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EPINEPHRINE-INDUCED CYCLIC AMP ACCUMULATION IN THE PSORIATIC EPIDERMIS

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Abstract. Although there are many reports concerning different β-adrenergic responsiveness in involved and uninvolved skin of psoriasis, previous experiments have been done mainly by using keratome-sliced skin, which contains unknown amounts of stratum corneum, dermis, skin appendages, etc. In order to determine the effect of epinephrine on the cyclic AMP level of 'pure' epidermis in psoriasis, a microdissection technique was employed. Basal levels of cyclic AMP in the involved epidermis were slightly higher than in the uninvolved epidermis (involved 1.9±0.3 pmoles/mg dry weight; uninvolved 1.3±0.3 pmoles/mg d.w.). This difference was not statistically significant ($p>0.1$). The response to epinephrine by the involved epidermis (8.4±1.0 pmoles/mg d.w.) was much lower than that in the uninvolved epidermis (23.3±4.3 pmoles/mg d.w.). The difference was statistically highly significant ($p<0.005$). Our data show that psoriatic involved epidermis per se had a reduced β-adrenergic responsiveness, which might be significantly involved in the pathophysiology of psoriasis.

Key words: Cyclic AMP; Epinephrine; Adenylate cyclase; Psoriasis

Defective β-adrenergic responsiveness in the involved skin of psoriasis has been reported by several investigators (11, 18, 19, 27). These experiments, however, were usually carried out on keratome-sliced skin, which contains unknown amounts of stratum corneum, dermis, skin appendages, etc., all of which would prevent accurate measurement of the pure epidermal cyclic AMP level (28). It is rather difficult to evaluate the results obtained from two such histologically different tissues as the involved and uninvolved skin in psoriasis, as discussed by Adachi et al. (1). Consequently, pure epidermal hormone responsiveness of adenylate cyclase remains unknown at this point, and this histological variance or heterogeneity might in part explain some of the differences in hormone responsiveness reported by us and other investigators (12, 31).

Recently Gommans et al. (4), using an isolated keratinocyte suspension, reported defective β-adrenergic responsiveness in the involved 'epidermis' of psoriasis. They isolated keratinocytes by trypsinization, however, which due to its proteolytic action might result in significant cell surface alteration and therefore, modulate the activity of epidermal adenylate cyclase which is an enzyme complex within the cell membrane. Actually, trypsin, at relatively higher concentrations, increases cyclic AMP levels of the skin and decreases β-adrenergic adenylate cyclase responsiveness (13).

In this communication, we report that pure epidermal β-adrenergic responsiveness in the involved skin of psoriasis is indeed lower than that in the uninvolved epidermis. Although decreased β-adrenergic responsiveness might not be specific to psoriasis and may be a concomitant of any rapidly proliferating epithelium (27), this defective response might be significantly involved in the pathophysiology of psoriasis, since cyclic AMP modulates many cellular events in relation to differentiation or proliferation of epidermis.

MATERIALS AND METHODS

Eleven psoriatic patients (5 males and 6 females) aged 16–70 years were investigated. All of these patients had been classified as having plaque-type psoriasis. No active treatment was given for at least a week. After local anesthesia with 0.5% xylocaine (without epinephrine), the skin samples were obtained by 7 mm punch from involved and uninvolved areas of psoriasis. Since the transitional zone between the involved and uninvolved area of psoriasis does not represent the characteristic response to epinephrine (12), the biopsy sites were carefully chosen to avoid these areas.

The biopsy samples thus obtained were washed three times in Hanks' balanced salt solution (HBSS) at 4°C and each piece was cut vertically with a razor blade into two portions, as shown in Fig. 1. Skin pieces were then pre-incubated in HBSS for 15 min at 37°C to standardize the cyclic AMP level (29). After the pre-incubation, each skin

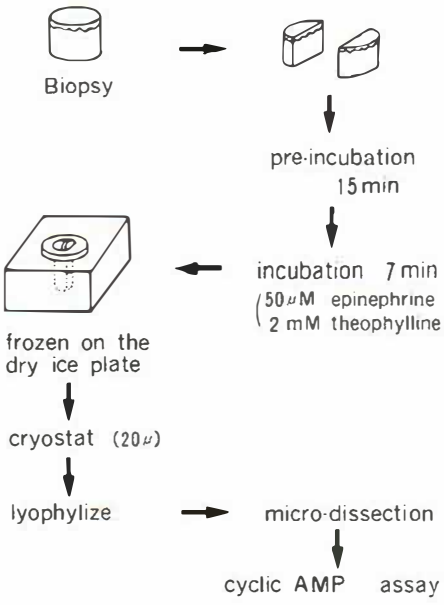

Fig. 1. Scheme of experimental procedure.

half-piece from involved and uninvolved areas of psoriasis was incubated for 7 min with 50 μM epinephrine and 2 mM theophylline in HBSS at 37°C. The concentration of epinephrine was previously shown to be sufficient for the maximal accumulation of cyclic AMP, which is reached after 7–10 min in the presence of phosphodiesterase inhibitor (theophylline) (30).

For control purposes, the other half-pieces were incubated in HBSS for 7 min, too. After the incubation, each skin piece was placed in and frozen on the cryostat adaptor, which had already been chilled on dry ice (see Fig. 1). The samples were frozen in less than 10 sec and the frozen samples were cut in a cryostat at 20 μm thickness, at right angles to the skin surface.

Epidermis and dermis were dissected under a stereomicroscope after lyophilization of the cryostat sections at −30°C. Keratin layers were also removed from the epidermis. The pure epidermal tissues thus obtained were weighed on a quartz microbalance (17), and tissues weighing 20–50 μg were placed in test tubes and the cyclic AMP content in these microdissected epidermis samples was determined.

Cyclic AMP content was determined by the radioimmuno-assay of Steiner et al. (23) without deproteinization according to the method of Honma et al. (10). With such small amounts of tissue, there was no significant difference in the cyclic AMP level determined in the presence vs. absence of deproteinization procedure (10, 15). Data were expressed per mg dry weight and each value was the mean of at least two microdissected samples. Statistical analysis was done by Student's *t*-test.

The reagents of cyclic AMP assay were obtained from Yamasa Shoyu Co., Ltd., Tokyo, Japan. Epinephrine was the product of Daiichi Pharmaceutical Co., Osaka, Japan, and theophylline was obtained from Tokyo Kasei Kogyo Co., Tokyo. Chemicals were prepared fresh before each experiment, and the pH of the medium was adjusted to 7.

RESULTS

Table I shows the effect of epinephrine on the cyclic AMP levels in the involved and uninvolved epidermis of psoriasis. Control values represent cyclic AMP levels of epidermis which was incubated in media, without epinephrine or theophylline. Theophylline alone had little effect on the cyclic AMP levels of epidermis (data not shown). As can be seen, both involved and uninvolved epidermis responded to epinephrine (plus theophylline), resulting in the accumulation of cyclic AMP. Although a marked variation in the cyclic AMP accumulation was noted from case to case, a decreased epinephrine-induced cyclic AMP accumulation in the involved epidermis was seen in all patients. The variability of epinephrine response was more marked in the uninvolved epidermis

Table I. *Effect of epinephrine on the epidermal cyclic AMP accumulation*

After the pre-incubation for 15 min, each sample from involved (I) and uninvolved (U) skin was incubated with 50 μM epinephrine in the presence of 2 mM theophylline for 7 min. Data are expressed as cyclic AMP pmoles/mg dry weight. 'Control' denotes the value of the skin piece which was incubated in the media without epinephrine or theophylline for 7 min

Case no.	1		2		3		4		5		6		7	
Sex	M		F		M		M		F		F		F	
Age	32		66		27		50		64		38		19	
Biopsy site	Arm		Leg		Back		Chest		Arm		Arm		Back	
	U	I	U	I	U	I	U	I	U	I	U	I	U	I
Control	2.3	2.0	1.0	2.1	1.3	1.6	0.4	0.25	1.3	2.5	1.1	2.3	2.5	2.9
Epinephrine + theophylline	42.3	5.4	16.8	8.8	9.9	4.6	16.3	6.0	53.8	11.9	18.1	6.8	5.7	4.5

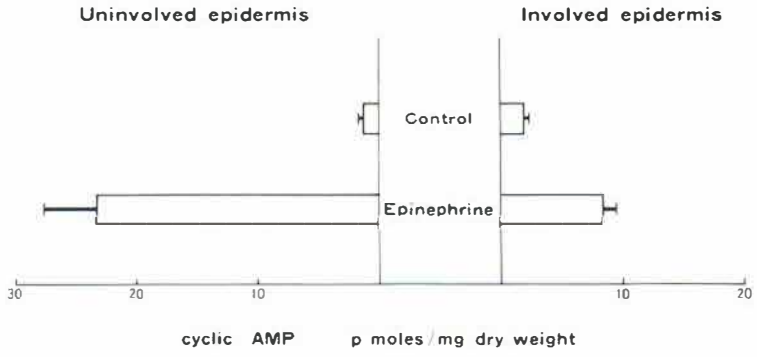

Fig. 2. Effect of epinephrine on the 'pure' epidermal cyclic AMP level in psoriasis. The averages (+ S.E.) for the epidermal cyclic AMP accumulation are computed from the data in Table I. For detailed experimental conditions, see Table I.

(range 5.7–53.8 pmoles/mg dry weight) than in the involved epidermis (range 4.5–13.2 pmoles/mg d.w.) but did not correlate with age of patients or biopsy sites.

The general tendency is summarized in Fig. 2. For the control (basal) values, the mean cyclic AMP level in the involved epidermis was 1.9±0.3 pmoles/mg d.w. and that of uninvolved epidermis was 1.3±0.3 pmoles/mg d.w. The difference was not statistically significant ($p>0.1$). For the epinephrine responses, the mean cyclic AMP level in the involved epidermis was 8.4±1.0 pmoles/mg d.w. and that of uninvolved epidermis was 23.3±4.3 pmoles/mg d.w. The difference was statistically highly significant ($p<0.005$). It was shown that psoriatic involved epidermis, when compared with the uninvolved epidermis, lost nearly 65% of its responsiveness to epinephrine.

DISCUSSION

Our data clearly indicate that psoriatic involved 'pure' epidermis responded defectively to epinephrine, resulting in a decreased cyclic AMP accumulation. The data are consistent with the previous results when using keratome-sliced skin (11, 12, 19, 27, 31) or trypsinized epidermal cell suspension (4); each system has its potential errors, such as dermal contamination (1, 28) and trypsin-induced receptor modifications (13). Application of a microdissection technique could prevent these errors and enabled us to compare pure epidermal epinephrine responses in both sites.

Another notable finding of our study was the marked variation in the epinephrine response, especially in the uninvolved epidermis (Table I). No data are available for comparison on the epinephrine response in the normal human epidermis. Although it is reported that in some tissues (6, 21) the density of β-adrenergic receptor declines significantly with age, there was no correlation in our study—at least for the psoriatic population.

The mechanism of decreased β-adrenergic response in the psoriatic plaque remains unknown at present. Besides that seen in psoriasis, the defective epidermal β-adrenergic response has been seen on several occasions (7, 13, 14, 24). For example, topical application of carcinogen or tumor promotors on mouse skin in vivo reduced isoproterenol-induced cyclic AMP accumulations (7, 24). Epinephrine-adenylate cyclase responsiveness decreased markedly during the long-term incubation in in vitro conditions (14). In the same system, hydrocortisone was shown to have a protective effect on the β-adrenergic adenylate cyclase system in epidermis (14). These observations suggest that the epidermal β-adrenergic adenylate cyclase system is relatively unstable or under physiological regulation and can be easily modified by both endogenous and exogenous manipulations. On the other hand, the histamine adenylate cyclase system in epidermis seems to be much more stable (13, 14).

	9		10		11	
M	F		F		M	
I	16		70		40	
Abdomen	Leg		Leg		Arm	
U I	U	I	U	I	U	I
0.5 0.6	0.6	1.5	0.8	2.0	2.8	3.3
4.2 8.6	31.3	12.2	25.4	10.7	22.7	13.2

It is interesting to note that the adenylate cyclase defect in the psoriatic plaque is relatively specific to the epinephrine system and histamine response was not reduced in the psoriatic involved epidermis (4, 12, 31). Previously we reported that the epidermal β-adrenergic adenylate cyclase system was markedly impaired after the trypsin treatment, whereas the histamine adenylate cyclase system was well preserved (13). The relatively specific β-adrenergic adenylate cyclase defect, therefore, might be due to the endogenous trypsin- or chymotrypsin-like neutral protease, which was suggested to be increased in the psoriatic plaque (9, 16).

The defective β-adrenergic adenylate cyclase response in the psoriatic plaque might then be significantly involved in the pathophysiology of psoriasis. Accumulating evidence suggests that the cyclic AMP system participates in the processes of many epidermal cell functions, such as cell proliferation, differentiation, etc. (8, 25). The epinephrine adenylate cyclase system is known to inhibit epidermal cell proliferation, at least in the G_2 phase of the cell cycle (3, 20, 32). Consequently, a decreased β-adrenergic adenylate cyclase response might be closely related to an increased cell proliferation rate, such as can be seen in psoriasis (5, 26). The carcinogen or tumor promotor-induced defective β-adrenergic response (7, 24) is a most interesting finding in this context, since topical application of these agents has been known to induce a hyperproliferative state of epidermis in vivo (2, 22).

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