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Calmodulin Activities Are Significantly Increased in Both Uninvolved and Involved Epidermis in Psoriasis

TOSHIHIRO MIZUMOTO, M.D., YOSHIO HASHIMOTO, M.D., MASAKI HIROKAWA, M.D.
NORITAKA OHKUMA, M.D., HAJIME IIZUKA, M.D., AND AKIRA OHKAWARA, M.D.

Department of Dermatology, Asahikawa Medical College, Asahikawa, Japan

In order to elucidate a part of calmodulin actions in the hyperproliferative state in human epidermis, calmodulin activities in the psoriatic and in the normal human epidermis were determined using calmodulin-deficient phosphodiesterase from bovine heart and purified pig skin epidermal calmodulin as a standard. Skin samples were obtained from 11 normal healthy controls and from both the uninvolved and involved regions of 8 nonconsanguineous psoriatic patients. Pure epidermal samples, prepared by the microdissection method, were used for calmodulin assays. Normal human epidermis contained 270 ± 13 ng/mg dry weight, whereas calmodulin activities were significantly increased in psoriatic epidermis, 412 ± 29 ng/mg dry weight for the uninvolved epidermis and 747 ± 46 ng/mg dry weight for the involved epidermis, respectively. These results suggest that calmodulin may play an important role in cell proliferation in human epidermis.

Calmodulin is known as a ubiquitous multifunctional calcium-dependent protein present in the cytosol of a wide variety of eukaryotic cells. Recent reports have revealed that calmodulin is involved in cell proliferation or cell division in several cell systems [1,2]. In addition, it was also observed that calmodulin levels were elevated in virally transformed cells [3,4] and neoplastic tissues of human mammary gland [5] and rat liver [6].

Since the demonstration of mouse epidermal calmodulin by Murray and Rogers in 1978 [7], its existence was also confirmed by pig skin [8] and human epidermis [9].

However, how epidermal calmodulin is implicated in the induction of the hyperproliferative and/or neoplastic state in human epidermal keratinocytes remains obscure. Recently it was shown that calmodulin levels were markedly elevated, approximately 30 times in the psoriatic lesion as compared to the uninvolved and normal epidermis [10]. Although this observation was later supported by Tucker et al [11], according to their data it was indicated that uninvolved epidermis in psoriasis contained somewhat higher amounts of calmodulin as compared to those in involved epidermis. In contrast with these observations, our previous data have shown that no critical difference of its activities was observed in either involved or uninvolved epidermis in psoriasis [12]. A possible reason for this discrepancy is that we used a Castroviejo keratome in the previous study to obtain epidermal samples as assay materials.

This study was undertaken to resolve this discrepancy, by using pure epidermal samples, prepared by the microdissection method.

MATERIALS AND METHODS

Purification of pig skin epidermal calmodulin has been reported elsewhere [8,13]. Calmodulin-deficient phosphodiesterase was prepared from bovine heart as previously described [8].

Skin samples for calmodulin determination were obtained from various sites from 11 normal healthy volunteers without any skin diseases (ages varied from 8–83 years) and 8 nonconsanguineous psoriatic patients (ages varied from 39–64 years) by excision biopsies under local anesthesia, using 0.5% lidocaine with no addition of epinephrine (Fujisawa Pharmaceutical Co. Osaka, Japan). All the skin specimens of psoriatic patients were taken from both plaque and uninvolved skin (5 cm distance from plaque). Each skin sample thus obtained was immediately frozen in liquid nitrogen and sliced at 16 μm thickness in the cryostat, set at −25°C, and then freeze-dried overnight with the lyophilizer. The horny layer and dermal constituent of these freeze-dried skin slices were removed under the stereomicroscope to provide pure epidermal samples for the calmodulin measurement as was precisely described previously by Adachi and Yamasawa [14]. The weight of these pure epidermal samples was measured by means of the quartz microbalance.

The procedure of calmodulin assay was essentially identical with the method described by Teo et al [15], using calmodulin-deficient phosphodiesterase from bovine heart and pig skin epidermal calmodulin as a standard. Each assay was performed in triplicate. Statistical analysis of the data was carried out by Student's t-test. Cyclic AMP and 5′-nucleotidase grade III from *Crotalus adamanteus* were obtained from Sigma Chemical Co. (St. Louis, Missouri). All other chemicals were of analytical grade and were purchased from Nakarai Chemicals, Ltd. (Kyoto, Japan).

RESULTS

Calmodulin prepared from pig skin epidermis migrated as a single band on sodium dodecyl sulfate-polyacrylamide gel electrophoresis and molecular weight was estimated at approximately 16,500–17,500 (data not shown). As can be seen in Fig 1, calmodulin-deficient phosphodiesterase prepared from bovine heart was activated by the purified epidermal calmodulin in the presence of calcium.

Calmodulin activities in individual skin samples were as follows. In normal human epidermis, calmodulin activity was 270 ± 13 ng/mg dry weight. In contrast to this, its activities were much higher in both the uninvolved and involved epidermis of psoriasis. It was shown to be 412 ± 29 ng/mg dry weight for the uninvolved epidermis ($p < 0.001$ when compared with normal human epidermis) and 747 ± 46 ng/mg dry weight for the involved epidermis ($p < 0.001$ when compared with the uninvolved epidermis) (Fig 2).

DISCUSSION

As mentioned previously, it has been shown by several authors [10,11] that calmodulin may play an important role in the induction of its hyperproliferative state in psoriatic epidermis. According to these reports, although calmodulin activities were elevated in the involved epidermis in psoriasis, there was a great difference in its activities in the uninvolved epidermis in psoriasis. While Van de Kerkhof and Van Erp reported that calmodulin levels were increased more than 30 times in the psoriatic lesion in comparison with those in normal and uninvolved epidermis [10], Tucker et al found that increases of calmodulin levels were approximately 6–8 times in both uninvolved and involved epidermis in psoriasis as compared to normal counterparts [11]. According to our previous study, however, there were no significant differences in calmodulin activities between involved and uninvolved epidermis in psoriasis, when a Castroviejo keratome was used to obtain epider-

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Reprint requests to: Toshihiro Mizumoto, M.D., Department of Dermatology, Asahikawa Medical College, 3-11 Nishikagura, Asahikawa, 078-11, Japan.

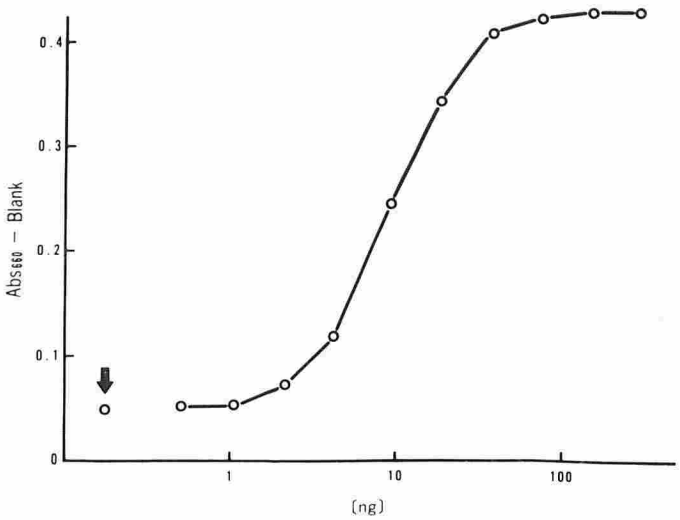

FIG 1. Standard curve of calmodulin assay for calmodulin-deficient cAMP phosphodiesterase. Standard assays were performed at pH 7.5 and 30°C for 30 min. The reaction mixtures of 0.5 ml contained, in addition to 50 μl of the phosphodiesterase and various amounts of pig skin epidermal calmodulin (*abscissa*), 60 mM each of Tris and imidazol, 6 mM magnesium acetate, 2 mM cAMP, 50 μM calcium chloride, and 0.2 units of 5′-nucleotidase. Inorganic phosphate released was determined as previously described [8]. Each *point* denotes a mean of duplicate assays. The *arrow* indicates 0 value.

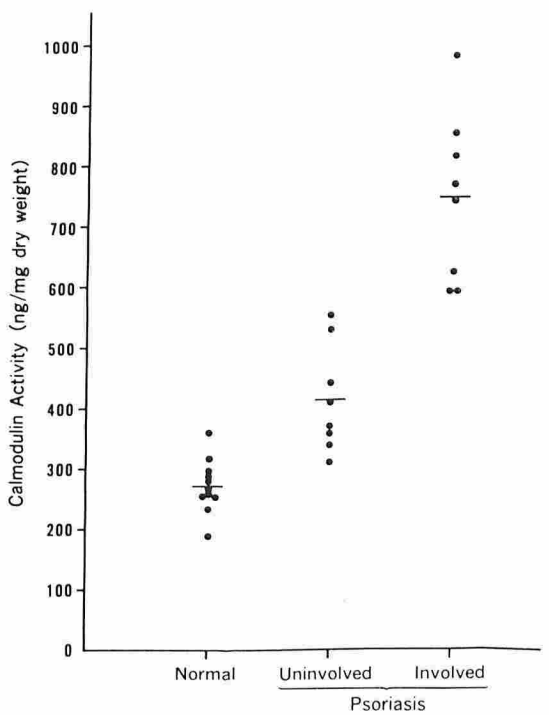

FIG 2. Calmodulin activities in the normal human and the psoriatic epidermis (uninvolved and involved). Each *point* denotes a mean of triplicate assays of individual skin samples.

mal assay materials [12]. As a possible explanation for the inconsistent values of calmodulin levels in the involved and uninvolved psoriatic epidermis found by these investigators, aside from the methodologic differences of calmodulin assay, it could be assumed to be due to the difference in skin samples examined. When skin samples were taken with a Castroviejo keratome or razor blade, a certain dermal contamination could not be avoided. Therefore, the microdissection method was employed in the present study in order to obtain pure epidermal samples. Similar evidence had already been presented by Iizuka et al [16] in the cyclic nucleotide-phosphodiesterase assay in psoriasis.

By using pure epidermal samples thus obtained, it was shown that calmodulin activities in the uninvolved and involved epidermis in psoriasis were increased as compared to those of normal human epidermis (Fig 2). It should be reemphasized from our data, that in determining the biologically active substances in epidermal keratinocytes, one should use pure epidermal samples as the analytical materials.

Although most of the epidermal phosphodiesterase is not regulated by calmodulin [8], in recent years the biologic role of calmodulin on the cell cycle has been suggested [2,17]. Even if our data of increased calmodulin activities in psoriatics are consistent with those assumptions, their precise interrelation remains unknown. In this connection, it is suggested that calmodulin might also play an appreciable role in the regulation of its cellular biologic process in human epidermal keratinocytes.

From the present results, we would like to draw the following conclusions: (1) normal human epidermis contains a significant amount of biologically active calmodulin, as was suggested by Peterson and Wuepper [9]; (2) psoriatic epidermis (both the uninvolved and involved) has higher activities of calmodulin than normal human epidermis; (3) calmodulin may also participate in the initiation of the hyperproliferative state of human epidermal keratinocytes, as was suggested in psoriasis.

Addendum

During the revision of this manuscript, Fairley et al reported an excellent study on calmodulin levels in normal and psoriatic epidermis (J Invest Dermatol 84:195–198, 1985). Their data were fundamentally coincident with our findings in this manuscript.

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