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Topical Application of Potent Glucocorticoids Augments Epidermal Beta-adrenergic Adenylate Cyclase Response in vivo

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The effects of topical application of glucocorticoids on the epidermal beta-adrenergic adenylate cyclase response were investigated. A significant increase in this receptor response was observed 24 h following topical application of potent glucocorticoid ointments (0.12% betamethasone-17-valerate, 0.05% clobetasol-17-propionate). The application of a relatively weak glucocorticoid, hydrocortisone-17-butyrate, revealed no augmentation effect. There was no significant difference in other adenylate cyclase responses (adenosine-, and histamine-) between control and glucocorticoid-treated epidermis. UVB irradiation is known to augment the beta-adrenergic response of epidermis. Comparison of the effects revealed that topical glucocorticoid treatment had less effect than UVB irradiation, and when the UVB irradiation was combined with glucocorticoid treatment, the beta-adrenergic augementation effect was not enchanced. Cyclic AMP phosphodiesterase activities were not significantly altered by the glucocorticoid-, UVB-, or combined treatments. Our data indicate that epidermal beta-adrenergic adenylate cyclase response is affected by topical application of 'potent' glucocorticoids in vivo. Although the effect is weaker than that induced by UVB irradiation, we believe the system might be a useful tool for dissecting the glucocorticoidal potency of topical preparations using the epidermal keratinocyte response in vivo. *Key words: Cyclic AMP; Epidermis.* (Received April 1, 1986.)

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Among the epidermal receptor adenylate cyclase systems, the response of the beta-adrenergic system has been known to be altered by many in vivo manipulations (1). Since the beta-adrenergic system is assumed to be one of the regulatory mechanisms of keratinocyte proliferation (2), alteration of the beta-adrenergic response would also result in an alteration of the catecholamine-dependent regulatory system of keratinocyte proliferation. Consistent with this, an altered (defective) beta-adrenergic response appears to be a useful (but no obligatory) observation in epidermal hyperplasia, including psoriasis (1, 3).

Previously, using a long term incubation system in vitro, we have shown that many antipsoriatic agents augment the beta-adrenergic response of epidermis, which include glucocorticoids, colchicine, and retinoids (4, 5). Our incubation system which contains no serum, however, was somewhat artificial, where the control skin gradually lost the beta-adrenergic response during the long term incubation (4). Thus it was criticized that our in vitro system might not reflect the physiologic of in vivo condition of epidermal keratinocytes.

Subsequently we found that UVB irradiation and topical PUVA treatment, both of which are well-known modalities of psoriasis therapy, also augment the beta-adrenergic response in vivo (6, 7). These findings prompted us to see whether other agents such as

those listed above have similar effects in vivo. In this communication we report the effect of topical application of glucocortioids on the epidermal beta-adrenergic adenylate cyclase system, and its relation to the effect of UVB irradiation in vivo.

MATERIALS AND METHODS

Glucocorticoid ointments

We used the following glucocorticoid ointments, which are commercially available and clinically used in Japan. (1) 0.1% hydrocortisone-17-butyrate, (Torii Pharmaceutical Co., Tokyo, Japan). (2) 0.12% betamethasone-17-valerate (Shionogi Pharmaceutical Co., Osaka, Japan). (3) 0.05% clobetasol-17-propionate (Japan Glaxo K. K., Tokyo, Japan).

Ultraviolet light source

For UVB irradiation, we used Toshiba-Eisai Dermaray equipment, M-DMR-1 (Tokyo, Japan) with 5 fluorescent lamps (FL-20-SE-30, Toshiba, Tokyo). These tubes emitted wave lengths of 280-370 nm with the main emission between 295–315 nm and peak emission at 305 nm. The power output of this instrument was 1.9 mW/cm^2 with a target distance of 30 cm as measured by a UV radiometer (Tokyo-kogaku, Tokyo).

Topical glucocorticoid and UVB treatment

Domestic white-haired pigs weighing 5–10 kg were anaesthetized with Nembutal (Dainippon, Osaka, Japan) intraperitoneally (dose 30 mg/kg). Fifteen minutes after the anaesthesia (after clipping the hair and washing the skin surface), four 5×5 cm areas were chosen and the following treatments were administered on each area: (1) topical application of glucocorticoid ointments; (2) UVB irradiation alone (230 mJ/cm^2) (this energy was equal to 2 minimal erythema doses); (3) topical application of glucocorticoid immediately following UVB irradiation; (4) no treatment as a control. The dose of topical glucocorticoid ointments was 40 mg/cm^2. After 24 h the treated pigs were anaesthetized again and skin specimens were obtained from the four areas by use of a Castroviejo keratome (Storz Instrument Co., St. Louis, Missouri) adjusted to 0.3 mm setting. Because of variations of the beta-

Table I. *Effects of various glucocorticoids on adenylate cyclase responses*

Experimental series	Cyclic AMP (pmoles/mg protein)	
	Control	Glucocortioid-treated
Hydrocortisone-17-butyrate		
No addition	1.5±0.1	1.5±0.1
Epinephrine	6.2±1.4	6.0±0.5
Adenosine	34.9±1.2	37.4±1.5
Histamine	38.2±3.8	44.6±2.3
Betamethasone-17-valerate		
No addition	1.3±0.1	1.3±0.1
Epinephrine	6.8±0.5	10.2±1.2*
Adenosine	30.8±2.9	29.3±2.1
Histamine	31.9±3.4	26.5±3.5
Clobetasol-17-propionate		
No addition	1.3±0.1	1.4±0.1
Epinephrine	4.6±0.4	13.2±1.5**
Adenosine	22.8±1.3	24.9±1.9
Histamine	32.1±3.3	31.0±4.6

Pig skin was obtained from glucocorticoid-treated and non-treated areas at 24 h following topical application. Skin squares were made and were incubated with epinephrine, adenosine, and histamine as described in the text. Each experimental series was performed on the same animal. Data are the means ± SE of 4 independent experiments. * $p < 0.02$ compared with control. ** $p < 0.01$ compared with control.

adrenergic response among different pigs (8), comparison of the responses was made on each experimental series using the same pig. Each experimental series was made at least 4 times using different pigs and the representative data are shown in the tables.

Cyclic AMP and phosphodiesterase assay

Each skin slice obtained by the Castroviejo keratome was cut into 5×5 mm squares, which were washed 3 times in RPMI 1640 medium and preincubated in the RPMI 1640 medium for 15 min at 37°C to standardize the cyclic AMP level (9). After the preincubation, 2 pieces of skin squares were randomly selected and were incubated with various adenylate cyclase stimulators as described previously (5). The concentrations of epinephrine, adenosine, and histamine added in the incubation medium were 50 μM, 2 mM, and 1 mM, respectively. The cyclic AMP content in skin squares was measured by radioimmunoassay using a Yamasa cyclic AMP assay kit (Yamasa Shoyu Co., Tokyo, Japan) after partial purification by the method of Yoshikawa et al. (9). The cyclic AMP phosphodiesterase activities in skin squares were measured by the method of Adachi et al. (10). The substrate cyclic AMP concentrations for low and high Km enzymes were 0.75 μM and 102 μM, respectively. Protein concentrations were measured by the method of Lowry et al. (11) using bovine serum albumin as a standard. The statistical significance of the data obtained was evaluated by Student's *t*-test.

RESULTS

Table I shows the effects of various glucocorticoid ointments on the adenylate cyclase responses of epidermis. At 24 h following topical application, skin specimens were obtained and adenylate cyclase responses were compared. 0.1% hydrocortisone-17-butyrate had no effect on the adenylate cyclase responses of epidermis. On the other hand, potent glucocorticoid ointments (0.12% betamethasone-17-valerate; 0.05% clobetasol-17-propionate) augmented the beta-adrenergic adenylate cyclase response after topical application. The augmentation effect was specific to the beta-adrenergic system; neither adenosine nor histamine responses were altered by each treatment. The basal cyclic AMP levels without the addition of adenylate cyclase stimulators were not affected by these 3 topical glucocorticoid treatments (Table I).

Comparison of the effect of glucocorticoids with UVB irradiation revealed that UVB-induced beta-adrenergic augmentation effect was more marked than that induced by topical glucocorticoid treatments (Table II). The combination of glucocorticoid and UVB irradiation resulted in a slight increase in the beta-adrenergic augementation compared

Table II. *Combination of topical glucocorticoids with UVB irradiation*

	Cyclic AMP (pmoles/mg protein)			
Experimental series 1	Control	Betamethasone-17-valerate	UVB	Both treatments
No addition	1.3±0.1	1.3±0.1	1.6±0.1	1.6±0.1
Epinephrine	6.8±0.5	10.2±1.2*	31.5±1.8**	33.9±3.9**
Experimental series 2	Control	Clobetasol-17-propionate	UVB	Both treatments
No addition	1.3±0.1	1.4±0.1	1.5±0.1	1.5±0.1
Epinephrine	4.6±0.4	13.2±1.5**	28.1±2.2**	30.2±1.2**

Pig skin was obtained from control (non-treated), glucocorticoid-treated, UVB-irradiated, and combination (glucocorticoid and UVB) areas at 24 h following the treatment. Skin squares were made and were incubated with epinephrine. Data are the means ± SE of 4 independent experiments from the same animal in each experimental series. * $p<0.05$ compared with control, ** $p<0.01$ compared with control.

with that induced by UVB alone. The difference, however, was statistically not significant (Table II). There was no significant difference in either low or high Km cyclic AMP phosphodiesterase activities by the glucocorticoid-, UVB-, and combination-treatments in vivo (Table III).

DISCUSSION

Our data indicate that topical application of glucocorticoid ointments augments the beta-adrenergic adenylate cyclase response of pig skin in vivo (Table I). The effect was specific to the beta-adrenergic response; adenosine- and histamine-responses were not affected. Further, the effect was apparently dependent on the glucocorticoidal potency in topical preparations; the effect was observed by the potent glucocorticoid ointments, betamethasone-17-valerate and clobetasol-17-propionate. Betamethasone-17-valerate is ranked as a potent glucocorticoid, and clobetasol-17-propionate is ranked as an extremely potent glucocorticoid preparation (12). A relatively weak glucocorticoid, hydrocortisone-17-butyrate, had no effect on the beta-adrenergic response of epidermis (Table I). These results are essentially consistent with those observed in the in vitro incubation system where glucocorticoids specifically augment the beta-adrenergic response, the effect of which also correlated to the generally accepted glucocorticoidal potency (4, 13).

UVB irradiation has been shown to augment the beta-adrenergic response in vivo (6). Thus in spite of the counteracting effect of glucocorticoids on UVB-induced inflammatory change (14), both modalities were shown to have similar effect on the beta-adrenergic response of epidermis (Table II). Comparison of the effects revealed that UVB irradiation has a stronger effect than the topical glucocorticoid treatment in vivo (Table II). Although the results in Table II also suggest that the therapeutic mechamisms of glucocorticoids and UVB may be similar, further studies would be required to determine the precise relationship, since numerous interactions between these two modalities would be expected in the in vivo condition.

Our results contrast with those of Gommans et al. (15) who reported that the topical application of another extremely potent glucocorticoid, fluocinolone acetonide, on human skin did not modulate the response of adenylate cyclase to epinephrine stimulation. Since they used trypsinization to prepare keratinocytes, and since the beta-adrenergic receptor

Table III. *Effects of topical glucocorticoid, UVB irradiation and combination treatments on cyclic AMP phosphodiesterase activity*

	Phosphodiesterase activity (pmoles/min/mg protein)	
	Low Km	High Km
Control	10.4±1.3	205.1±27.5
Betamethasone-17-valerate	9.5±0.9	232.8±21.3
UVB	10.0±1.2	195.9±15.3
Combination	12.5±1.8	202.3±21.9

Pig skin was obtained from control (non-treated), glucocorticoid (betamethasone-17-valerate) treated, UVB-irradiated, and combination (glucocorticoid and UVB) areas at 24 h following the treatment. Skin squares were made and phosphodiesterase activities were measured as described in the text. Data are the means ± SE of 4 independent experiments from the same animal. Essentially the same results were obtained when clobetasol-17-propionate was used instead of betamethasone-17-valerate (data not shown).

system is sensitive to trypsinization (16), their preparative system might have modified the beta-adrenergic response pattern during the trypsinization. On the other hand, species differences in the cyclic AMP response pattern have been reported by several investigators (17). No data are available at present to show whether human epidermis responds to topical glucocorticoid preparations in the same way as pig epidermis.

Because of their mainstay position in the treatment of many inflammatory skin disorders including psoriasis, the evaluation of topical glucocorticoidal potency has been a matter of interest. The vasoconstrictor assay would probably be the most sensitive and reproducible system in vivo (18), and is by definition dependent on the dermal vascular response. Our system, as well as the epidermal phospholipase A_2 inhibition system (19), might provide another tool to dissect the glucocorticoidal potency in topical preparations. Studies are under way using a wide range of topical glucocorticoids to expand and confirm these findings in our laboratory.

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