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CYCLIC AMP ACCUMULATION IN PSORIATIC SKIN: DIFFERENTIAL RESPONSES TO HISTAMINE, AMP, AND EPINEPHRINE BY THE UNINVOLVED AND INVOLVED EPIDERMIS

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Using the uninvolved and involved skin from psoriatic patients, we investigated the effects of histamine and AMP (or adenosine) in vitro on the intracellular cyclic AMP levels. Both agents activated adenylate cyclase of the uninvolved and involved resulting in the accumulation of cyclic AMP. Without a cyclic nucleotide phosphodiesterase (PDE) inhibitor, these responses were biphasic and the maximal accumulation was observed in 5 min. With the PDE inhibitor both responses were markedly potentiated and high levels of cyclic AMP were observed for more than 20 min.

The response to histamine by the involved skin was much greater than that by the uninvolved. The degree of the response to adenosine was approximately equal. In accordance with our previous work, the response to epinephrine by the involved skin was much less than that by the uninvolved. Thus adenylate cyclases of involved skin from psoriatic patients exhibit a markedly diminished response to epinephrine while at the same time exhibiting a markedly enhanced response to histamine. This precludes the possibility that the unresponsiveness to epinephrine can be due to a generalized inability of the epidermal psoriatic plaque cell to make a functioning cell membrane.

It has been suggested that altered cyclic nucleotide levels might be involved in the pathogenesis of psoriasis [1–4]. Voorhees et al [2] reported decreased cyclic AMP levels in psoriatic epidermis, however, this was not confirmed by Härkönen, Hopsu-Hava, and Raij [5], or by our data using the microdissected pure epidermis [6,7]. The significance of changes in endogenous cyclic nucleotide levels, or an altered cyclic AMP/cyclic GMP ratio [3] in psoriasis remains unclear at this point.

It also has been suggested that the receptor specific adenylate cyclase system might be defective in psoriatic epidermis, i.e., the accumulation of cyclic AMP after in vitro stimulation with epinephrine and other β-adrenergic agonists [8–10] and prostaglandin E (PGE) [11,12] was shown to be lower in the involved lesion than in the uninvolved skin of psoriasis patients.

Elsewhere we have discussed the fact that whatever the findings may be in regard to cyclic nucleotide levels in an established lesion of psoriasis, such data on the levels alone does not shed any light on the changes which might have occurred at the onset of the lesion. At present this area is still completely unknown [13].

Previous data from our laboratory have shown that in addition to epinephrine [14] and PGE [11] receptors, pig epidermal adenylate cyclase has 2 other independent receptors, histamine H_2 [15,16] and adenosine and adenine nucleotides [17,18]. Consequently in this study we investigated the response of psoriatic epidermis to these 2 other stimulators of adenylate cyclase and compared the responses in the uninvolved and involved skin for each stimulator.

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Abbreviations:
PDE: cyclic nucleotide phosphodiesterase
PGE: prostaglandin E

MATERIALS AND METHODS

Epidermal samples were obtained from 8 adult male patients with well-developed psoriatic lesions. No active treatment was given for at least 7 days before taking the skin. In 1 patient (case 4) who had a concomitant asthma attack, epinephrine inhalation was given until 30 min before taking the skin. No patient had systemic antihistamine for at least a week. Skin was taken from the back except for 2 cases (case 1 and case 2 from the forearm) with a Castroviejo keratome.

In most cases (cases 1, 2, 3, 4, 7, 8) sheets of skin were taken in 1 piece going from uninvolved skin into the involved area with the keratome set to cut at 0.3 mm. This depth of slice is below the epidermis in the normal appearing area, but sometimes does cut off the bottom of the epidermal ridges in the involved area. In cases 5 and 6 the involved area was cut separately with the keratome set at 0.5 mm to obtain below the epidermal ridges. Neither systemic nor local anesthesia was given to avoid the possible effect on cyclic AMP level of skin. The removed skin was kept in Hank's balanced salt solution at 4°C and used within 1 hr. Incubation was done as described previously [14,15] after preincubation for 15 to 20 min at 37°C [19]. Cyclic AMP levels in the epidermis were measured by Gilman's protein binding method [20] with slight modification [19]. Cyclic AMP levels in the incubation media were measured by the same binding assay after chromatographic purification. Namely, the media in which skin tissues had been incubated were applied to Dowex AG 1 × 8 formate columns and the cyclic AMP fractions were eluted by 2 N formic acid. The eluates were freeze-dried, reconstituted with small amounts of water, further purified with $ZnSO_4$-$Ba(OH)_2$ precipitation, and finally subjected for the protein binding assay. Protein was measured by the method of Lowry, et al [21].

Chemicals and drugs were all prepared fresh before each experiment and the pH of the media was adjusted to 7. Epinephrine was the product of Parke Davis (Detroit, Michigan). All of the other chemicals were purchased from Sigma Chemical Company (St. Louis, Missouri).

RESULTS

Figure 1 shows that the addition of histamine increased the cyclic AMP level in both uninvolved and involved epidermis, but the cyclic AMP level was much higher in the involved epidermis. The responses were short lived and the peak was observed in about 5 min. The addition of the phosphodiesterase inhibitor (theophylline) markedly potentiated the effect of histamine in both uninvolved and involved epidermis, and even at 20 min after the addition of histamine and theophylline, the cyclic AMP level was still high. With the addition of both agents the level of cyclic AMP was also higher in the involved epidermis. A time course study of histamine activation was repeated with skin from case 3 (0.3 mm depth for both uninvolved and involved skin). Although the maximal accumulation of cyclic AMP was much less than that in case 5 (Fig 1), the peak again was reached in 5 min and followed by decline.

Figure 2 is the effect of AMP in the uninvolved and involved epidermis. Since theophylline is a competitive inhibitor for the

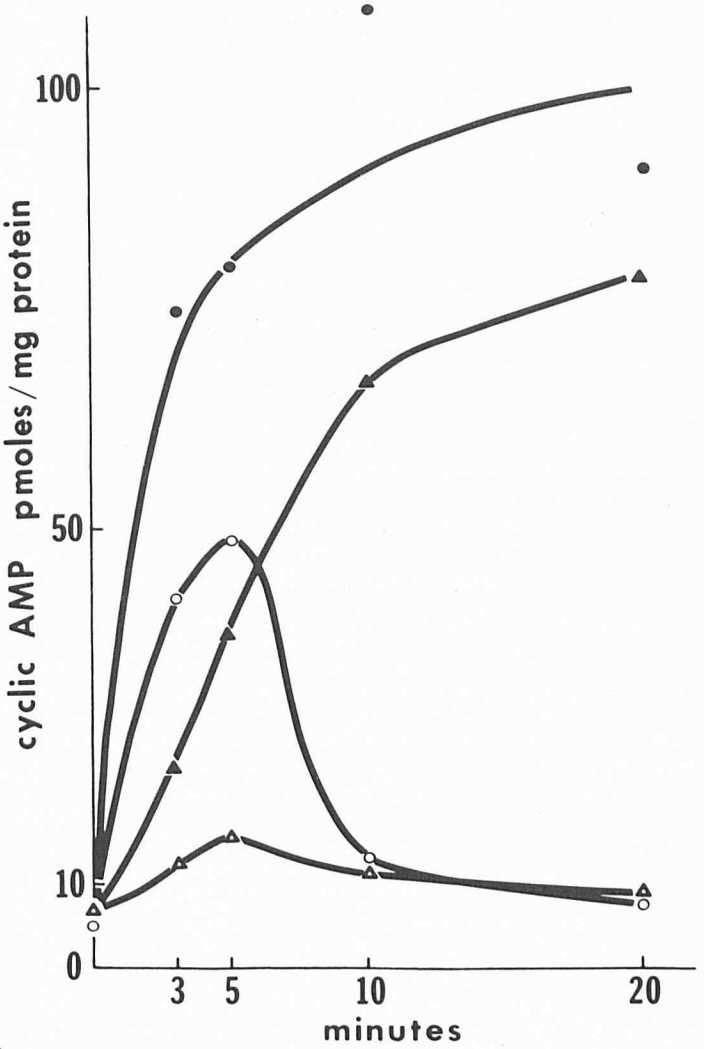


FIG 1. Time course of the effect of histamine in the uninvolved (0.3 mm depth) and involved (0.5 mm depth) epidermis (case 5). △ = Uninvolved, histamine (1 mM); ▲ = uninvolved, histamine (1 mM) + theophylline (5 mM); ○ = involved, histamine (1 mM); and ● = involved, histamine (1 mM) + theophylline (5 mM).

adenosine and adenine nucleotides receptor [17], papaverine was used as the phosphodiesterase inhibitor in this case. Essentially the same pattern of cyclic AMP accumulation was observed after AMP stimulation as was seen after histamine stimulation except for the fact that the difference between involved and uninvolved skin was much less marked. The phosphodiesterase inhibitor also potentiated the effect of AMP in both uninvolved and involved epidermis, and after stimulation no difference was seen between involved and uninvolved skin.

Table I shows the effects of histamine and AMP at different concentrations with the PDE inhibitor. The cyclic AMP accumulation appeared to be dependent on the concentration of histamine added with the saturation concentration being between 100 μM and 1 mM. The data suggests that 1 mM of AMP was sufficient to cause the maximal accumulation of cyclic AMP, our previous study with pig skin had also shown the maximal stimulation of AMP at 1 mM [17]. Either PDE inhibitor alone gave minimal effect on the cyclic AMP level. Because of numbers of observation points were not sufficient, the apparent Km values were not calculated. Apparently the responses to variable substrate levels of histamine and AMP are approximately the same between the uninvolved and involved epidermis: hence the Km values may be assumed to be similar between the uninvolved and involved skin.

Table II shows the effects of histamine, AMP (or adenosine) and epinephrine on the cyclic AMP levels in the uninvolved and involved epidermis. Although the responses to 3 stimulators were markedly variable from case to case, the uninvolved skin

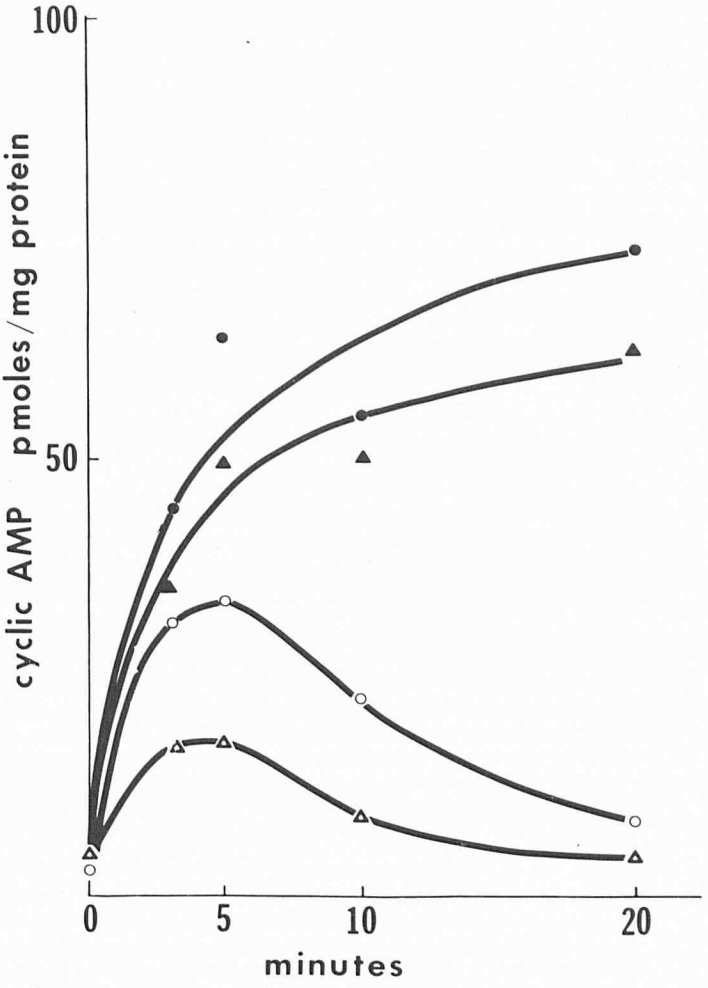


FIG 2. Time course of the effect of AMP in the uninvolved (0.3 mm depth) and involved (0.5 mm depth) epidermis (case 5). △ = Uninvolved, AMP (1 mM); ▲ = uninvolved, AMP (1 mM) + papaverine (100 μM); ○ = involved, AMP (1 mM); and ● = involved, AMP (1 mM) + papaverine (100 μM).

TABLE I. *Effect of histamine (A) and AMP (B) concentrations*

A. Incubation was done for 7 min with various concentrations of histamine + 5 mM theophylline. Skin was taken from case 6 (uninvolved 0.3 mm depth, involved 0.5 mm depth).

	Cyclic AMP (pmoles/mg protein)	
	Uninvolved	Involved
No Addition	2.2	3.1
1 μM Histamine	3.5	3.5
10 μM	5.0	4.5
100 μM	8.4	9.0
1 mM	9.0	10.7

B. Incubation was done for 7 min with various concentrations of AMP and 100 μM papaverine. Skin was taken from case 8 (uninvolved 0.4 mm depth, involved 0.4 mm depth). ND = not determined.

	Cyclic AMP (pmoles/mg protein)	
	Uninvolved	Involved
No Addition	1.1	0.7
1 μM	ND	3.9
10 μM	3.6	5.8
100 μM	14.6	9.3
500 μM	ND	16.0
1 mM	22.1	19.2

TABLE II. *Effects of histamine, AMP and epinephrine on the epidermal cyclic AMP accumulation*

After the pre-incubation (15–20 min), the skin from uninvolved (U) and involved (I) were incubated with 3 stimulators for 7 min. Data are expressed as cyclic AMP pmoles/mg protein. Concentrations of the drugs added to the media were: Histamine = 1 mM, Adenosine or AMP = 1 mM, Epinephrine = 50 μM. Theophylline (5 mM) was added to the media for the histamine and epinephrine experiments, papaverine (100 μM) was added for the AMP (adenosine) experiment. 00 = 0 time immediately after the pre-incubation. Control = 7 min later with no additions.

Case	1		2		3		4[a]		5		6		7		8	
Biopsy site	Forearm		Forearm		Back		Back		Back		Back		Back		Back flank	
	U	I	U	I	U	I	U	I	U	I	U	I	U	I	U	I
Keratome depth (mm)	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.5	0.3	0.5	0.3	0.3	0.4	0.4
00	0.2	1.8	2.0	2.5	1.8	3.1	2.3	2.9	2.3	3.0	0.2	0.5	1.9	4.8	3.0	1.6
Control	0.1	1.8	1.0	4.6	1.2	2.8	1.9	2.5	1.8	1.9	0.2	1.1	0.1	3.8	ND	ND
Histamine	8.0	16.3	5.6	19.1	7.4	10.1	7.3	9.5	52.6	71.0	5.7	6.2	3.5	20.9	4.0	15.0
AMP or Adenosine	24.0	25.8	19.3	23.3	35	11.4	27.2	32.1	74.5	83.3	7.7	9.0	19.9	38.4	12.9	13.1
Epinephrine	59.6	30.8	68.6	19.4	149	14.5	14.4	10.3	58.3	37.8	35.0	4.0	ND	ND	21.1	6.0

[a] Asthmatic patient, epinephrine inhalation was used until 30 min prior taking the skin.

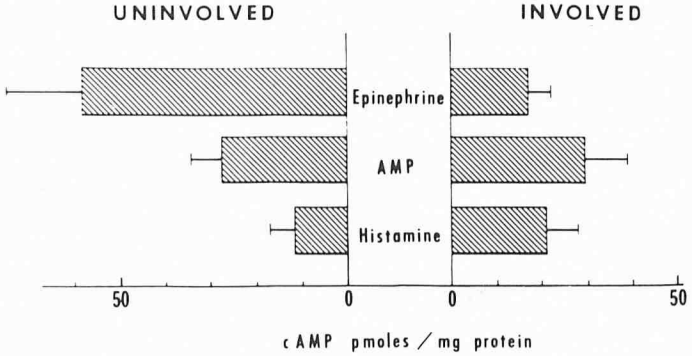


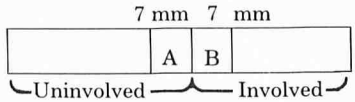
FIG 3. Differential responses to histamine, AMP and epinephrine by the uninvolved and involved skin from psoriatic patients. The averages (± SE) for the epidermal cyclic AMP accumulation are computed from data in Table II. For detailed experimental condition c.f. Table II.

consistently showed better response to epinephrine and weaker response to histamine. The response to AMP or adenosine by the uninvolved and involved was about equal except for case 3 and 7 where each uninvolved and involved skin showed better response respectively. This general tendency is summarized in Fig 3. The difference in the histamine response between the uninvolved and involved skin was analyzed by a paired Student *t*-test and was statistically significant ($p < 0.01$). The difference in response to epinephrine is marked and significant: the involved skin lost nearly 75% of it's responsiveness. The results are in accordance with our previous data [9]. To exclude the possibility that the cyclic AMP-leakage into medium during the incubation of psoriatic skin with epinephrine and theophylline, thereby the involved psoriatic skin showed apparently lower response to epinephrine, we also measured the cyclic AMP levels in the incubation media in which psoriatic tissues had been incubated with epinephrine and theophylline for 7 min. For this purpose, we purified cyclic AMP in the media by column chromatography as described in "Methods" in order to avoid the possible interference by substances in the media. The results clearly show that up to 7 min of the incubation period, the leakage of cyclic AMP in the medium is none or negligible.

Table II also suggests that the basal cyclic AMP levels (00 and controls) in the involved epidermis are higher than those in the uninvolved. Since these values are those after the pre-incubation and do not represent an endogenous (steady state) cyclic AMP level, the significance of this difference awaits elucidation. The cyclic AMP level which has increased transiently due to ischemia effect [19,22] after biopsy gradually declines during the pre-incubation period.

Table III shows the effect of epinephrine at the transitional zone of a psoriatic plaque. Due to the limited area of the transitional zone, only the epinephrine response was investigated. Clearly, the involved epidermis responded weakly as

TABLE III. *Effect of epinephrine at the transitional zone*



A: Uninvolved transitional zone. B: Involved transitional zone.

Skin was taken as a single sheet and each strip of 7 mm width from the boundary was used as the transitional zone. The arrow indicates clinical separation between the involved and uninvolved. The cyclic AMP value with no addition of epinephrine was 0.4–3.6 pmoles/mg protein. Incubation period was for 7 min with 50 μM epinephrine and 5 mM theophylline.

Case	Cyclic AMP pmoles/mg protein			
	Uninvolved		Involved	
	Uninvolved	Uninvolved Transitional (A)	Involved Transitional (B)	Involved
1	92.0	63.6	60.8	ND[a]
2	82.4	78.7	36.1	17.7
3	149	141	56.9	14.5
8	ND	52.0	10.6	6.0

[a] ND = not determined

compared with the uninvolved epidermis, and the transitional responses were in-between. Thus, the uninvolved epidermis adjacent to psoriatic lesion responded less than the uninvolved but better than the lesional epidermis at the edge did. Also, the lesional epidermis at the border responded better than the involved skin (c.f. a diagram in Table III).

DISCUSSION

Our data show that histamine and adenosine (or AMP) cause cyclic AMP accumulation in psoriatic human skin (uninvolved and involved). Since these effects were highly potentiated by the addition of phosphodiesterase inhibitors, it seems they act on the adenylate cyclase as is the case in pig skin [15,17]. These histamine and AMP responses by human skin appears generally to be somewhat weaker than those of pig skin. Since we did not study the response of normal human epidermis, the question of whether this difference is due to a species difference or due to the disease remains unclear.

The major purpose of this study was to see if different responses to the 3 adenylate cyclase stimulators occurred between uninvolved and involved skin. Variability in degrees of responsiveness between the patients was observed in the 8 cases (Table II). The cause of this variability remains unclear at this time, but it is interesting to note that in case 4 (who had epinephrine inhalation 30 min before the biopsy), the epinephrine response was very weak and there was no difference in response between the uninvolved and involved epidermis. In this case, the previous exposure to the epinephrine may have induced refractoriness to the epinephrine response [23,24]. The

variability might also be in part explained by contamination of different dermal components. The thickness of the involved psoriatic epidermis is quite variable [25]. In most of our cases we could obtain whole layers of epidermis from the uninvolved area by a keratome with a 0.3 mm setting, but this depth was sometimes not deep enough to get whole epidermis from the involved area. The thickness of the skin is variable with the angle of the cutting blade, the pressure applied, and the speed of cutting [26].

In spite of the variability of responses from patient to patient, if we compare the responses in each case, the epinephrine response is markedly different from the histamine and AMP response, i.e., the epinephrine response (and probably the PGE response [11,12]) was much higher in the involved epidermis. It should be noted that this tendency was the same even when we compare the effect of agents on skin cut so as to minimize the effect of different dermal components (case 5, 6). The physiological significance of this apparent discrepancy between the receptor specific responses remains to be solved, however, these results suggest that the total catalytic activity of the psoriatic plaque might not be defective even though the epinephrine receptor system of adenylate cyclase is severely defective in the lesion. This is compatible with the results of Härkönen, Hopsu-Hava, and Raij [5], who showed that the response of adenylate cyclase to sodium fluoride was similar in normal and psoriatic epidermis.

As far as the epinephrine effect at the transitional zone is concerned, our data suggest that the zone does not represent the characteristics of the purely involved or purely uninvolved epidermis. It would be of interest to study the transition zone in a lesion known to be evolving or clearing.

In summary, these results indicate that the 4 known sites for activation of adenyl cyclase are affected in quite specific fashion when the normal-appearing skin become a psoriatic plaque. The plaque loses almost 75% of its responsiveness to epinephrine, loses about 50% of its responsiveness to PGE, is unchanged in response to AMP or adenosine, and gains 80% in responsiveness toward histamine. Although these changes are not known to be specific for psoriasis, they do make it unlikely that the unresponsiveness to epinephrine is due to a generalized inability of the cell membrane to make proper, functioning receptors.

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