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

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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 processes 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 effector that may be involved in promoting cell proliferation and other events that oppose those believed 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 nucleotides. In this study the levels of cyclic AMP were measured in the involved epidermis (IE) and uninvolved 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 nucleotide 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 nucleotides 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 abnormalities in involved epidermis (IE) as compared with uninvolved epidermis (UE): marked glycogen accumulation (1); decreased tissue specialization (differentiation) (2); and increased rate of proliferation (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 generally recognized that beta adrenergic agonists promote glycogenolysis 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: \((G_2+M)\) (5). Taking the foregoing into account our original postulate was that the abnormalities 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 calculated 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 principle (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 uninvolved 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 3H-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\% ± 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\% ± 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-phosphodiesterase 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 expressed 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 differences 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 involving the analysis of this cyclic nucleotide. The changes in epidermal cell cyclic GMP concentration were of a greater magnitude and only 12 patients were utilized. Statistical significance 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 ($\bar{X}$) ± 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

<table>
<thead>
<tr>
<th>Picomoles of cAMP per</th>
<th>Psoriasis</th>
<th>N</th>
<th>% Decrease</th>
<th>Student's t-test</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Uninvolved</td>
<td>Involved</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mg Protein</td>
<td>12.3 ± 1.2</td>
<td>7.0 ± 0.7</td>
<td>25</td>
<td>43</td>
</tr>
<tr>
<td>Ug DNA</td>
<td>1.1 ± 0.1</td>
<td>0.7 ± 0.1</td>
<td>25</td>
<td>36</td>
</tr>
<tr>
<td>Mg Wet Weight</td>
<td>1.2 ± 0.1</td>
<td>1.0 ± 0.1</td>
<td>25</td>
<td>17</td>
</tr>
</tbody>
</table>
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 reported 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**

<table>
<thead>
<tr>
<th>Femtomoles of cGMP Per</th>
<th>Psoriasis</th>
<th>N</th>
<th>% Increase</th>
<th>Student's t-test</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Uninvolved</td>
<td>Involved</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mg Protein</td>
<td>114.6 ± 23.0</td>
<td>236.5 ± 34.8</td>
<td>9</td>
<td>106</td>
</tr>
<tr>
<td>Ug DNA</td>
<td>6.1 ± 1.0</td>
<td>11.8 ± 3.4</td>
<td>12</td>
<td>93</td>
</tr>
<tr>
<td>Mg Wet Weight</td>
<td>2.6 ± 0.4</td>
<td>5.5 ± 1.6</td>
<td>12</td>
<td>112</td>
</tr>
</tbody>
</table>
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 significant 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 fibroblasts in vitro. In each of the cases cited the initiation of proliferation (i.e., of cells in Go or early G1) 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 reported that histamine, a substance found to stimulate the G2→M phase of the epidermal cell cycle (5), promotes striking increases (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 compartments—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 consequences 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 concept 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 proliferation and a failure on the part of the cell to differentiate because 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 postulated 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.

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