

CYCLIC ADENOSINE MONOPHOSPHATE, ADENYL CYCLASE
AND CYCLIC NUCLEOTIDE PHOSPHODIESTERASE
IN PSORIATIC EPIDERMIS

With a Comment on Urinary cAMP

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Abstract. The concentration of cyclic adenosine 3',5'-monophosphate (cAMP) and the activity of adeny cyclase and cyclic nucleotide phosphodiesterase were measured in the plaque and healthy epidermis of psoriatic persons and compared with the values for normal human epidermis. Daily urinary excretion of cAMP was also measured in psoriatic and control subjects. The concentration of cAMP was found to be slightly elevated in the psoriatic plaque when compared with the healthy areas of the same person. There was no difference in the urinary cAMP excretion in normal and psoriatic persons. Adenyl cyclase activity (with or without sodium fluoride) was slightly lower, and cyclic nucleotide phosphodiesterase considerably lower, in psoriatic plaque than in healthy epidermis. Contrary to earlier reports, the response of adenyl cyclase to sodium fluoride was found to be similar in normal and psoriatic epidermis.

Cyclic adenosine 3',5'-monophosphate (cAMP) is known to regulate a number of intracellular metabolic pathways, e.g. glycolysis and lipolysis, by activating phosphorylase and lipases. It is also thought to play a role in the regulation of cell division (8). cAMP is produced from ATP by an enzyme, adenyl cyclase, situated in the plasma membrane. The enzyme of several tissues is known to be activated by adrenalin, other catecholamines and sodium fluoride (20).

Mier & Urselmann (13) demonstrated the presence of adenyl cyclase in whole skin homogenate of the guinea-pig and found that it was activated by fluoride but not by adrenalin. Using similar techniques, the same investigators did not find any differences in adenyl cyclase activity between homogenates of normal and atopic human skin (14). Vorhees et al. (23) and Duell et al. (5) found

that an adenyl cyclase preparation obtained from the epidermis of a newborn rat was stimulated by isoproterenol, while adrenalin had hardly any stimulatory effect. On the other hand, Bronstadt et al. (3) showed that adrenalin augments the formation of cAMP in hamster skin in vitro. They found no formation of this metabolite in skin from which the epidermis had been scraped, and concluded that cAMP is formed only in the epidermis. Rao et al. (18), however, found that cultured human skin fibroblasts contained adenyl cyclase with properties resembling those of other tissues and the capacity to form cAMP. The concentration of this nucleotide in tissues is regulated not only by the rate of synthesis, but also by the rate of breakdown. The enzyme that cleaves cAMP is cyclic nucleotide phosphodiesterase. Phosphodiesterase in mouse skin homogenate was detected by Mier & Urselmann (15) and enzymes with similar characteristics have been found in other mammalian tissues. In a later study by the same authors (7) no difference was observed in the activity of phosphodiesterase in atopic and normal human whole-skin homogenates.

Psoriasis has been suggested to originate from a defect in the adenyl cyclase-cAMP cascade (23). The psoriatic skin lesion is characterized by a 10- to 12-fold increase in the rate of cell division, with low phosphorylase activity and glycogen accumulation in the epidermis (1). Theoretically, these changes could be due to a low concentration of cAMP in the epidermal cells, since an increase in cAMP level retards epidermal cell divi-

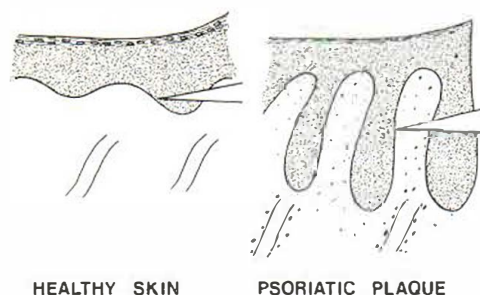


Fig. 1

sion and initiates glycogen breakdown. This theory is supported by the experimental evidence that adrenalin (4), isoproterenol, and cAMP are known to inhibit epidermal cell proliferation (16). The effect of the hormones is simulated and potentiated by theophylline (25), which inhibits phosphodiesterase and thus prevents degradation of cAMP. It is also mimicked by dibutyryl cAMP, a lipid-soluble derivative of cAMP (25).

It remains to be seen whether, in fact, the level of inherent cAMP in the psoriatic epidermis is lower than normal, and if so, whether there is a defect in the rate of synthesis or an increase in the breakdown of cAMP. Recently, Vorhees et al. (24) reported low cAMP levels in psoriatic plaque epidermis, and Halprin (6) has suggested that adenylyl cyclase is defective in the psoriatic epidermis.

In this study we report our findings at the level of intracellular cAMP, adenylyl cyclase, and cyclic nucleotide phosphodiesterase in normal and psoriatic human epidermis. At the same time, values for the urinary secretion of cAMP by psoriatic and normal control persons are given, since systemic as well as local changes are often present in psoriasis.

MATERIAL AND METHODS

Epidermal samples

Patients suffering from psoriasis as well as non-psoriatic controls were selected from the in-patient department of the Dermatological Clinic, Turku. None of the subjects had received any systemic or local treatment on the biopsy area during the preceding 2 weeks. The epidermal samples were taken with a keratome from large typical psoriatic plaques on the body or thighs after removing all scales with pincers. Control samples were taken from the corresponding contralateral area or from the immediate vicinity of the plaque. The keratome slices were immediately placed on dry ice and kept at -75°C .

Acta Dermatovenereol (Stockholm) 54

until analysed. Light-microscope examination of the sections revealed that the sections were cut so that about one-half of the volume of the dermal papillae was included both in control and involved biopsies, as seen in Fig. 1.

Urine samples

Urine from psoriatic and from non-psoriatic patients was collected for 24 hours and stirred well, the volume was measured and 50-ml aliquots were stored at -20°C until analysed. No coffee or tea was allowed during the period of collection.

Cyclic adenosine monophosphate (cAMP)

Frozen skin samples were weighed on a torsion balance in a cold room at -20°C . For cAMP determination, samples weighing ca. 5 mg were extracted by homogenization with 500 μl of 6% ice-cold trichloroacetic acid and assayed with the radioimmunoassay kit produced by the Schwarz/Mann Chemical Company (Orangeburg, New York, USA).

Urinary cyclic AMP was measured either according to the method of Raji et al. (17) or with the radioimmunoassay kit. The first method is based on separation of cAMP from other nucleotides by column chromatography, followed by hydrolysis with cyclic nucleotide phosphodiesterase and enzymic conversion to ATP³² which was counted by Cerenkov radiation.

Adenylyl cyclase (AC)

For enzyme activity measurements, the tissue samples were homogenized in 4 vol of 50 mM Tris-HCl buffer, pH 7.5, containing 2 mM dithiothreitol at $0-2^{\circ}\text{C}$. Fifty- μl portions of homogenates equivalent to 10–15 mg of tissue were assayed for adenylyl cyclase according to a modification of the method by Krishna, Weiss & Brodie (10). Incubations were carried out at 30°C for 20 min, with 1 mM (^3H)-ATP (spec. act. 60 mCi/mmol) as substrate in 500 μl of medium containing 50 mM Tris-HCl buffer (pH 7.5), 3 mM MgCl_2 , 10 mM theophylline, 5 mM phosphoenolpyruvate, 20 $\mu\text{g}/\text{ml}$ of pyruvate kinase and 2 mM dithiothreitol. Activity was also measured in the presence of 10 mM sodium fluoride or 0.1 mM adrenalin. Proportionality with time was observed in the presence and absence of these activators. Separation of (^3H)-cyclic AMP from (^3H)-ATP was facilitated by increasing the length of the Dowex AG50 W-X4 column to 8 cm and eluting with 1 mM potassium phosphate buffer, pH 7.0.

Cyclic nucleotide phosphodiesterase (PDE)

Cyclic nucleotide phosphodiesterase was assayed by a method based on the same principle as that of Breckenridge & Johnston (2). Twenty μl of the homogenate equivalent to 4–6 mg of tissue was added to 100 μl of reagent consisting of 50 mM Imidazol-HCl buffer (pH 7.5), 5 mM MgCl_2 , 0.02% bovine serum albumin, 0.01% Triton X-100, 100 $\mu\text{g}/\text{ml}$ of 5-nucleotidase (Sigma), and 5 mM cAMP. Incubation was carried out at 30°C for 20 min and terminated by adding ice-cold trichloroacetic acid to a final concentration of 6%. The inorganic phosphate liberated was measured according to Lowry &

Table I. Cyclic adenosine monophosphate in normal and psoriatic epidermis

Tissue	cAMP ($\mu\text{mol/kg}$ wet weight)
Normal (11)	0.94 ± 0.17
Psoriatic	1.62 ± 0.36 **
Healthy (13)	
Lesion (14)	

** $P < 0.01$.

The values are averages ± 1 S.E.M. The number of specimens assayed is included in parentheses.

Lopez (11). Protein was determined according to Lowry et al. (12).

Statistics

Statistical analyses of the results were carried out either by the *t*-test according to Jonge (9) or by a matched-pair *t*-test (19) in cases where skin specimens from the same patient were compared.

RESULTS

cAMP in epidermis

In the psoriatic patients the cAMP concentration in healthy skin areas did not differ significantly from the level in the normal controls (Table I), whereas in the lesions the concentrations were about twice as high as in the normal controls. This difference was significant ($P < 0.01$). The concentration in the lesions was also higher than in the healthy skin area of the psoriatic patient; this difference was not significant.

cAMP in urine

Urinary cAMP excretion, estimated from samples of 24-hour urine, was the same in the psoriatic as in control patients (Table II). With the same methods, the same level has been found in a larger series of patients (2.0 – 6.5 $\mu\text{mol}/24$ h, in preparation). There was no correlation between the age of the patient and urinary cAMP excretion.

Adenyl cyclase

Adenyl cyclase activities in normal skin, healthy psoriatic skin and psoriatic plaques are shown in Table III. It appears that the enzyme activities are roughly the same in normal controls and in skin of healthy psoriatics. Significantly lower values were found in the psoriatic plaques, when estimated with or without fluoride, as compared

Table II. Cyclic adenosine monophosphate in normal and psoriatic urine

Urine	cAMP ($\mu\text{mol}/24$ h)
Normal (6)	4.40 ± 0.79
Psoriatic (26)	4.00 ± 0.37
Men (18)	4.28 ± 0.45
Women (8)	3.38 ± 0.67

The values are averages ± 1 S.E.M. The number of urine samples analysed are given in parentheses. No significant differences were found between the groups.

with healthy areas in the same person or with normal control skin specimens. A response to sodium fluoride was clearly demonstrable and in this respect normal skin [10.2 ± 0.8 (S.E.M.)-fold increase], healthy psoriatic skin (6.0 ± 1.2 -fold increase) and plaque enzymes (9.3 ± 1.5 -fold increase) scarcely differed.

Cyclic nucleotide phosphodiesterase

Cyclic nucleotide phosphodiesterase activity measured in normal control skin and healthy and plaque skin of psoriatics is recorded in Table IV. No difference was found between normal skin and healthy psoriatic skin. The psoriatic plaque, on the other hand, had a strikingly lower activity.

DISCUSSION

The cAMP level recorded by us in the normal skin was the same as that found by Voorhees et al. (24). This level is in the range reported for other mammalian tissues (22). That healthy skin

Table III. Adenyl cyclase activity in normal and psoriatic epidermis

Tissue	Adenyl cyclase (pmol/mg prot./min)	
	No activator	10 mM NaF
Normal	0.25 ± 0.03 (4)	2.48 ± 0.21 (4)
Psoriatic	0.44 ± 0.07 (4) *	2.31 ± 0.19 (5) **
Healthy		
Lesion		

* $P < 0.05$
** $P < 0.025$ } matched-pair *t*-test.

*** $P < 0.01$, *t*-test according to Jonge.

The values are averages ± 1 S.E.M. The number of specimens analysed are given in parentheses.

Table IV. Cyclic nucleotide phosphodiesterase in normal and psoriatic epidermis

The values are averages $\pm$ 1 S.E.M. The number of specimens assayed are given in parentheses

Tissue	Phosphodiesterase (nmol/mg prot./min)
Normal (11)	2.10 ± 0.47
Psoriatic	} **
Healthy (11)	1.86 ± 0.46 }
Lesion (12)	0.52 ± 0.13 }

** $P < 0.01$.

* $P < 0.02$, matched-pair *t*-test.

areas of psoriatics and normal epidermis of controls had the same level of cAMP as was expected. It is also in agreement with the findings of Voorhees et al. (24).

Our finding of comparatively high levels of cAMP in the psoriatic as compared with the normal epidermis does not agree with the findings of Voorhees et al. (24), who reported 36% lower values in psoriatic plaques (on a wet weight basis). The report of Voorhees et al. appears to be based on careful work and their methods seem reliable, although, as the authors themselves admit, Brooker's isotope method may be subject to some errors. The radioimmunoassay developed by Steiner et al. (21, 22) and used by us is at least equally reliable. Thus the differences between our chemical methods and those of Voorhees et al. do not seem to explain the differences in the values obtained, especially since the results in normal patients are identical.

Voorhees et al. (24) interpreted the 36% decrease in the mean cAMP level as support for the theory that the high rate of mitosis in the psoriatic epidermis is due to lack of the mitotic inhibition caused by cAMP in normal epidermis. However, their data merit closer inspection. In psoriatics the cAMP values in healthy epidermis varied from 0.3 to 3.3 and in plaque from 0.2 to 2.3 pmol/mg, i.e. the ranges are about tenfold and for the most part overlapping. Moreover, in 5 of their 25 patients the cAMP level in the psoriatic plaque epidermis was higher than in the healthy skin area, i.e. the result is quite the reverse and in accord with our findings. Therefore, we think that it is too early to conclude from those studies that cAMP is the sole inhibitor of mitosis in the

epidermis and that the psoriatic plaque is a direct consequence of a low local level of cAMP.

Voorhees et al. (24) stress the importance of the appropriate keratome setting in obtaining the biopsies for assay, and we fully agree that differences in the biopsy technique may lead to differences in results. This is especially true of assays made on whole-thickness epidermal homogenate, for the exact pattern of cAMP distribution in the various epidermal layers is not known. Voorhees et al. (24) used the keratome setting of 0.10 mm to isolate normal epidermis and a setting of 0.25–0.50 mm to isolate psoriatic plaque including the elongated rete pegs and dermal papillae from skin. Our biopsies were considerably more shallow in order to eliminate dermal contamination which would have resulted in erroneously low cAMP values. It is possible that the difference in the technical procedures alone may explain the variance between two sets of data.

cAMP is known to be liberated from cells into plasma and excreted into urine; in fact, cAMP and cGMP (cyclic guanosine 3',5'-monophosphate) are the only nucleotides which have been found in high concentrations in urine, the former being the more abundant (22). It was therefore of interest to study the urinary excretion of cAMP. However, the similarity of the values for normal and psoriatic patients suggests that the psoriatic epidermis does not liberate cAMP in greater amounts than does normal skin. In the sera of patients with psoriasis the levels of several cellular enzymes, e.g. nucleic acid phosphodiesterase, are raised (26), and this has been considered a sign of the "leakier" nature of psoriatic as compared with normal epidermal cells.

Recently, Halprin (6) found that the adenylyl cyclase of the psoriatic plaque is less sensitive to activation by sodium fluoride (and adrenalin) than is the adenylyl cyclase in the surrounding normal skin. This would lead to decreased levels of cAMP in the epidermal cells, if it is assumed that the cAMP level is physiologically regulated by adrenalin. Such a reversible defect in the enzyme molecule could be explained only by a mutation in the gene regulating the synthesis of the enzyme itself or of some factor modifying its function. Our preliminary results do not support this theory, either, since adenylyl cyclase from the normal and psoriatic plaque epidermis was equally activated by sodium fluoride. No stimulation by

adrenalin could be expected, since homogenization is known to destroy this property. We are currently modifying our techniques to obtain the experimental conditions described by Halprin and so eliminate differences in technique.

We found low levels of cyclic nucleotide phosphodiesterase in psoriatic plaque epidermis compared with healthy skin of psoriatics and of normal controls. No previously published data on the activity of this enzyme are available for comparison. The low levels of this enzyme would explain why the concentration of cAMP was relatively high in the psoriatic plaque. It should be remembered, however, that the activity of this enzyme is always considerably higher than that of the synthesizing enzyme, adenylyl cyclase. We do not know, however, to what extent adenylyl cyclase is active in living cells.

Our results are based on a limited number of samples, and further studies are required to establish the histological variation of the properties measured. Further studies are unlikely to alter our basic conclusions on the concentration of epidermal cAMP, adenylyl cyclase and phosphodiesterase in psoriasis. Studies on other cellular control mechanisms may afford more insight into the biology of the psoriatic epidermis.

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