Acta Dermatovener (Stockholm) 60: 95–98, 1980

DIFFERENCES IN RESPONSE OF PSORIATIC EPIDERMIS IN CYCLIC AMP ACCUMULATION AGAINST CERTAIN ADENYL CYCLASE AGONISTS

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Abstract. Epidermal slices of pig skin and psoriatic human skin were used in a study on responses of adenyl cyclase to epinephrine, histamine and adenosine. In pig skin, histamine stimulated adenyl cyclase slightly more than epinephrine. The histamine concentration eliciting the maximum cyclic AMP accumulation was 5×10^{-4}M, which was 20 times greater than that of epinephrine. The optimal concentration of adenosine seemed to be an additional 10 times higher (5×10^{-3}M). When involved and uninvolved epidermal specimens from 18 psoriatic patients were incubated with these three agents, cAMP formation with histamine was unchanged or slightly increased in involved epidermis, while the response to epinephrine was markedly depressed and to adenosine moderately so. These findings suggest further that there is a selective defect in the membrane enzyme system in psoriatic epidermis.

Key words: Cyclic AMP; Epinephrine; Histamine; Adenosine; Psoriasis

Defects in epinephrine-sensitive and prostaglandin E sensitive adenyl cyclases in psoriatic epidermis have been reported (10, 11, 1) and a role in the pathogenesis of psoriasis has been proposed. There is also evidence to suggest that a membrane abnormality exists in involved psoriatic epidermis (8, 7). Recently Iizuka and his associates demonstrated histamine (H_2) and adenosine-sensitive adenyl cyclase systems in the epidermis (2, 3). According to their results, the response to histamine is markedly increased in involved epidermis in psoriasis, while the response to adenosine remains unchanged compared with that of uninvolved epidermis (5). As we have obtained slightly different results, these are now presented.

MATERIALS AND METHODS

Fresh pig skin was obtained from a local slaughterhouse, and epidermal sheets were removed using a Castroviejo Keratotome set at 0.2 mm. Histological observation revealed these specimens to contain the entire epidermis with a small contamination from the upper dermis.

Lesional and adjacent non-lesional epidermis was also taken in the same way from 18 psoriatic patients who were either untreated or who had had donor sites shielded from topical 8-Methoxypsoralen application plus long-wave ultraviolet light irradiation. To obtain the patients' cooperation, donor sites were prepared with local anesthesia prior to the procedure and consequently all samples were washed in two changes of ice-cold Hanks' media for at least one hour before use. Samples from uninvolved skin contained a smaller dermal component than those from pig skin. Epidermal sheets were cut into squares approximately 5×5 mm in size. Skin from psoriatic patients was cut into three portions: a marginal 5 mm piece within the lesion, a 5 mm piece of uninvolved epidermis just outside the lesion, and a third, more distant one.

After the preincubation in Hanks' media for 15 min at 37°C, one or two pieces of each epidermal sheet were incubated for 5 min with either epinephrine, histamine, or adenosine. Details of this procedure have been reported previously. Cyclic AMP accumulation in epidermis after this incubation is due to adenyl cyclase activation (12). Following the incubation, each specimen was quickly frozen between flat surfaces of dry ice, homogenized with 0.5 ml of 10% ice-cold trichloroacetic acid (TCA) and centrifugated. Supernatant TCA was extracted by shaking vigorously with 1 ml of water-saturated ethyl ether. The ether phase was discarded and this process was repeated three times. The aqueous phase was then freeze-dried for storage at −20°C. Cyclic AMP was determined by radioimmuno-assay of Steiner et al. (9). The reagents of this assay were obtained from Yamasa Shoyu Co., Ltd., Japan. Freeze-dried extracts were redissolved in 100 μl water and part or all of each sample (according to cAMP content) determined in a total reaction mixture of 300 μl. Protein content was measured by the method of Lowry et al. (6) on the TCA precipitate with a bovine serum albumin standard.

RESULTS

Pig epidermis. Fig. 1 illustrates the responses of pig epidermal adenyl cyclases to epinephrine, histamine and adenosine. Since the effect of epinephrine at the various concentrations has already been studied (12), the incubation with fixed

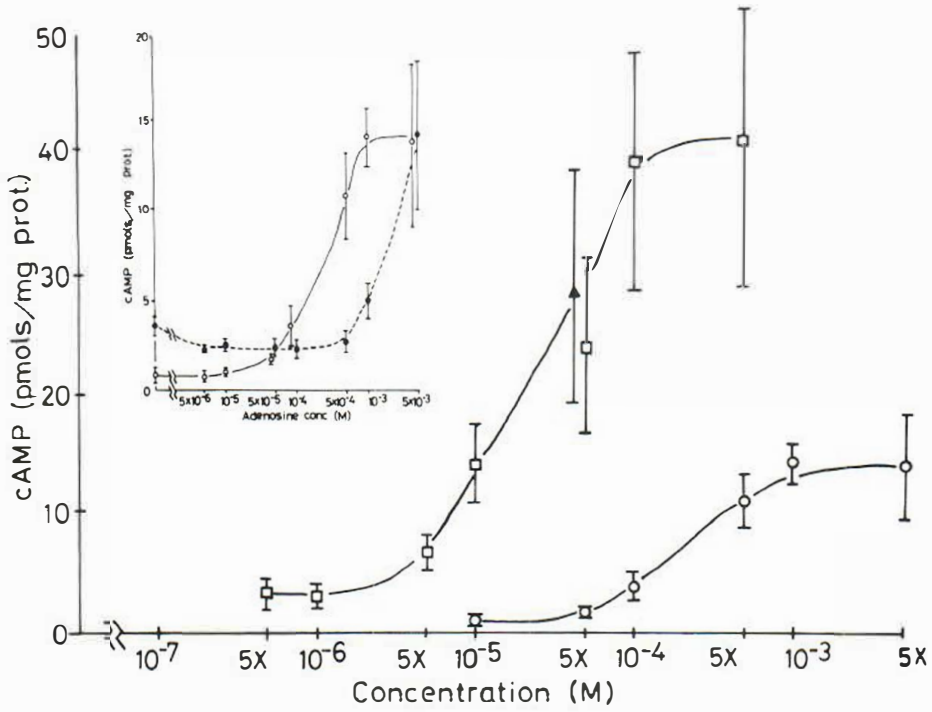

Fig. 1. Effect of epinephrine, histamine and adenosine on cAMP accumulation in pig epidermis. ▲: epinephrine; □—□: histamine; ○—○: adenosine. Data are shown as a mean ±S.E.M. in 5 experiments carried out simultaneously for the selected concentrations of histamine, adenosine and 5×10^{-5} M epinephrine. Theophylline (10 mM) was added to the reaction mixtures for epinephrine and histamine, but not for adenosine. *Inset:* Cyclic AMP accumulation in pig epidermis at various concentrations of adenosine in the absence (○—○) or presence (●---●) of 10 mM theophylline. Means ± S.E.M. of 4 to 5 experiments.

epinephrine concentration was run together to establish whether our previous data was compatible with this result. Histamine produces a maximum activation at a 20 times higher concentration than epinephrine, with a slightly larger cAMP accumulation. The optimal concentration of adenosine is 10 times higher than that of histamine. The smaller cAMP accumulation with adenosine is due to the absence of 10 mM theophylline which is known to inhibit the adenosine effect (3) as is seen in an inset in Fig. 1.

Epidermis from psoriatic patients. Specimens were obtained from the marginal part of a psoriatic lesion (I) and surrounding uninvolved skin (I and III) (see Materials and Methods) and cAMP accumulation were studied with epinephrine (5×10^{-5} M), histamine (5×10^{-4} M) and adenosine (5×10^{-3} M). The results are expressed as a mean + S.E.M. (Fig. 2). A markedly diminished response to epinephrine is observed in the involved epidermis, as has been shown previously (11).

As regards response to adenosine and histamine, involved and uninvolved psoriatic epidermis do not differ so markedly as to epinephrine, although the activation by adenosine tends to be decreased in the former. The response to histamine may even be slightly increased in involved epidermis. Since no clear differences were seen in histamine responsiveness, involved and uninvolved epidermal specimens from 4 psoriatic patients were reacted with different concentrations of histamine (Fig. 3). Because of the limited number of samples and large individual variations, these data do not conclusively demonstrate increased histamine responsiveness in involved epidermis, though it is suggested at several histamine concentrations with an almost identical optimal concentration (5×10^{-4} M), but much smaller cAMP accumulation compared with pig epidermis (Fig. 1). This study was also carried out using epidermis from 2 non-psoriatic subjects, one of whom showed a similar response, while the other showed much greater response.

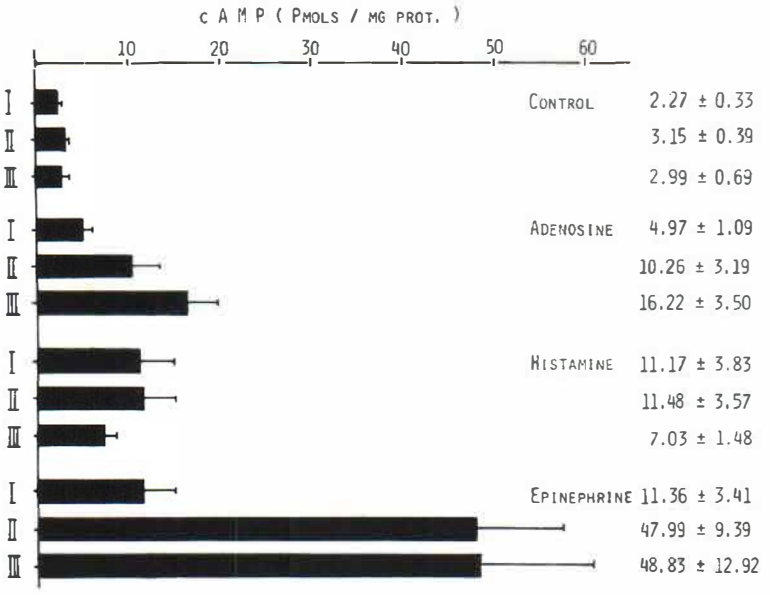

Fig. 2. Accumulation of cAMP in the involved and uninvolved epidermis of 18 psoriatic patients after incubation with either adenosine 5×10^{-3} M, histamine 5×10^{-4} M, or epinephrine 5×10^{-5} M.
All incubation media except those with adenosine contain 10 mM theophylline in Hanks' media. I: involved skin; II, III: uninvolved skin (see Materials and Methods).

DISCUSSION

Previous studies with the same experimental system have revealed that porcine and human epidermis have four independent adenyl cyclase systems, each of which reacts with a specific agonist: epinephrine, prostaglandin E, histamine, or adenosine (11, 1, 2, 3, 5). Our results using pig and psoriatic human epidermis confirmed three of these.

One of our observations is the remarkable difference between pig and human epidermis in the response to histamine. Pig epidermis showed much larger accumulations of cAMP than did human epidermis, while in the response to epinephrine or adenosine, similar levels of cAMP were observed (Figs. 1 and 2). Three interpretations of this observation may be made. The difference may reflect species differences in the histamine response, as many organs in various experimental animals have shown. Secondly, these differences may be caused by a slightly greater dermal contamination in the pig skin samples, with more mast cells and capillary elements contaminating the reaction system. Finally, epidermal cells from patients with psoriasis may, in fact, exhibit a depressed histamine response. At present, we have no clear answer for the last two possibilities, since we are technically unable to obtain thinner epidermal samples, and only 2 non-psoriatic subjects were studied.

With respect to differences between involved and uninvolved psoriatic epidermis, the marked decrease in the epinephrine response in involved epidermis was again observed. However, our results with adenosine and histamine are not identical with those of Iizuka et al. (5), who observed similar responses to adenosine in involved and uninvolved epidermis, while the response to histamine was significantly increased in involved epidermis. Our data with histamine showed only a slight increase in

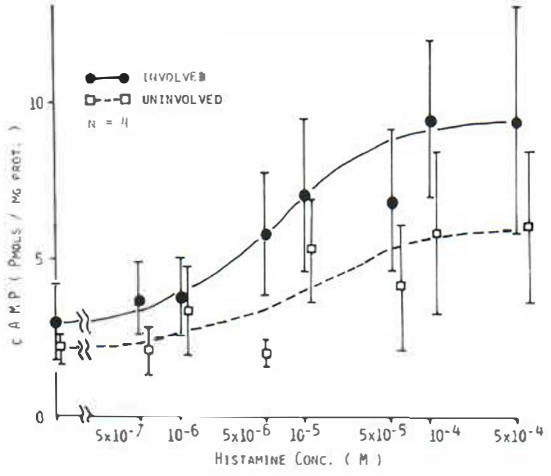

Fig. 3. Response to several concentrations of histamine by involved and uninvolved epidermis in psoriatic patients. (●—●: involved skin, □—□: uninvolved). Average± S.E.M. of four cases.

the involved tissue. However, this difference could be due to the factors. First, their samples were 0.3 to 0.5 mm thick, including more germinative cells and, at the same time, more dermal components particularly infiltrating PMN leukocytes which are known to have H_2 receptors. Secondly, our samples corresponded to the transitional zone of their sample, where a diminished epinephrine reactivity has been noted (5). In the studies with adenosine, one further difference exists—the absence of cAMP phosphodiesterase inhibitor from our system. In a recent report, Iizuka et al. showed a slight increase in phosphodiesterase activity in involved psoriatic epidermis (4). Although it is hard to believe that such a small increase could be the reason for our different observation with adenosine, because phosphodiesterase activity is much greater than that of adenyl cyclase—even in psoriatic epidermis—this point must be marked for future study. Therefore it can be said that the defect in the membrane enzyme system of psoriatic epidermis has not been confirmed and requires further study, although data so far strongly suggest that the epinephrine-sensitive adenyl cyclase system is affected in psoriatic epidermis.

ACKNOWLEDGEMENT

We thank Miss. M. Mizuno, B.A. for her excellent technical assistance and P. R. Bergstresser, M.D. for his valuable suggestions in preparing the manuscript. This study was supported in part by a grant from the Lydia O'Leary Foundation of Japan.

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Received July 11, 1979

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