INCREASED CYCLIC GMP AND DECREASED CYCLIC AMP LEVELS
IN THE HYPERPLASTIC, ABNORMALLY DIFFERENTIATED
EPIDERMIS OF PSORIASIS

John J. Voorhees, Marek Stawiski and Elizabeth A. Duell,

Department of Dermatology,
University of Michigan Medical School,
Ann Arbor, Michigan 48104

and

Mari K. Haddox and Nelson D. Goldberg,

Department of Pharmacology,
University of Minnesota Medical School,
Minneapolis, Minnesota 55455

Summary

The genetic skin disease psoriasis has been
examined as a model system that may provide an
understanding of the control of normal epidermal
specialization (differentiation) and the perturbed
regulatory processes in proliferative diseases.
The excessive glycogen accumulation, increased
proliferation and decreased tissue specialization
characteristic of psoriasis involve cellular pro-
cesses that have been shown to be regulated by
cyclic AMP in other cells and tissues. It has also
been suggested that cyclic GMP is a cellular ef-
fector that may be involved in promoting cell pro-
liferation and other events that oppose those be-
lieved to be mediated by cyclic AMP. It was
postulated, therefore, that the epidermis of the
psoriasis lesion might exhibit an imbalance in the
cellular concentrations of these two cyclic nucleo-
tides. In this study the levels of cyclic AMP were
measured in the involved epidermis (IE) and unin-
volved epidermis (UE) from 25 psoriasis patients.
The concentrations of cyclic AMP were found as
reported previously using a different analytical
procedure, to be significantly lower in IE based
on protein and DNA. A comparison of the levels
of cyclic GMP in IE versus UE of 12 other psoriasis
patients showed the levels of this cyclic nucleo-
tide to be significantly increased in IE based on
protein, DNA and wet weight. We suggest that this
imbalance in the ratio of these two cyclic nucleo-
tides may have pathophysiological relevance to the
initiation and/or the maintenance of the psoriasis
lesion.

Introduction

Several common skin diseases, including the prototypic
genetic disease psoriasis, have three characteristic abnormali-
ties in involved epidermis (IE) as compared with uninvolved epi-
dermis (UE): marked glycogen accumulation (1); decreased tissue
specialization (differentiation) (2); and increased rate of pro-
liferation (3). Each of these cellular processes has been shown
to be influenced dramatically in other tissues by agents that
stimulate cellular adenosine 3',5'-monophosphate (cyclic AMP)
generation and by cyclic AMP itself. For example, it is gener-
ally recognized that beta adrenergic agonists promote glyco-
genolysis in liver and a number of other tissues by way of a rise
in cellular cyclic AMP levels (4). It seems very likely that
cyclic AMP serves as a regulator of these processes in epidermal
cells as well. It has also been found that beta adrenergic
stimulation in association with a rise in the concentration of
tissue cyclic AMP slowed the flow of epidermal cells through at
least a portion of the cell cycle: $(G_2 \rightarrow M)$ (5). Taking the fore-
going into account our original postulate was that the abnormali-
ties in the lesions of common proliferative skin diseases, as
exemplified by psoriasis, might derive from or be associated with
decreased levels of cyclic AMP in IE versus UE (6).

This hypothesis was first tested by determining the levels
of cyclic AMP in IE versus UE of epidermis from psoriasis patients
using the analytical procedure for cyclic AMP determination
described by Brooker, et al (7). The results of these studies
showed that cyclic AMP concentrations were lower in IE than UE;
the decreases were determined to be highly significant when cal-
culated on the basis of either wet weight, DNA or protein content
of the sample (8).

To further critically test our postulate a second study was initiated in which we sought to confirm these previously detected decreases by using a second cyclic AMP assay based upon a different analytical prinicple (9). During this second study we became aware of the hypothesis of Goldberg, et al (10) that guanosine 3',5'-monophosphate (cyclic GMP) might be associated with promoting a number of cellular events that are antagonistic to those thought to be mediated by cyclic AMP. Hadden, et al (11) reported that mitogen induced lymphocyte proliferation was associated with strikingly increased cellular levels of cyclic GMP and proposed that cyclic GMP may be an intracellular effector involved in promoting the proliferative process. Accordingly, our study was extended in order to determine whether an imbalance might exist in the levels of both cyclic nucleotides in IE versus UE of psoriasis.

The purpose of the present communication is to present the results of these studies which demonstrate that cyclic AMP levels are significantly decreased and cyclic GMP levels significantly increased in IE versus UE of psoriasis patients. A scheme for epidermal growth control is proposed modeled after Goldberg, et al (10) and Hadden, et al (11) which derives from the "Yin-Yang" or "dualism" hypothesis of biological regulation.

Materials and Methods

Patient Selection

After obtaining informed consent, biopsies were taken from patients with well-developed lesions of large plaque psoriasis. The psoriasis patients selected had not used antipsoriasis medications either topically or systemically for a minimum of approximately two weeks prior to biopsy. The biopsy specimens

obtained from 17 male and 8 female patients ages 20 to 62 years
were acceptable on a histological basis for cyclic AMP analysis
and by the same criterion samples acceptable for cyclic GMP
analysis were obtained from 5 male and 7 female patients ages 36
to 65 years. Biopsies from involved areas and clinically unin-
volved control sites were taken from either one or two of the
following locations: the trunk, buttocks or thighs. Although the
distance between IE and UE biopsy sites was usually several
centimeters, the minimum acceptable proximity of the two sites
was 2.5 cm.

Epidermal Biopsies

        IE and UE areas were cleansed with 3% hexachlorophene
(pHisoHex) and gentle brushing. The hexachlorophene was washed
from the surface by an ethanol lavage. This cleansing technique
minimized but did not totally eliminate the wide variation in
amount of psoriasis scale among patients. Each biopsy site was
anesthetized by the local infiltration of 8 ml of 1% lidocaine
(Xylocaine) hydrochloride without epinephrine. Anxious patients
or those experiencing any detectable degree of discomfort at the
onset of the procedure were not biopsied.

        Epidermal strips were obtained using a keratome device
modified as previously reported (8) for the special purpose
described. Each epidermal strip was approximately 1.5 cm wide and
3 cm long. Two such strips (one IE and one UE) were taken. UE
was removed with a keratome shim setting of 0.10 mm, whereas IE
required a setting between 0.275 and 0.425 mm. The most common
keratome setting for the histologically acceptable biopsies was
0.35 mm.

        Criteria for histologic acceptability have been previously
described in detail (8). Briefly, the keratome cleavage is at

or very near the dermoepidermal junction. Approximately 25% of the IE specimen as estimated histologically is dermis (mesenchyme) having fibroblasts, endothelial cells and scattered inflammatory cells. In UE specimens about 15% of the tissue is relatively acellular (in comparison with IE) dermis.

From psoriasis patients the IE strip was always removed before the UE strip. The average time required for the keratome to cut through IE was 5 sec; for UE approximately 8 sec was required. Biopsy strips from IE and UE were obtained in a manner that permitted half the specimen to drop directly into isopentane-free liquid nitrogen, a narrow transverse piece to drop into neutral buffered formalin for examination by light microscopy and the remaining portion to drop directly into the liquid nitrogen. The procurement of the transverse piece of tissue for histology required 3 sec for IE and 5 sec for UE. The wet weights of IE and UE strips were determined immediately while the specimens were frozen.

Assay of Cyclic AMP

The frozen epidermal strips were powdered in liquid nitrogen and while in the frozen state, homogenized (Ten-Broeck, glass-on-glass homogenizer) with 5.0 ml of 6% trichloroacetic acid (TCA) (4°C) containing 200 cpm/ml of ^{3}H-cyclic AMP (Schwarz Bio Research, 16.3 curies/mmole) to permit estimation of recoveries. A 0.2 ml aliquot of the homogeneous suspension was removed for protein analysis (12) and the remainder was centrifuged (17,000g, 30 min, 4°C). DNA was extracted from the pellet with 3% perchloric acid (PCA) by a modification of the method of Hennings and Elgjo (13) and assayed by the method of Burton (14). TCA was removed from the supernatant fraction by 3 successive extractions with five volumes of water-saturated diethyl ether.

Cyclic AMP was partially purified free of interfering tissue metabolites by chromatography on Bio Rad AG1-X2 (5 x 80 mm columns, 200-400 mesh) in the chloride form. After washing the column containing the sample with distilled water, the cyclic AMP was eluted with a solution of hydrochloric acid, pH 2.0. The recovery of cyclic AMP from the extracts was approximately 70% $\pm$ 2.5% for either the uninvolved or involved epidermal samples. After lyophilization of the samples, the cyclic AMP containing residue was dissolved in 0.2 ml of 0.025M acetate buffer, pH 4.4 and duplicate samples of 5, 10 and 15 µl aliquots were assayed in a final reaction volume of 50 µl according to the method of Gilman (9). Hydrolysis of random samples with phosphodiesterase according to a modification (8) of the method of Otten, Johnson and Pastan (15) resulted in the disappearance of 95% $\pm$ SE 3.4% (N=8) of what was detectable in the assay as cyclic AMP and no correction was therefore made for noncyclic AMP material.

Assay of Cyclic GMP

Cyclic GMP analysis was performed by the fluorometric enzymatic cycling technique of Goldberg and O'Toole (16). The procedure involved purification of the acid extracts (after removal of TCA with ether) first on Dowex-1 formate columns (17) and subsequently by thin layer chromatography (TLC) on silica gel (16). Portions of the eluates from the TLC purification were used to estimate recovery, noncyclic GMP (i.e., non-phospho-diesterase hydrolyzable) reactive material, and cyclic GMP content. The latter two measurements were performed in triplicate.

Experimental and Statistical Design

In the absence of a stable denominator the data have been ex-pressed in terms of wet weight, protein and DNA content of the

samples in an attempt to arrive at the most accurate approximation
possible. The results obtained using wet weight give the most
variable estimates, probably because of edema and dead scale dif-
ferences in IE as compared to UE. From the present and previous
study (8) we have found that in IE there is 24% more assayable
protein and 6.7% more assayable DNA per mg wet weight than in UE
obtained from 50 patients.

Because the changes in cyclic AMP levels were relatively
small, 25 patients were included in the portion of the study in
volving the analysis of this cyclic nucleotide. The changes in
epidermal cell cyclic GMP concentration were of a greater mag-
nitude and only 12 patients were utilized. Statistical signifi-
cance was analyzed by Student's t-test for paired data using a
one sided hypothesis. Calculations of data and statistical
significance in Tables 1 and 2 were performed on an IBM 360/67
computer. All the data are expressed as mean $(\overline{X})$ ± 1 standard
error (SE) of the mean.

Results

Table 1 shows that the concentration of cyclic AMP in IE is
less than that found in UE. The absolute percent decrease differs
depending upon the manner used to express the data; decreases of
43%, 36% or 17% were calculated when the amount of cyclic AMP was
expressed on the basis of protein, DNA or wet weight, respectively.
The differences between the means used to calculate the percents
are highly significant (p<.005) when protein and DNA are the
denominators. On the basis of wet weight the decrease in cyclic
AMP concentration is minimal (17%) and only somewhat suggestive
on a statistical basis (.10>p>.09).

TABLE I

DECREASED CYCLIC AMP LEVELS IN QUICK FROZEN KERATOMED SAMPLES
OF EPIDERMIS FROM PSORIASIS PATIENTS

Picomoles of cAMP per	Psoriasis		N	% Decrease	Student's t-test
	Uninvolved	Involved			
Mg Protein	12.3 ± 1.2	7.0 ± 0.7	25	43	$p<0.001$
Ug DNA	1.1 ± 0.1	0.7 ± 0.1	25	36	$0.005>p>0.001$
Mg Wet Weight	1.2 ± 0.1	1.0 ± 0.1	25	17	$0.10>p>0.09$

These data indicate that the mean cyclic AMP concentration was lower in IE versus UE of psoriasis. Nevertheless, it should be pointed out that although 22 of the 25 patients studied exhibited lower cyclic AMP levels in IE than UE, the levels of 3 patients (i.e., based on protein or DNA) were 20% higher in IE specimens. With wet weight as a denominator, 9 patients had cyclic AMP levels 28% higher in IE than in UE. However, as shown in the materials and methods section, the concentration of metabolites in IE versus UE is probably most reliably determined when based on DNA content rather than on wet weight of the tissue sample.

Table 2 shows that the concentration of cyclic GMP in IE is greater than in UE. The absolute percent increase again depends on the denominator used to express the data; calculated by the 3 different methods increases of 106%, 93% or 112% were obtained using protein, DNA or wet weight, respectively. The differences between the means used to calculate the percents are highly significant ($p < .005$) in terms of DNA and wet weight. When the denominator is protein the 106% increase has moderate significance statistically ($p < .02$).

Although the mean cyclic GMP level found in the 12 psoriasis patients was higher in IE than in UE, the cyclic GMP level of IE in one patient was lower than that of UE in terms of DNA and wet weight, the only two methods used to determine the concentration of cyclic GMP in the epidermis of this and two other patients.

Discussion

The results reported here in conjunction with previously re-ported data (8) strongly suggest that there is an imbalance in the levels of cyclic AMP and cyclic GMP in the involved epidermis of

TABLE 2

INCREASED CYCLIC GMP LEVELS IN QUICK FROZEN KERATOMED SAMPLES
OF EPIDERMIS FROM PSORIASIS PATIENTS

Femtomoles of cGMP Per	Psoriasis		N	% Increase	Student's t-test
	Uninvolved	Involved			
Mg Protein	114.6 ± 23.0	236.5 ± 34.8	9	106	$0.02 > p > 0.01$
Ug DNA	6.1 ± 1.0	11.8 ± 3.4	12	93	$0.005 > p > 0.001$
Mg Wet Weight	2.6 ± 0.4	5.5 ± 1.6	12	112	< 0.001

psoriasis. The data, however, might best be viewed as strongly
suggestive rather than as conclusive because of uncertainties
(see material and methods) inherent in the biological material
under investigation. These include: 1) lack of an entirely
satisfactory data base upon which the cyclic nucleotide content
of the tissue can be expressed, 2) the presence of variable
amounts of contaminating mesenchymatous tissue, which contains
tissue elements of a different character than epidermis proper,
and 3) the possibility that the cyclic nucleotide levels may be
stratified differently in IE than UE as are the cells in these
two morphologically dissimilar tissues. In view of these problems
the data have been expressed in terms of protein, DNA and wet
weight in order to obtain a consensus.

The results of experiments conducted to date consistently
indicate that cyclic AMP levels are decreased in the IE of
psoriasis. This conclusion is based upon statistically signifi-
cant decreases seen in the cyclic AMP levels of IE versus UE in 2
of 3 denominators in 25 patients (Table 1) and upon previous work
where a significant decrease ($p < 0.001$) was found using any one of
the 3 denominators (8).

The IE of psoriasis shows significant increases in the
cellular levels of cyclic GMP (Table 2) which are larger than the
decreases found in cyclic AMP levels. The finding of a greater
concentration of cyclic GMP in the more rapidly proliferating
epidermis of IE is reinforced by the observations of Hadden, et al
(11) concerning mitogen induced proliferation of lymphocytes in
which the initiation of proliferation is associated with a
temporally discrete increase of over 10-fold in cellular cyclic
GMP levels. In addition, Goldberg, et al (18) have found that
insulin and tetradecanoyl phorbol acetate (TPA), a potent skin

mitogen and tumor promoter (19), induce proliferation of fibro-
blasts in vitro. In each of the cases cited the initiation of
proliferation (i.e., of cells in G_0 or early G_1) is associated
with a temporally discrete increase of 10-40-fold in cellular
cyclic GMP levels extending over a period of minutes (mitogen or
insulin) or seconds (TPA) (18). It has also been recently re-
ported that histamine, a substance found to stimulate the $G_2 \rightarrow M$
phase of the epidermal cell cycle (5), promotes striking in-
creases (i.e., 16-fold) in cyclic GMP concentration of mouse
epidermal slices within minutes after exposure to the histamine
(20).

     An interpretation of the increases of only approximately 100%
seen in IE of psoriasis in comparison with the dramatic effects
produced by histamine is that the concentration of cyclic GMP may
be increased in a limited number of cells (i.e., those undergoing
proliferative stimulation). Also, the cells in the proliferative
compartment of IE are cycling asynchronously. Lastly, the
measurements of cyclic GMP in IE and UE are made in tissue samples
which contain both the proliferating and differentiating compart-
ments—a problem which does not exist in fibroblasts in vitro. A
similar interpretation can be offered for the modest decreases in
cyclic AMP levels in IE versus UE since the decreases are not as
dramatic as those found in transformed fibroblasts (21,22).

     To explain the possible involvement of cyclic GMP and cyclic
AMP in the control of epidermal cell function and the conse-
quences that may result from an imbalance in the levels of these
two cyclic nucleotides we have developed a working hypothesis
based on the "Yin-Yang" or "dualism" concept of biological control
proposed by Goldberg, et al (10). According to the dualism con-
cept the two cyclic nucleotides are thought to provide opposing

regulatory influences in bidirectionally controlled systems (18).
Within this framework it is proposed that in psoriasis the
elevated cyclic GMP levels provide a stimulus for continued
epithelial cell proliferation and an inhibitory influence on the
differentiative process. The reduced cyclic AMP concentration
may constitute a permissive influence with regard to prolifera-
tion and a failure on the part of the cell to differentiate be-
cause of the active role this cyclic nucleotide is proposed to
play in the differentiative process (23). The model is compatible
with the hypothesis for the control of cell proliferation postu-
lated by Hadden, et al (11) wherein increased cellular cyclic GMP
levels represent an active signal to induce cell proliferation
while elevation of or sustained high steady-state levels of
cyclic AMP may limit or inhibit the initiation of cell division.

References

1. K. M. HALPRIN and A. OHKAWARA, J. Invest. Derm. 46, 51 (1966).

2. B. LAGERHOLM, In: Psoriasis, Proceedings of the International
    Symposium, Stanford University, 1971, E. M. Farber and A. J.
    Cox (eds.), p. 161, Stanford University Press, Stanford,
    California (1971).

3. G. D. WEINSTEIN and J. L. MCCULLOUGH, Ann. Rev. Med. 24, 345
    (1973), Annual Reviews Inc., Palo Alto, California.

4. G. A. ROBISON, R. W. BUTCHER and E. W. SUTHERLAND, Cyclic AMP,
    p. 130, Academic Press, New York (1971).

5. J. J. VOORHEES, E. A. DUELL, L. J. BASS, J. A. POWELL and
    E. R. HARRELL, J. Invest. Derm. 59, 114 (1972).

6. J. J. VOORHEES and E. A. DUELL, Arch. Derm. 104, 352 (1971).

7. G. BROOKER, L. J. THOMAS, JR. and M. M. APPLEMAN, Biochemistry
    7, 4177 (1968).

8. J. J. VOORHEES, E. A. DUELL, L. J. BASS, J. A. POWELL, and
    E. R. HARRELL, Arch. Derm. 105, 695 (1972).

9. A. G. GILMAN, Proc. Nat. Acad. Sci. USA 67, 305 (1970).

10. N. D. GOLDBERG, M. K. HADDOX, D. K. HARTLE and J. W. HADDEN,
    In: Proceedings of the Fifth International Congress on
    Pharmacology, Karger, Basil, Switzerland, 1973, in press.

11. J. W. HADDEN, E. M. HADDEN, M. K. HADDOX and N. D. GOLDBERG,
    Proc. Nat. Acad. Sci. USA 69, 3024 (1972).

12. O. H. LOWRY, N. J. ROSEBROUGH, A. L. FARR and R. J. RANDALL,
    J. Biol. Chem. 193, 265 (1951)

13. H. HENNINGS and K. ELGJO, Cell and Tissue Kinet. 3, 243 (1970).

14. K. BURTON, In: Methods in Enzymology 12B, 163, L. Grossman
    and K. Moldave (eds.), Academic Press, New York (1968).

15. J. OTTEN, G. S. JOHNSON and I. PASTAN, Biochim. Biophys. Res. Comm. 44, 1192 (1971).

16. N. D. GOLDBERG and A. G. O'TOOLE, In: Methods of Biochemical Analysis, D. Glick (ed.) 20, p. 1, Interscience Publishers, New York (1971).

17. F. MURAD, V. MANGANIELLO and M. VAUGHAN, Proc. Nat. Acad. Sci. USA 68, 736 (1971).

18. N. D. GOLDBERG, M. K. HADDOX, R. ESTENSEN, C. LOPEZ and J. W. HADDEN, In: Cyclic AMP in Immune Response and Tumor Growth, L. M. Lichtenstein, C. W. Parker and W. Braun (eds.), Springer-Verlag, New York, 1973, in press.

19. E. HECKER, In: Molekularbiologie des Malignen Wachstums, p. 105, Springer-Verlag, Mosbach/Baden, Berlin (1966).

20. J. J. VOORHEES, N. H. COLBURN, M. STAWISKI, E. A. DUELL, M. K. HADDOX and N. D. GOLDBERG, In: Control of Proliferation in Animal Cells, Cold Spring Harbor Laboratory Publishers, New York, 1974, in press.

21. J. OTTEN, J. BADER, G. S. JOHNSON and I. PASTAN, J. Biol. Chem. 247, 1632 (1972).

22. J. R. SHEPPARD, Nature New Biology 236, 14 (1972).

23. K. N. PRASAD and J. R. SHEPPARD, Exptl. Cell Res. 73, 436 (1972).