Epidermal Cyclic GMP is Increased in Psoriasis Lesions

KENJI ADACHI, M.D., PH.D., TAKASHI AOYAGI, M.D., OSUMU NEMOTO, M.D., KENNETH M. HALPRIN, M.D., AND VICTOR LEVINE, B.S.C.

Veterans Administration Hospital and Department of Dermatology, University of Miami School of Medicine, Miami, Florida, U. S. A.

Cyclic GMP levels in epidermis of normal subjects and of psoriatic patients were measured with a highly sensitive radioimmunoassay method. Technical improvements for the assay are 2-fold: (1) skin samples were frozen in vivo before biopsy and local injection of any anesthetic was avoided to overcome ischemia effect which could lower cyclic GMP artificially; (2) epidermis was microdissected to avoid contamination of dermis and keratin layers. The results show that on a per mg tissue dry weight basis the cyclic GMP levels are about 200 fmol in the involved lesional epidermis and 70 fmol in the uninvolved or normal epidermis. Similarly increases in the cyclic GMP levels in the lesional epidermis are observed when the data are expressed either on a DNA or protein basis. The cyclic GMP level in normal epidermis from non-psoriatic subjects is the same as that in the uninvolved epidermis of psoriasis patients.

Cyclic GMP has been implicated in cell proliferation and differentiation (for the most recent reviews c.f. references 1, 2 and 3). Hadden et al [4] reported that in a lymphocyte system mitogens such as concanavalin A and phytohemagglutinin caused marked increases in cyclic GMP. Since then, there have been many reports that cyclic GMP stimulates DNA synthesis in thymic lymphoblasts and fibroblasts [5-9]. However Miller et al [10] found contradictory data in that cyclic GMP rose as growth of cultured 3T3 cells slowed down and that the addition of serum to resting cell caused a prompt fall in both cyclic AMP and cyclic GMP.

In vivo levels of cyclic GMP were found to be increased in psoriasis [11,12] skin tumor promotion [13-15] adenocarcinoma of the human colon [16], experimental hepatomas [17-19], renal cortical tumors [20], and also rapidly growing neonatal kidney [21].

Although a high cyclic GMP level is generally found to be associated with cell proliferation, Hickie et al [22] reported that in several Morris hepatomas of varying growth rates cyclic GMP levels were not necessarily related to the growth rates. Also, in skin tumor promotion by phorbol myristate acetate (PMA) cyclic GMP level did not occur immediately or shortly after the PMA treatment (30 sec to 30 min) [13,14]. Also, the fact that tumor promotion can induce ornithine decarboxylase without changing the cyclic GMP level [13] weakens the hypothesis that the primary role of cyclic GMP is on epidermal proliferation.

In view of the limited (for the cyclic GMP in skin, c.f. "Discussion") and sometimes conflicting information available concerning the possible role of cyclic GMP on cell proliferation, we have undertaken a study of the cyclic GMP levels in vivo in normal and psoriatic epidermis.

Manuscript received October 29, 1979; accepted for publication April 8, 1980.

This work was supported in part by grants AM 17179 from the National Institute of Health, the Dermatology Foundation of Miami and the A. O. Wellman Fund.

Reprint requests to: K. Adachi, M.D., Dermatology Service, VA Hospital, Miami, Florida 33125.

Abbreviations:
PMA: phorbol myristate acetate

MATERIALS AND METHODS

Experimental procedures were essentially the same as those in our previous studies on microassays of cyclic AMP levels in epidermis [23] except for the use of cyclic GMP antiserum and antigen. Again care was taken not to cause local ischemia and even local injection of anesthetic was avoided. Skin was frozen in vivo and then removed without thawing as described previously. The epidermal samples free from dermis and keratin layers were microdissected from frozen-dried skin slices and weighed on Mettler micro balance (usual sample weight ranged from 50 to 100 mg). The cyclic GMP levels in the samples were measured by the radioimmunoassay method of Steinr, Parker, and Kipnis [24] after acetylation [25] with micro-modification described by Ferrendelli et al [26]. Cyclic GMP antiserum and 125I-cyclic GMP antigen were purchased from Collaborative Research, Inc. (Waltham, Ma). Detailed technical procedures were described in our previous communication.

RESULTS

The results of cyclic GMP measurements are summarized in Table I. The in vivo cyclic GMP levels in normal and the uninvolved epidermis of psoriatic subjects are essentially the same. Regardless of the basis for comparison, i.e. per a weight, protein, or DNA basis, the lesional involved epidermis has a much higher concentration of cyclic GMP than the normal or uninvolved epidermis does.

DISCUSSION

Voorhees et al [11] contributed the first data on cyclic GMP levels in psoriatic epidermis in 1973 and recently Marcelo et al [12] reported the results of their re-investigation with an improved sensitive radioimmunoassay. Our present study presents further technical improvements, i.e. (1) use of microdissection to minimize the contamination of dermis and keratin layers and (2) in vivo freezing before biopsy to avoid possible ischemia effect, which could result in artificially low values. The previous and current cyclic GMP data summarized in Table II demonstrates general agreement in that the involved skin (epidermis) of psoriasis contains at least twice as much cyclic GMP as does noninvolved epidermis.

One discrepancy is that in the previous study by Marcelo et al, the cyclic GMP level in normal epidermis falls just in between the involved and uninvolved skin of psoriasis, whereas in our study, values for the uninvolved and normal epidermis are essentially the same. This discrepancy may be partially explained by an increased variability in dermal contamination in the keratome sliced skin (c.f. cyclic GMP levels expressed per DNA show more consistent agreement among the three). Furthermore one may note that cyclic GMP levels in present study are higher than those previously reported. This is probably due to our avoiding the ischemia effect. In the previous two studies skin was anesthetized by local injection of xylocaine before keratoming. The local injection of even saline could cause local ischemia [23], which artificially elevates the in vivo level of cyclic AMP [13,14,27,28] and simultaneously lowers that of cyclic GMP [29].

The present study clearly supports the association of a high cyclic GMP content and a high rate of cell proliferation. This does not clarify its role as the cause, the result, or just an associated epiphenomenon.
Table I. Cyclic GMP levels in human epidermis in vivo

<table>
<thead>
<tr>
<th>Cyclic GMP (fmol) per</th>
<th>Normal (n = 16)</th>
<th>Psoriasis</th>
<th>Psoriasis</th>
</tr>
</thead>
<tbody>
<tr>
<td>mg dry wt.</td>
<td>68.6 ± 3.8</td>
<td>73.1 ± 5.0</td>
<td>191 ± 21</td>
</tr>
<tr>
<td>mg protein</td>
<td>83.6 ± 4.0</td>
<td>88.5 ± 6.5</td>
<td>226 ± 23</td>
</tr>
<tr>
<td>µg DNA</td>
<td>4.76 ± 0.47</td>
<td>4.90 ± 0.37</td>
<td>10.4 ± 1.1</td>
</tr>
</tbody>
</table>

* × increase = involved

<table>
<thead>
<tr>
<th></th>
<th>Uninvolved (n = 10)</th>
<th>Involved (n = 10)</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>mg dry wt.</td>
<td>66.2 ± 3.5</td>
<td>73.3 ± 5.0</td>
<td>p &lt; 0.001</td>
</tr>
<tr>
<td>mg protein</td>
<td>84.6 ± 4.0</td>
<td>91.8 ± 6.5</td>
<td>p &lt; 0.001</td>
</tr>
<tr>
<td>µg DNA</td>
<td>4.45 ± 0.4</td>
<td>4.90 ± 0.37</td>
<td>p &lt; 0.001</td>
</tr>
</tbody>
</table>

* p values are for the involved vs either normal or uninvolved.

Table II. Cyclic GMP levels in psoriasis—A review

<table>
<thead>
<tr>
<th>Normal Subject</th>
<th>Psoriasis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Voorhees et al</td>
<td>2.6 (115, 6.1)</td>
</tr>
<tr>
<td>Marcelo et al</td>
<td>15 (112, 6.6)</td>
</tr>
<tr>
<td>1979 [12]</td>
<td></td>
</tr>
<tr>
<td>Adachi et al</td>
<td>21 (84, 4.8)</td>
</tr>
<tr>
<td>(this study)</td>
<td></td>
</tr>
</tbody>
</table>

Cyclic GMP levels are expressed as fmol per wet weight (the first figures before parenthesis), fmol per mg protein (the first figure in the parenthesis), and fmol per µg DNA (the second in the parenthesis).

For comparison, our data on a dry weight basis were converted to wet weight (by dividing by 3.3).

REFERENCES