%
	Mercaptoethanol	55%
	Mg^{++}, NaF, theophylline > Caffeine and mercaptoethanol	16%
Plus:	Magnesium chloride (20 mM)	65%
	Mercaptoethanol (20 mM)	59%
	Epinephrine	108%

The enzyme activity assayed in the complete system as described in the text is taken as 100%. Each figure represents an average of 8 determinations.

the amount of particulate fraction used and the time of incubation up to at least 1 hour. Like other mammalian adenyl cyclases, the follicle adenyl cyclase requires the presence of Mg^{++} and caffeine, NaF and theophylline for maximal activity (Table 1). The assay of cyclic AMP phosphodiesterase indicated that the addition of caffeine, NaF and theophylline increases cyclic AMP production by inhibiting the phosphodiesterase.

In Table 2, the effects of various sex hormones on adenyl cyclase of the hair follicles are summarized. Undoubtedly this enzyme is dependent on a sex hormone. The marked inhibition of the enzyme by dihydrotestosterone but not by testosterone strongly supports the view that the former is the tissue active male hormone in hair follicles. Recent studies by Bruchovsky and Wilson (7) strongly suggest that dihydrotestosterone is the active androgen in the target organs. A marked increase in adenyl cyclase activity is observed upon addition of estrone; a fact that coincides with the clinical observation that scalp hair growth is influenced by female hormones (2).

Table II

Effects of sex hormones on adenyl cyclase activities of hair follicles

Subject / Hormone Added	#A Exp. 1	2	3	#B Exp. 1	2	#C Exp. 1	Ave. Inc. (+) or Dec. (-)
None (base value)	1.14	1.14	1.70	1.48	1.18	1.12	100%
Testosterone	1.06	1.14	1.17	1.55	0.80	1.24	91%
Dihydrotestosterone	0.40	0.52	0.83	0.71	0.52	0.80	49%
DHA	-	-	1.56	-	-	-	-
Androstenedione	-	-	1.45	-	-	-	-
Estrone	2.41	1.84	-	2.25	1.85	2.33	177%
Estradiol-17β	-	1.06	-	-	1.31	-	-
Pregnenolone	-	1.10	-	-	1.09	-	-
Progesterone	-	1.04	-	-	1.21	-	-

The activity is expressed as mμmoles/hr/mg protein. Each figure is an average of 5 to 8 determinations with S.E. of means being 3 to 8%. Concentration of each hormone added was 100 μg/ml reagent mixture.

Table 3 shows the response of the adenyl cyclase of hair follicles to different concentrations of dihydrotestosterone and estrone. It appears that a concentration of about 0.1 μg/ml reagent mixture gives maximal activation. Both hormones show, however, no effect on cyclic AMP phosphodiesterase activities at this concentration. Thus, the data presented represent enzymatic evidence for the hormonal control of hair follicle metabolism.

Inhibition of adenyl cyclase of hair follicles by dihydrotestosterone is a particularly significant finding, since the development of pattern baldness

Table III

Effects of dihydrotestosterone and estrone on adenyl cyclase activities at different concentrations

Conc. (μg/ml Rgt.) / Hormone	0	0.01	0.1	1	10	100
Dihydrotestosterone	1.42	1.22	0.77	0.72	0.68	0.70
Estrone		1.33	1.98	2.46	2.28	2.19

The activity is expressed as mμmoles/hr/mg protein. Each figure is an average of 5 to 8 determinations.

(which clinically is a transformation of large stout terminal hairs to short thin vellus hairs) has been believed to be due to the direct action of elevated testosterone levels. We now propose the following molecular mechanism for the genesis of this type of baldness: testosterone is converted to dihydrotestosterone (8), a tissue active androgen, which inhibits adenyl cyclase in the follicles and causes a lowering of the intracellular cyclic-AMP level. This conceivably inhibits energy production (1) and protein (enzyme) synthesis (1), and may cause premature termination of the growing stage of the hair follicles. Years of repetition of these processes probably transform the terminal type to the vellus type follicles. Although we have not yet conclusively proved the hypothesis, the initial role played by adenyl cyclase in the hair follicles appears to be significant in the balding process of the human scalp.

Acknowledgement

This work was supported in part by Grant FR 00163 of the National Institutes of Health and by funds from Grant AM 08445. Publication No. 486 from the Oregon Regional Primate Research Center.

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