Acta Derm Venereol (Stockh) 1987; 67: 295–300

Melittin-induced Alteration of Epidermal Adenylate Cyclase Responses

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Kajita S, Iizuka H. Melittin-induced alteration of epidermal adenylate cyclase responses. Acta Derm Venereol 1987; 67: 295–300.

Using an in vitro pig skin-slice incubation system, we investigated the effect of melittin, a phospholipase A_2 (PLA_2) stimulator, on the adenylate cyclase-cyclic AMP (cAMP) system. Significant decreases of various epidermal (beta-adrenergic, adenosine, and histamine) adenylate cyclase responses were observed as early as 1 h following the melittin treatment (50 μg/ml). The effect of melittin was concentration-dependent and the minimal concentration of melittin was 10 μg/ml for the inhibition of the beta-adrenergic adenylate cyclase response, whereas more than 50 μg/ml concentration was required for the inhibition of the adenosine and histamine adenylate cyclase responses. There was no significant difference in either low or high Km cAMP phosphodiesterase activity between control and melittin-treated skin. The beta-adrenergic augmentation effect by various chemicals (colchicine and Ro10-1670, an active form of Ro10-9359) were suppressed by the simultaneous addition of melittin in the incubation medium. Our data indicate that melittin affects not only on the beta-adrenergic adenylate cyclase system but also on the adenosine and histamine adenylate cyclase systems. However, the beta-adrenergic system was shown to be more sensitive to melittin than the other receptor adenylate cyclase systems. *Key words: Melittin; Cyclic AMP; Epidermis.* (Received October 16, 1986.)

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It has been reported that arachidonic acid metabolites, which are produced by activation of PLA_2, are increased in psoriatic hyperproliferative epidermis (1, 2). Defective beta-adrenergic adenylate cyclase response is observed in psoriasis (3, 4) and it has been suggested that PLA_2 might play an important role on the adenylate cyclase-cAMP system of epidermis (5, 6). We have shown that chemicals and manipulations for the treatment of psoriasis augment the beta-adrenergic response of epidermis and that this beta-adrenergic augmentation might be related to their therapeutic efficacies (7–12). Among these chemicals, we recently reported that the beta-adrenergic augmentation effect induced by glucocorticoids depends on protein synthesis mechanism (13), and might be revealed through the synthesis of PLA_2 inhibitory protein (lipocortin) (5). However, there has been no evidence suggesting relationship between PLA_2 and other chemicals (colchicine and Ro10-1670) which also affect the epidermal beta-adrenergic response.

Melittin, a basic polypeptide from bee venom, is a PLA_2 stimulator, which has numerous effects on cell membrane (14). Further phorbol ester, another stimulator of PLA_2, has been known to affect the adenylate cyclase response of epidermis resulting in the decreased beta-adrenergic response as well as the induction of epidermal cell proliferation (15, 16). Thus it might be suggested that melittin treatment would also decrease the beta-

Abbreviations: cAMP = cyclic adenosine 3′, 5′-monophosphate, Ro10-1670 = all-E-9-(4-methoxy-2, 3, 6-trimethyl-phenyl)-3, 7-dimethyl-2, 4, 6, 8-nonatetraenoate, Ro10-9359 = ethyl (all-E)-9-(4-methoxy-2, 3, 6-trimethyl-phenyl)-3, 7-dimethyl-2, 4, 6, 8-nonatetraenoate, PLA_2 = phospholipase A_2, DMSO = dimethyl sulfoxide, UVB = middle wave ultraviolet light.

adrenergic response. However, some investigators report that the beta-adrenergic adenylate cyclase system is enchanced by the melittin treatment in other tissues (17). Because of the apparent inconsistent results among various tissues, and because of the possible significance of PLA_2 activity in epidermal inflammation such as in psoriasis, we investigated the detailed effect of melittin on the adenylate cyclase system of pig epidermis, which include the relation to the effect of colchicine and Ro10-1670.

MATERIALS AND METHODS

The experimental procedures were the same as previously described (7). Domestic pigs weighing about 10 kg were anesthetized with Nembutal (Dainippon, Osaka, Japan) intraperitoneally (30 mg/kg). Fifteen minutes after the anesthesia, skin slices were taken from the backs of pigs using a Castroviejo keratome (Storz, Instrument Co., St. Louis, Missouri) adjusted 0.3 mm setting. The skin slices were then cut into squares (5×5 mm) and washed three times in RPMI 1640 medium, and the skin squares were floated with their keratin layers up in 10 ml of RPMI 1640 medium with added antibiotics (100 U/ml penicillin, 0.1 mg/ml streptomycin, and 0.25 μg/ml fungizone) and various concentrations of melittin in the presence or absence of the chemicals (colchicine or Ro10-1670). The incubations were done at 37°C in an atmosphere of 5% CO_2 in air. Since Ro10-1670 is relatively insoluble in ethanol, it was dissolved in DMSO and added in the incubation medium. The final concentration of DMSO was 0.1% unless otherwise stated. Melittin and colchicine were freely dissolved in water and added in the incubation medium. After an appropriate time, the skin squares were transferred and floated in new RPMI 1640 medium for cAMP accumulation studies. The skin squares were preincubated at 37°C for 15 min to standardize cAMP level, and after the preincubation 2 squares were randomly selected and floated in RPMI 1640 medium containing various adenylate cyclase stimulators. The concentrations of epinephrine, adenosine, and histamine added for the cAMP accumulation were 50 μM, 2 mM, and 1 mM, respectively. Previously it was shown that the concentrations of these chemicals were sufficient for the maximal accumulation of cAMP, which was attained after 5 min without cAMP phosphodiesterase inhibitors (18). After the incubation at 37°C in water bath, skin squares were quickly frozen between 2 plates of dry ice. The cAMP content in these skin squares was measured by radioimmunoassay using a Yamasa cAMP assay kit (Yamasa Shoyu Co., Tokyo) after partial purification by the method of Yoshikawa et al. (19).

The cAMP phosphodiesterase activities in skin squares were measured by the method of Adachi et al. (20). The substrate cAMP concentrations for low and high Km enzymes were 0.75 μM and 102 μM, respectively. Protein concentration was measured by the method of Lowry et al. (21). Chemicals and drugs were all prepared fresh before each experiment and pH of the medium was adjusted to 7. The statistical significance of the data obtained was evaluated by Student's *t*-test.

RPMI 1640 medium was purchased from Gibco (Grand Island, New York). Penicillin-streptomycin-fungizone mixture was obtained from M.A. Bioproducts (Walkersville, Maryland). Melittin was obtained from Sigma Chemical Co. (St. Louis, Missouri). Colchicine was purchased from Boehringer Mannheim Gmbh (F.R.G.) and Ro10-1670 was a generous gift from Nippon Roche K.K. (Tokyo, Japan).

RESULTS

Fig. 1 shows the time course of the effect of melittin on the adenylate cyclase responses. The skin squares were treated with melittin at 50 μg/ml concentration for various incubation periods. All of adenylate cyclase responses of the melittin-treated skin were shown to be decreased compared with the control skin following 1 h of incubation (Fig. 1). Continuous melittin treatment resulted in progressive decrease of all these responses, which reached around the basal cAMP level at 24 h (data not shown). The decrease of the beta-adrenergic response when compared with the control skin was 65%, whereas that of the adenosine or histamine response was 46% or 32%, respectively. The decrease of the adenosine and histamine responses by the melittin treatment for 1 h were recovered and returned almost to the control levels following the washing and additional 24 h incubation with melittin-free medium as shown in Fig. 2. However, a significant decrease of the beta-adrenergic response was still remained (Fig. 2). The effect of various concentrations of

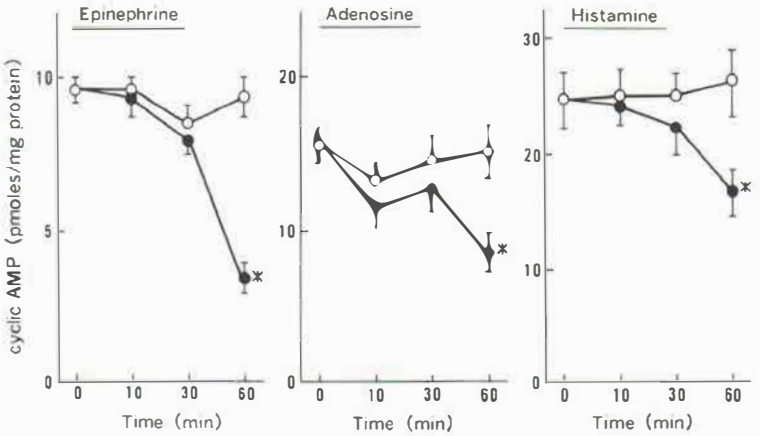

Fig. 1. Time course of the effect of melittin on the adenylate cyclase responses. Skin squares were incubated with (●—●) or without (○—○) melittin (50 μg/ml) for the indicated time and were then incubated with epinephrine (50 μM), adenosine (2 mM), and histamine (1 mM) for the cyclic AMP accumulation. Data are the means ± SE (n=4). * p<0.01 compared with control.

melittin was shown in Fig. 3. Inhibitory effect of melittin on the beta-adrenergic adenylate cyclase response was revealed at 10 μg/ml, whereas more than 50 μg/ml concentration was required for the detection of the inhibitory effect on the adenosine and histamine adenylate cyclase responses. Table I shows the combination effect of melittin and colchicine or Ro10-1670. Both colchicine and Ro10-1670 augmented the beta-adrenergic response, however, the simultaneous addition of melittin and colchicine or Ro10-1670 abolished their augmentation effect (Table I). Both low and high Km cAMP phosphodiesterase activities were not significantly different between control and melittin-treated skin as shown in Table II.

DISCUSSION

Our results indicate that there is a definitie difference in the sensitivity to melittin, a PLA_2 stimulator, among various adenylate cyclase systems. As shown in Figs. 1 and 3, the beta-adrenergic adenylate cyclase system was much more sensitive than the adenosine or histamine adenylate cyclase system.

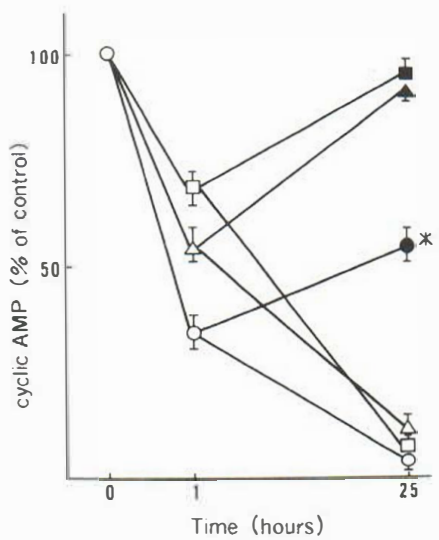

Fig. 2. Reversal of the inhibitory effects of melittin on the adenylate cyclase responses. Skin squares were incubated with melittin (50 μg/ml) for the first 1 h. Then skin squares were divided into two groups and each group was incubated for the additional 24 h in a new RPMI medium with or without melittin. At the indicated time, the skin squares were incubated with adenylate cyclase stimulators as in Fig. 1. Data are expressed as % of the control. ●—●, epinephrine response of the skin incubated with melittin-free medium; ○—○, epinephrine response of melittin-treated skin; ▲—▲, adenosine response of the skin incubated with melittin-free medium; △—△, adenosine response of melittin-treated skin; ■—■, histamine response of the skin incubated with melittin-free medium; □—□, histamine response of melittin-treated skin. * p<0.01 compared with control.

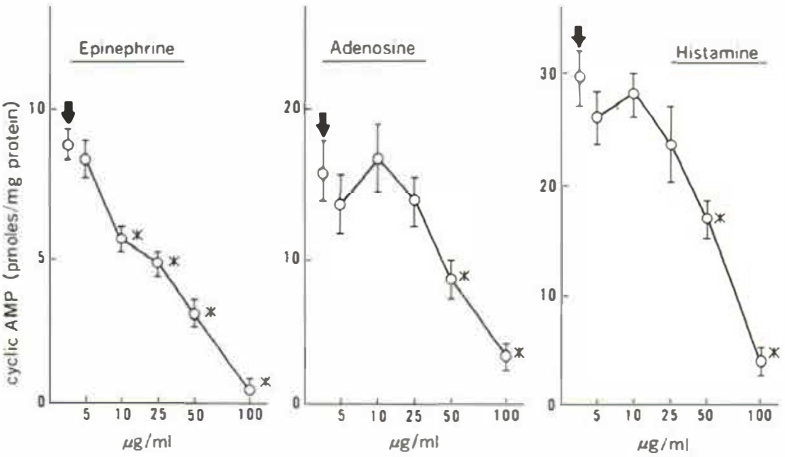

Fig. 3. Concentration effects of melittin on the adenylate cyclase responses. Skin squares were incubated with various concentrations of melittin for 1 h and were then incubated with adenylate cyclase stimulators as in Fig. 1. Data are the means ±SE (n=4). Arrows indicate the control skin responses. * p<0.01 compared with control.

There are several contradictory reports regarding the melittin-induced alterations of the adenylate cyclase response which depend on cell systems (17, 22, 23). Epidermis was shown to be another tissue where melittin decreased adenylate cyclase responses. Regarding the beta-adrenergic adenylate cyclase response, Bobik et al. (23) reported that the concentration of melittin more than 2.5 μg/ml was required to reveal its inhibitory effect on the cardiac cells. In our study, the minimal concentration required for the inhibition of the beta-adrenergic response was 10 μg/ml (Fig. 3). This inhibitory effect appears to be not due to the melittin-induced irreversible toxicity since the inhibitory response of the adenosine and histamine returned almost to the control level after the incubation with melittin-free

Table I. *Combined effect of melittin with colchicine or Ro10-1670 on the beta-adrenergic response*

	Cyclic AMP (pmoles/mg protein)
Experimental series 1	
Control	3.2±0.1
Melittin	2.3±0.3
Colchicine	17.3±1.4*
Both agents	3.3±0.2
Experimental series 2	
Control	4.4±0.7
Melittin	2.6±0.5
Ro10-1670	9.4±0.9*
Both agents	3.9±0.3

Skin squares were incubated with colchicine (1 μM) or Ro10-1670 (10 μM) with or without melittin (50 μg/ml) for 24 h, and were then incubated with epinephrine (50 μM) for the cyclic AMP accumulation. Each experimental series was performed using pig skin obtained on the same occasion. Data are the means ± SE (n=4). * p<0.01 compared with control.

Table II. *Effects of melittin on the cyclic AMP phosphodiesterase activity*

	Phosphodiesterase activity (pmoles/min/mg protein)	
	Low Km	High Km
Control	12.3±1.6	292.2±20.6
Melittin	12.7±1.0	284.5±21.5

Skin squares were incubated with melittin (50 μg/ml) for 1 h, and phosphodiesterase activities were measured as in the text. Data are the means ± SE (n=4). Essentially the same results were obtained after 24 h incubation (data not shown).

medium as shown in Fig. 2. The beta-adrenergic response, however, still remained low following the same treatment.

We have shown that glucocorticoids (7), colchicine (8), and retinoids (10) augment the beta-adrenergic adenylate cyclase response of epidermis. In order to see whether colchicine or retinoids reveal their beta-adrenergic augmentation effect through the alteration of PLA_2 activity (as glucocorticoids do), the skin was incubated with the combined medium of melittin and colchicine or Ro10-1670. As shown in Table I, both colchicine and Ro10-1670 augmented the beta-adrenergic response, whereas the addition of melittin markedly suppressed both beta-adrenergic augmentation effects following a 24 h incubation. Although it has been suggested that glucocorticoids, colchicine, and retinoids work independently on the beta-adrenergic adenylate cyclase system (8, 10), melittin revealed no differential inhibitory effect on the beta-adrenergic augmentation induced by these chemicals.

The physiological relationship between the effect of melittin (activation of PLA_2) and modification of epidermal adenylate cyclase systems remains to be determined. It has been reported that PLA_2 activity is increased in the uninvolved psoriatic epidermis (24), where the beta-adrenergic response is not decreased. Further UVB irradiation, which is known to activate PLA_2 activity, augmented the beta-adrenergic response both in vivo (9) and in vitro (9, Iizuka et al., unpublished). Thus PLA_2 activation does not appear to be the obligatory component of the beta-adrenergic augmentation effect. Our present study, however, indicates that PLA_2 activation (at least by the melittin) is associated with the relatively specific beta-adrenergic defect as well as the loss of regulatory mechanism by various stimulators of the beta-adrenergic response. Further study would be required regarding the precise relation between phospholipid metabolism and adenylate cyclase activity, since both regulatory systems are supposed to be important modulators of keratinocyte biological activity.

ACKNOWLEDGEMENTS

The technical assistance by Miss Y. Abe, Miss F. Nakamura and the secretarial assistance by Miss A. Naeka are highly appreciated.

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