Increased Cholera Toxin-, and Forskolin-induced Cyclic AMP Accumulations in Psoriatic Involved Versus Uninvolved or Normal Human Epidermis

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Psoriatic involved epidermis reveals variously altered receptor-adenylate cyclase responses; among them the most prominent is defective beta-adrenergic adenylate cyclase response, which is normally the major receptor-adenylate cyclase system of human epidermis. It is known that activation of hormone-stimulated adenylate cyclase, a membrane-bound enzyme complex, requires functional coupling of at least 3 distinct subunits: 1) receptor subunit (R), 2) guanine nucleotide binding protein (G), and 3) catalytic subunit (C). The precise nature of the beta-adrenergic defect in the psoriatic epidermis, however, remains to be determined, especially in terms of G and C function. Using the involved and uninvolved skin from psoriatic patients, we investigated effects of cholera toxin (which monitors G-C interaction) and forskolin (which monitors C function) on the adenylate cyclase system of epidermis, which were compared with those of normal human epidermis. Both agents increased cyclic AMP levels of involved, uninvolved, and normal human epidermis. Marked accumulations were observed in the presence of cyclic nucleotide phosphodiesterase inhibitor, isobutylmethylxanthine (IBMX); without the phosphodiesterase inhibitor, the effect of each agent was minimal. Comparison of the effects of cholera toxin revealed that the psoriatic involved epidermis accumulates much more cyclic AMP than the uninvolved epidermis (involved: 193 ± 65; uninvolved: 117 ± 54 pmoles/mg protein/5 h). Similarly forskolin-induced cyclic AMP accumulations of the involved epidermis were much more than those of uninvolved epidermis (involved: 374 ± 152; uninvolved: 101 ± 41 pmoles/mg protein/2 h). Those of normal human epidermis were not significantly different from those of uninvolved epidermis (cholera toxin: 99 ± 36 pmoles/mg protein/5 h; forskolin: 84 ± 22 pmoles/mg protein/2 h). Our results indicate that G and C function and their interaction is not defective (but rather increased) in the psoriatic involved epidermis. This suggests that the defective beta-adrenergic response of psoriatic involved epidermis reflects defective R or R-G interaction of the epidermal adenylate cyclase system. *J Invest Dermatol 91:154–157, 1988*

Epidermis contains four independent receptor adenylate cyclase systems: 1) beta-adrenergic-, 2) prostaglandin E-, 3) adenosine-, and 4) histamine-receptor adenylate cyclase systems [1]. Among them the beta-adrenergic- and prostaglandin E-adenylate cyclase responses are defective in the psoriatic involved epidermis [2–5]. Because the beta-adrenergic response is the major receptor adenylate cyclase system of normal human epidermis [6,7], and this receptor response is defective in many experimentally-induced hyperproliferative conditions of keratinocytes, it has been suggested that the finding is associated with pathophysiology of the psoriatic hyperproliferative epidermis [8]. The precise mechanism of the defective beta-adrenergic adenylate cyclase response remains unknown at present.

It has been known that hormone-stimulated adenylate cyclase activity reflects the interaction of at least 3 distinct subunits embedded in the lipids of the plasma membrane [9,10]. These are 1) hormone receptor (R), 2) guanine nucleotide binding protein (G), and 3) catalytic subunit (C). Upon agonist (hormone) binding to R, hormone-receptor complex interacts with G (R-G interaction), and the activated G then interacts with C (G-C interaction). This results in the activation of C which catalyzes the conversion of Mg-ATP to cyclic AMP; C of adenylate cyclase is essentially inactive in the absence of G. Thus regarding the beta-adrenergic (as well as prostaglandin E) defect of psoriatic involved epidermis, there are several possibilities; these include defect of each component (R,G, or C) as well as the defective interaction among them (R-G interaction, G-C interaction).

Cholera toxin and forskolin are unique activators of adenylate cyclase, which work on G and C, respectively, and activate each component [11–14]. Thus, using these two chemicals we can estimate the posterior part of adenylate cyclase activation. Forskolin-induced cyclic AMP accumulation directly monitors C function of adenylate cyclase [11], and cholera toxin-induced cyclic AMP accumulation monitors both G and C function, because cholera toxin requires both G and C as well as their interaction for the cyclic AMP accumulation [15]. In the present study, in order to dissect the precise nature of the altered receptor adenylate cyclase responses of psoriatic involved epidermis, we investigated the effects of these two chemicals on the adenylate cyclase system of psoriatic involved and uninvolved epidermis and compared with those of normal human epidermis.

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Abbreviations:
C: catalytic subunit
G: guanine nucleotide binding protein
IBMX: 3-iso-butyl-1-methylxanthine
R: recepter unit
TPA: 12-o-tetradecanoylphorbol-13-acetate

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MATERIALS AND METHODS

Epidermal samples were obtained from backs of 9 adult male patients with well-developed psoriatic lesions. No active treatment was given for at least a week before taking the skin. After 0.5% lidocaine anesthesia, sheets of skin were obtained by keratome biopsy. In most cases the sheets were taken in one piece going from uninvolved into the involved with the keratome set at 0.3 mm. The border between two areas was marked before taking the skin. Normal epidermal samples were obtained from 5 adult males during skin graft operation (thickness 0.3 mm). No dermatologic abnormality was observed at the obtained sites (thigh). The depth of 0.3 mm slice was below the epidermis in the normal and uninvolved area, but sometimes did not cut off the bottom of the epidermal ridges in the involved area. In two cases of psoriatic patients the involved area was cut separately with the keratome set at 0.4 mm to obtain below the epidermal ridges.

The skin sheets were washed 3 times in RPMI 1640 medium, cut into 5 × 5 mm squares, and the skin squares were then floated with their keratin layers up in 10 ml RPMI 1640 medium. Following preincubation at 37°C for 20–30 min to standardize the cyclic AMP level [16], 2–4 skin squares were floated on 3 ml RPMI 1640 medium with various chemicals to be tested. Forskolin was dissolved in ethanol and the final concentration of ethanol was less than 0.5% (v/v). As control for forskolin experiments, only ethanol was added to the medium. Cholera toxin was freely soluble in water. Incubations were performed at 37°C in 5% CO_2 in air. Neither antibiotics nor serum was added to the incubatiion medium. Following the incubation, each skin square was frozen between two plates of dry ice. The cyclic AMP content in each skin square was measured by radioimmunoassay using Yamasa cyclic AMP assay kit (Yamasa Shoyu Co., Tokyo, Japan). No deproteinization procedure was performed during the cyclic AMP assay; the presence of protein had little effect in our cyclic AMP assay system. The recovery of the added known amount of cyclic AMP during the procedure was about the same between the involved, uninvolved, and normal skin (~75%). Protein concentration was measured according to the method of Lowry et al [17] with bovine serum albumin as a standard. The statistical significance of the data obtained was evaluated by Student's t-test.

RPMI 1640 medium was obtained from Biken (Osaka). Cholera toxin from vibrio cholerae (C-3012) was obtained from Sigma (St Louis, MO). Forskolin, 7-deacetyl-7-o-hemisuccinic acid, was the product of Calbiochem (La Jolla, CA). All other chemicals were purchased from Nakarai Chemicals Ltd. (Kyoto, Japan).

RESULTS

Figure 1 shows the representative time course of the effects of cholera toxin (Fig 1*A*) and forskolin (Fig 1*B*) on the psoriatic involved and uninvolved epidermis. The cyclic AMP content gradually increased during the incubation. The addition of IBMX in the incubation medium markedly augmented the effects of cholera toxin and forskolin; without the cyclic AMP phosphodiesterase inhibitor, the effects of each agent were minimal. There was a marked difference in cholera toxin-induced and forskolin-induced cyclic AMP accumulations between psoriatic involved and uninvolved epidermis. Psoriatic involved epidermis accumulates much more cyclic AMP by the addition of each chemical. Normal control skin revealed essentially similar time course in response to cholera toxin and forskolin (data not shown). Although the magnitude of cyclic AMP accumulation was varied from case to case (especially in psoriatic involved epidermis), the time course pattern was strikingly similar among all cases examined (3 psoriatic and 2 normal cases).

The effects of cholera toxin and forskolin were concentration-dependnent and the most marked effect was observed at around 100–300 μg/ml and 100–500 μM, respectively (Fig 2*A*, 2*B*). Again the effects of cholera toxin and forskolin were more marked in the psoriatic involved epidermis. Normal control skin revealed similar concentration curve to those of psoriatic involved and uninvolved epidermis (data not shown). This general tendency was observed in all cases examined for the concentration study (2 psoriatic and 2 normal cases).

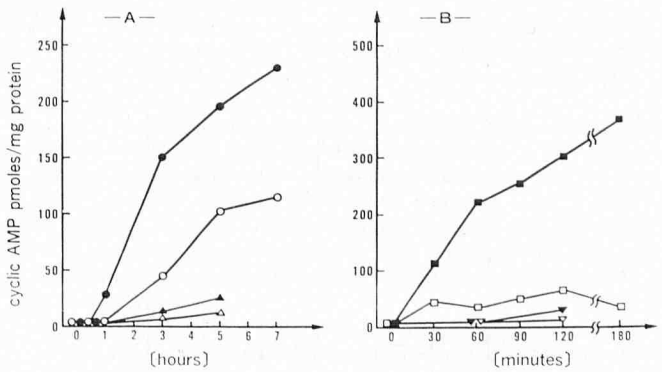

Figure 1. Time course of the effect of cholera toxin *(A)* and forskolin *(B)* on the cyclic AMP levels of psoriatic involved and uninvolved epidermis. Representative data of one of three psoriatic cases examined are shown. Skin squares were incubated with cholera toxin (100 μg/ml) or forskolin (100 μM) in the presence or absence of 1 mM IBMX for the indicated time. ○–○: Uninvolved, cholera toxin + IBMX. ●–●: Involved, cholera toxin + IBMX. △–△: Uninvolved, cholera toxin alone. ▲–▲: Involved, cholera toxin alone. □–□: Uninvolved, forskolin + IBMX. ■–■: Involved, forskolin + IBMX. ▽–▽: Uninvolved, forskolin alone. ▼–▼: Involved, forskolin alone.

Figure 3 shows the results of 9 psoriatic patients and 5 normal controls. Effects of IBMX, cholera toxin, and forskolin were compared between the psoriatic involved, uninvolved, and normal human epidermis. The basal levels of cyclic AMP without the treatment by these stimulators were all around 1–1.5 pmoles/mg protein. Although the responses to IBMX, cholera toxin, and forskolin were variable from case to case, the involved epidermis revealed markedly increased IBMX (Fig 3*A*), cholera toxin (Fig 3*B*), and forskolin (Fig 3*C*) effects compared with uninvolved and normal epidermis. Although the responses of the uninvolved epidermis appeared to be more than those of normal control epidermis, the differences were statistically not significant. The markedly increased cyclic AMP accumulations in the psoriatic involved epidermis were also observed in the two cases where the involved epidermis was obtained separately by 0.4 mm thickness (data included in Fig 3).

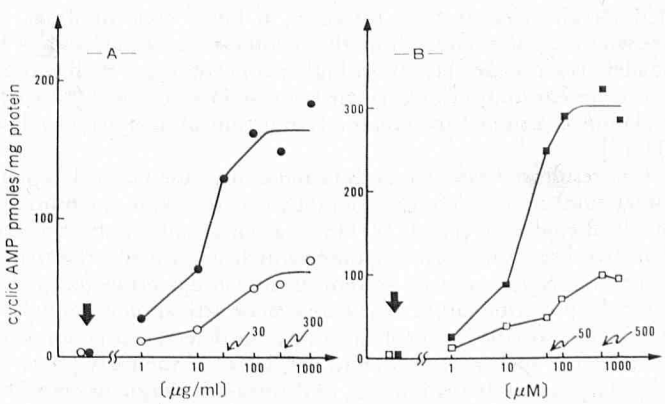

Figure 2. Concentration effects of cholera toxin *(A)* and forskolin *(B)* on the cyclic AMP levels of psoriatic involved and uninvolved epidermis. Representative data of one of two psoriatic cases examined are shown. Skin squares were incubated with various concentrations of cholera toxin *(A)* and forskolin *(B)* in the presence of 1 mM IBMX. The incubation time for the effects of cholera toxin and forskolin was 5 and 2 h, respectively. *Arrows* indicate 0 value. ○–○: Uninvolved, cholera toxin. ●–●: Involved, cholera toxin. □–□: Uninvolved, forskolin. ■–■: Involved, forskolin.

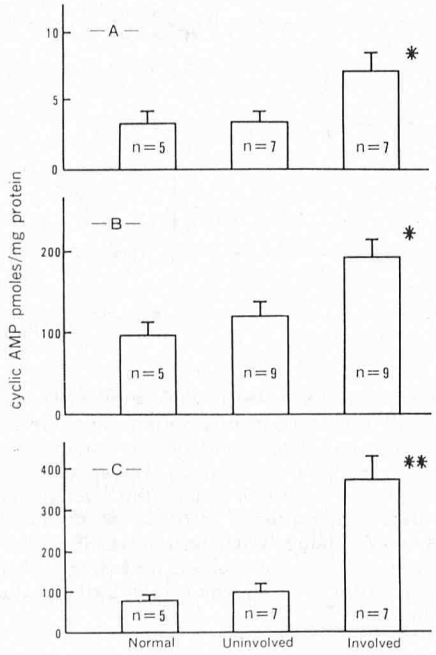

Figure 3. Effects of IBMX *(A)*, cholera toxin *(B)*, and forskolin *(C)* on the cyclic AMP levels of normal and psoriatic (uninvolved and involved) epidermis. Skin squares from 9 psoriatic patients and 5 normal controls were incubated with IBMX (1 mM), cholera toxin (100 μg/ml), and forskolin (100 μM) for 3, 5, and 2 h, respectively. IBMX (1 mM) was added to the incubation medium for cholera toxin and forskolin effects. Data are expressed as cyclic AMP pmoles/mg protein + SE. n = number of cases (IBMX and cholera toxin effects were performed in 7 out of 9 psoriatic cases). * = $p < 0.05$ compared with uninvolved and normal epidermis. ** = $p < 0.01$ compared with uninvolved and normal epidermis.

DISCUSSION

Our experimental procedure was essentially the same as that of Takeda et al [13,14]. In contrast to Ref 14, phosphodiesterase inhibitor, IBMX, markedly augmented the forskolin-induced cyclic AMP accumulations (Fig 1*B*). This is consistent with that forskolin and IBMX work through different mechanisms: forskolin through adenylate cyclase activation (C activation) and IBMX through phosphodiesterase inhibition. Similarly, cholera toxin-induced cyclic AMP accumulation was markedly augmented by the addition of IBMX (Fig 1*A*), the finding consistent with that of Takeda et al [13]. Besides C activation, forskolin, at lower concentrations, is known to reveal its effect through the interaction with G and/or R of adenylate cyclase [11,18]. At higher concentrations such as used in the present study (100 μM), forskolin-induced cyclic AMP accumulation is assumed to monitor C function of adenylate cyclase [11,19].

Our results indicate that both G and C function (as well as their interaction) are not defective (but rather increased) in the psoriatic involved epidermis (Fig 1-3). This is a remarkable contrast to the defective beta-adrenergic response, which is normally the major receptor adenylate cyclase system of human epidermis [6,7]. Because the posterior part of adenylate cyclase activation is not defective in the psoriatic involved epidermis, the defective beta-adrenergic response appears to be due to the defect of the early phase of adenylate cyclase activation, i.e., of R (beta-adrenergic receptor) or of R-G interaction. This should be a marked defect, because the beta-adrenergic defect is consistently observed [2–4] even in the presence of increased G-C and C function (Fig 3). The increased G and C function of psoriatic involved epidermis might reveal a compensatory augmented status of epidermal adenylate cyclase activity, because the effect of IBMX (which reflects the basal adenylate cyclase activity) was also increased in the psoriatic involved epidermis (Fig 3). Obviously the altered adenylate cyclase response to cholera toxin and forskolin is not the genetic epidermal finding of psoriatic patients, because the responses of the uninvolved epidermis were not significantly different from those of normal human epidermis (Fig 3). Similar findings have been reported regarding the beta-adrenergic response; the epinephrine response of the uninvolved epidermis was about the same as that of normal [2,6].

Although the nature of the early phase defect of adenylate cyclase activation in the psoriatic involved epidermis remains to be determined, several possibilities have been suggested. These include defective R [20] as well as defective G function, possibly through ras oncogene product [21,22]. Because the defective beta-adrenergic response is also observed in TPA-induced hyperproliferative epidermis, where the defective R-G interaction has been proposed [23], it might be suggested that hyperproliferative epidermis generally reveals the beta-adrenergic defect through a similar mechanism. Further study would be required to dissect the nature of the beta-adrenergic defect in the psoriatic as well as experimentally-induced hyperproliferative epidermis, because this receptor response has been suggested to be one of the most important regulatory mechanisms of keratinocyte proliferation, which can be modified by several anti-psoriatic agents including glucocorticoids, retinoids, etc. [24,25].

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