Acta Derm Venereol (Stockh) 1991; 71: 6–10

Effect of 1,25-Dihydroxyvitamin D_3 on Adenylate Cyclase and Protein Kinase C in Pig Epidermis

HIROKO KOIZUMI, CHIKAKO YASUI and AKIRA OHKAWARA

Department of Dermatology, Hokkaido University School of Medicine, Sapporo, Japan

1,25-dihydroxyvitamin D_3 ($1,25(OH)_2D_3$) is suggested to be involved in the regulation of keratinocyte proliferation and differentiation. Recent evidence also indicates its potential value for the treatment of psoriasis, where the alteration of various transmembrane signalling systems has been well documented. Using porcine epidermis, we investigated the effect of $1,25(OH)_2D_3$ on adenylate cyclase and protein kinase C systems, both of which are markedly altered in the psoriatic hyperproliferative epidermis. The effect was compared with that of another anti-psoriatic agent, hydrocortisone. Neither $1,25(OH)_2D_3$ nor hydrocortisone revealed any effect on cyclic AMP levels or adenylate cyclase responses of epidermis. Long-term (24 h) hydrocortisone treatment, however, resulted in increased β-adrenergic-, and prostaglandin E-adenylate cyclase responses. $1,25(OH)_2D_3$-treatment had no effect on the epidermal adenylate cyclase responses following 24 h of incubation. The addition of both $1,25(OH)_2D_3$ and hydrocortisone to the incubation medium resulted in the attenuation of the hydrocortisone-induced β-adrenergic-, and prostaglandin E-adenylate cyclase responses of the epidermis. Neither agent had any effect on the cholera toxin-induced and forskolin-induced cyclic AMP accumulations of the epidermis. Neither $1,25(OH)_2D_3$ nor hydrocortisone had any effect on the epidermal protein kinase C activity. It has been suggested that various anti-psoriatic agents might reveal their effect through the modulation of the adenylate cyclase system. Since $1,25(OH)_2D_3$ had no effect when it was added singly to the incubation medium and rather inhibited hydrocortisone-induced adenylate cyclase stimulation, it is suggested that $1,25(OH)_2D_3$ reveals its therapeutic efficacy through the mechanism, probably independently of the adenylate cyclase system. ***Key words: Cyclic AMP; Psoriasis; Hydrocortisone.***

(Accepted May 14, 1990.)

Acta Derm Venereol (Stockh) 1991; 71: 6–10.

Hiroko Koizumi, Department of Dermatology, Hokkaido University School of Medicine, Kita 15 Nishi 7, Kita-ku, Sapporo, Japan.

1,25-dihydroxyvitamin D_3 ($1,25(OH)_2D_3$), the active form of vitamin D_3, is synthesized from provitamin D_3, successively, in epidermis, liver and kidney (1). The compound has long been known to regulate the blood calcium level by enhancing intestinal calcium transport and bone mineral mobilization. Recent evidence, however, indicates the presence of a specific receptor for $1,25(OH)_2D_3$ in almost all tissues, including the epidermis (2–4), suggesting that $1,25(OH)_2D_3$ might have other biological functions. In keratinocytes, $1,25(OH)_2D_3$ inhibits cell proliferation and stimulates terminal differentiation (2,5). Interestingly, the active forms of the vitamin D_3 derivative, including $1,25(OH)_2D_3$, are therapeutically efficacious for psoriasis, where increased keratinocyte proliferation and defective keratinization have been well-documented (6–9).

Among the alterations in the psoriatic keratinocytes are the modified transmembrane signalling systems, which can be characterized by decreased β-adrenergic and prostaglandin E-adenylate cyclase responses as well as decreased protein kinase C activity (10,11). Using a porcine skin floating culture system in vitro, we investigated the effect of $1,25(OH)_2D_3$ on the adenylate cyclase and protein kinase C system of the epidermis. The effects were compared with those of hydrocortisone, which is a known stimulator of the β-adrenergic-, and prostaglandin E-adenylate cyclase responses of the epidermis (12,13). Since the effect of $1,25(OH)_2D_3$ on cultured keratinocytes is lessened when they are incubated in serum-containing medium (5), possibly due to the presence of vitamin D-binding protein and vitamin D metabolites in serum, the serum-free incubation system was employed in the present study.

MATERIAL AND METHODS

Chemicals

RPMI medium 1640 and Hanks' balanced salt solution were from Gibco Laboratories Ltd. (Grand Island, New York). Epinephrine was the product from Daiichi Pharmaceutical Co. Ltd. (Tokyo, Japan). Prostaglandin E_1 was

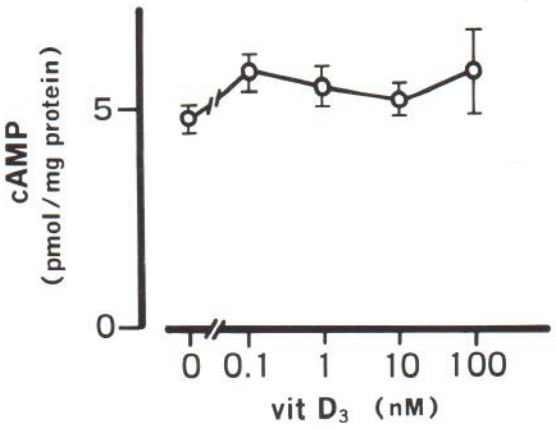

Fig. 1. 1,25(OH)$_2$D$_3$ concentration effect on β-adrenergic adenylate cyclase response. Porcine skin slices were floated and incubated in RPMI medium 1640 containing various concentrations of 1,25(OH)$_2$D$_3$, at 37°C in an atmosphere of 5% CO_2 in air. After 24 h of incubation, the skin slices were incubated with 50 μM epinephrine for 5 min at 37°C. After stopping the reaction on dry ice, the cyclic AMP concentration was determined by radio-immunoassay using a Yamasa cyclic AMP assay kit. The data represent means ± S.E. of four determinations.

purchased from Funakoshi Pharmaceutical Co. Ltd. (Tokyo). Hydrocortisone, 3-isobutyl-1-methyl-xanthine (IBMX), 12-*o*-tetradecanoyl phorbol-13-acetate (TPA), histone III-S and phosphatidylserine were from Sigma Chemical Co. (St. Louis, Mo.). [γ-^{32}P]ATP was purchased from Amersham Co. 1,25(OH)$_2$D$_3$ was supplied from Chugai Pharmaceutical Co. Ltd. (Tokyo). Other chemicals were freshly purchased from Wako Pure Chemical Industries Ltd. (Osaka, Japan).

Samples preparation and cyclic AMP accumulation

Epidermal slices were obtained from the pigs weighing about 10 kg, using a Castroviejo keratome adjusted at 0.2 mm depth. The epidermal slices were cut into 5×5 mm squares with a razor blade, and rinsed three times in Hanks' balanced salt solution. They were then floated on the buffer with keratin layer up.

In short-term experiments, the skin slices were preincubated at 37°C for 20 min in Hanks' balanced salt solution in the water bath. Then the specimens were incubated at 37°C for 5 min in the buffer with 50 μM epinephrine, 30 μM prostaglandin E$_1$, 1 mM adenosine, or 0.1 mM histomine in which agents to be tested were also included. Skin slices were quick-frozen between two plates of dry ice and cyclic AMP concentration was determined.

In long-term experiments, the skin slices were floated on RPMI medium 1640 with added antibiotics (0.1 mg/ml streptomycin, 100 units/ml penicillin, 0.25 μg/ml fungizone) containing the agents to be tested. The incubation were performed at 37°C in an atmosphere of 5% CO_2 in air. After a 24-h incubation, the skin slices were transferred to the new RPMI 1640 medium and incubated for cyclic AMP accumulation studies. The specimens were incubated for 5 min at 37°C on the water bath with either 50 μM epinephrine or 30 μM prostaglandin E$_1$ plus 1 mM IBMX. After stopping the reaction on dry ice, the cyclic AMP concentration was determined.

Cyclic AMP and phosphodiesterase assay

Cyclic AMP content in these skin slices was measured by radio-immunoassay using a Yamasa cyclic AMP assay kit (Yamasa Shoyu Co., Tokyo) as previously described (14). Cyclic AMP phosphodiesterase activity was measured *ad modum* Adachi et al. (15).

Preparation of protein kinase C

Porcine skin slices were immediately homogenized with a conified glass homogenizer with 20 mM Tris-HCl buffer, pH 7.5, containing 0.33 M sucrose, 2 mM EDTA, 0.5 mM EGTA and 2 mM phenylmethyl sulfonyl fluoride at 4°C. The homogenate was ultracentrifugated at 105,000 *g* for 60 min at 4°C. The supernatant was applied on the DE52 column equilibrated with the same buffer except for sucrose. The fraction of protein kinase C was eluted with 0.08 M NaCl (16).

Protein kinase C activity

Protein kinase C activity was assayed as previously described (17). Protein kinase C activity was assayed by measuring the incorporation of ^{32}P from [γ-^{32}]ATP into histone III-S. The standard reaction mixture in a final volume of 50 μl contained: 1 μmol Tris-HCl, pH 7.5; 250 nmol magnesium acetate; 10 μg histone III-S; 15 nmol $CaCl_2$; 0.5 nmol [γ-^{32}P]ATP (0.2–0.8 μCi/nmol); and the enzyme. One μg phosphatidylserine and 100 nM TPA were added for the stimulation of protein kinase C.

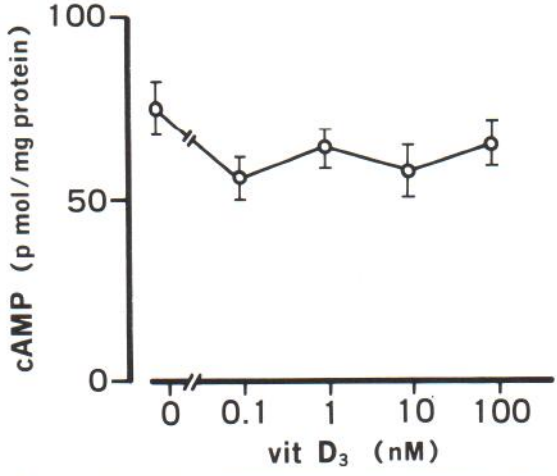

Fig. 2. 1,25(OH)$_2$D$_3$ concentration effect on prostaglandin E adenylate cyclase response. Pig skin slices were incubated in RPMI 1640 with various concentrations of 1,25(OH)$_2$D$_3$ at 37°C in an atmosphere of 5% CO_2 in air. After 24 h of incubation, the skin slices were incubated with 30 μM prostaglandin E$_1$ and 1 mM IBMX for 5 min at 37°C. After stopping the reaction, the cyclic AMP concentration was determined. The data represent means ± S.E. of four determinations.

Table I. *Beta-adrenergic and prostaglandin E response after 24 h incubation with the agents*

Data are expressed as the mean cyclic AMP concentration (pmol/mg protein) ± S.D. $n = 4$. * $p < 0.05$ compared with the control.

	Cyclic AMP (pmol/mg protein)		
	Basal	Epinephrine (50 μM)	PGE_1 + IBMX (30 μM) (1 mM)
Control	1.8±0.5	6.8±1.8	33.8±2.7
Hydrocortisone, 100 μM	1.8±0.2	12.8±2.8*	46.4±6.1*
$1,25(OH)_2D_3$, 10 nM	2.1±0.4	5.6±1.0	32.2±7.3
Hydrocortisone + $1,25(OH)_2D_3$	1.0±0.4	9.9±3.6	44.0±13.5

Protein concentration was measured *ad modum* Lowry et al. (18). Each examination was carried out with four determinations, and each was repeated more than three times independently. Statistical significance was determined by Student's *t*-test.

RESULTS

The single addition of $1,25(OH)_2D_3$ (or hydrocortisone) to the incubation medium had no effect on the cyclic AMP levels of porcine epidermis and the compound had no effect on the stimulatory receptor-(β-adrenergic-, prostaglandin E-, adenosine- and histamine-) adenylate cyclase responses of epidermis, either (data not shown). Figs. 1 and 2 show the effect of various concentrations of $1,25(OH)_2D_3$ on β-adrenergic and prostaglandin E-adenylate cyclase response. The epidermis was incubated with $1,25(OH)_2D_3$ (100 pM – 100 nM) for 24 h. β-adrenergic and prostaglandin E-adenylate cyclase responses were not stimulated by $1,25(OH)_2D_3$.

Porcine skin squares were incubated with various concentrations of $1,25(OH)_2D_3$ or 100 μM hydrocortisone for 24 h, and the adenylate cyclase responses were then compared (Table I). The basal level of cyclic AMP was unaffected by these treatments. The β-adrenergic-, and prostaglandin E-adenylate cyclase responses were increased by the hydrocortisone treatment, whereas $1,25(OH)_2D_3$-treatment had no effect on these receptor responses of epidermis. The addition of both $1,25(OH)_2D_3$ and hydrocortisone to the incubation medium resulted in the marked attenuation of the hydrocortisone-induced stimulation of the receptor-adenylate cyclase responses of epidermis (Table I). Neither $1,25(OH)_2D_3$ nor hydrocortisone revealed any effect

Table II. *Phosphodiesterase activity in pig epidermis after 24 h incubation with the agents*

The cyclic AMP phosphodiesterase activities were measured with the substrate cyclic AMP levels 0.5 and 102 μM for low and high K_m enzymes, respectively. The incubation times for the enzyme activities were 15 (for low K_m) and 40 min (for high K_m). Data are expressed as mean phosphodiesterase activity (pmol/min/mg protein) ± S.E. $n = 6$.

	pmol activity (pmol/min/mg protein)	
	Low K_m	High K_m
Control	4.4±0.2	45.4±5.4
Hydrocortisone, 100 μM	4.1±0.2	51.8±3.3
$1,25(OH)_2D_3$, 10 nM	4.4±0.1	39.4±5.3
Hydrocortisone + $1,25(OH)_2D_3$	4.5±0.3	50.7±4.9

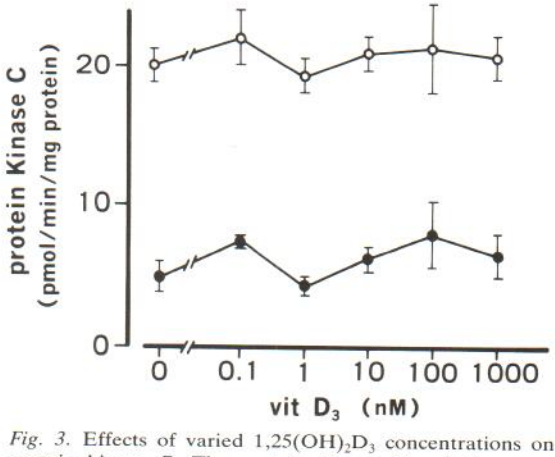

Fig. 3. Effects of varied $1,25(OH)_2D_3$ concentrations on protein kinase C. The protein kinase C activities were assayed with the indicated concentration of $1,25(OH)_2D_3$ in the presence (○–○) or absence (●–●) of 1 μg phosphatidylserine and 100 nM TPA. The data represent means ± S.E. of four determinations.

on cholera toxin-induced, and forskolin-induced cyclic AMP accumulation of epidermis, either in short-term or in long-term experiments (data not shown). No alteration of cyclic AMP phosphodiesterase activity was induced by 1,25(OH)$_2$D$_3$ or by hydrocortisone treatment (Table II).

Fig. 3 shows the effect of various concentrations of 1,25(OH)$_2$D$_3$ on the partially purified pig epidermal protein kinase C. 1,25(OH)$_2$D$_3$ (100 pM – 1 μM) had no effect on the protein kinase C activity. Nor did hydrocortisone (1–100 μM) have any effect on the epidermal protein kinase C activity (data not shown).

DISCUSSION

Our results indicate that the single addition of 1,25(OH)$_2$D$_3$ to the incubation medium had no effect on the adenylate cyclase system of the epidermis (Figs. 1, 2, Table I). Neither receptor-adenylate cyclase response, nor cholera toxin-induced, or forskolin-induced cyclic AMP accumulations were modified by the 1,25(OH)$_2$D$_3$ treatments. Cholera toxin and forskolin activates stimulatory guanine nucleotide binding protein (Gs) and the catalytic unit of the adenylate cyclase (C), respectively (19). It is known that all the stimulatory receptor adenylate cyclase systems exert their effects by activating Gs. Thus 1,25(OH)$_2$D$_3$ by itself was shown to have no effect on any component of the stimulatory epidermal adenylate cyclase systems.

It has been reported that various anti-psoriatic agents augment the β-adrenergic-, and prostaglandin E-adenylate cyclase responses of the epidermis (12,13,20,21). Those phenomena have been suggested to be closely associated with their clinical efficacy of psoriasis, where the defective adenylate cyclase response has been well documented (10).

1,25(OH)$_2$D$_3$ had no stimulatory effect on the adenylate cyclase responses; rather, it inhibited hydrocortisone-induced stimulation of the epidermal adenylate cyclase (Table I). Moreover, the compound had no effect on the cyclic AMP phosphodiesterase activity (Table II) or the protein kinase C activity (Fig. 3). Therefore it is unlikely that the chemical exerts its pharmacological effect through the modulation of these transmembrane signalling systems. As regards the protein kinase C activity, despite the decreased enzyme activity in the psoriatic epidermis (11), the modulation of the protein kinase C activity by the antipsoriatic agents in vitro has not been successful in our laboratory.

It has to be mentioned that not all the hyperproliferative epidermis has a decreased β-adrenergic response (22), and that not all the anti-psoriatic agents augment the β-adrenergic or prostaglandin E-adenylate cyclase responses of the epidermis (13,21). For example, chemicals which directly inhibit DNA synthesis of the epidermis (methotrexate, hydroxyurea, etc.) have been shown to have no β-adrenergic augmentation effect (21). The significance of the modulation of adenylate cyclase in psoriasis remains to be determined.

Recent evidence indicates that the regulatory mechanism of keratinocyte proliferation and differentiation might be under the control of intracellular, mutually-related complicated systems (23,24). Furthermore, despite the reports regarding the clinical efficacy of active vitamin D$_3$ in psoriasis (6–9), there is evidence that 1,25(OH)$_2$D$_3$ might be effective only for a limited population of psoriatics (25). Recently the resistance of cultured psoriatic keratinocytes to 1,25(OH)$_2$D$_3$-inducible growth inhibition has been reported (26). Thus the mechanism of anti-psoriatic effect of 1,25(OH)$_2$D$_3$, which is now considered to be a hormone rather than a vitamin by virtue of its specific receptor system (3), requires further investigation in order to be clarified.

REFERENCES

1. Holick MF. The cutaneous photosynthesis of previtamin D$_3$: a unique photoendocrine system. J Invest Dermatol 1981; 76: 51–58.
2. Hosomi J, Hosoi J, Abe E, Suda T, Kuroki T. Regulation of terminal differentiation of cultured mouse epidermal cells by 1,-25-dihydroxyvitamin D$_3$. Endocrinol 1983; 113: 1950–1957.
3. Kuroki T. Possible functions of 1,25-dihydroxyvitamin D$_3$, an active form of vitamin D$_3$, in the differentiation and development of skin. J Invest Dermatol 1985; 84: 459–460.
4. Horiuchi N, Clemens TL, Shiller AL, Holick MF. Detection and developmental changes of the 1,25-(OH)$_2$-D$_3$ receptor concentration in mouse skin and intestine. J Invest Dermatol 1985; 84: 461–464.
5. Smith EL, Walmorth NC, Holick MF. Effect of 1,-25-dihydroxyvitamin D$_3$ on the morphologic and biochemical differentiation of cultured human epidermal keratinocytes grown in serum-free condition. J Invest Dermatol 1986; 86: 709–714.
6. Morimoto S, Yoshikawa K, Kozuka T, et al. An open study of vitamin D$_3$ treatment in psoriasis vulgaris. Br J Dermatol 1986; 115: 421–429.
7. Kato T, Rokugo M, Terui T, Tagami H. Successful

treatment of psoriasis with topical application of active vitamin D_3 analogue, 1,24-dihydroxycholecalciferol. Br J Dermatol 1986; 115: 431–433.

8. Smith EL, Pincus SH, Donovan L, Holick MF. A novel approach for the evaluation and treatment of psoriasis. J Am Acad Dermatol 1988; 19: 516–528.
9. Kragballe K, Beck HI, Sogaard H. Improvement of psoriasis by a topical vitamin D_3 analogue (MC903) in a double-blind study. Br J Dermatol 1988; 119: 223–230.
10. Iizuka H, Adachi K, Halprin KM, Levine V. Cyclic AMP accumulation in psoriatic skin: Differential response to histamine, AMP, and epinephrine by the uninvolved and involved epidermis. J Invest Dermatol 1978; 70: 250–253.
11. Horn F, Marks F, Fisher GJ, Marcelo CL, Voorhees JJ. Decreased protein kinase C activity in psoriatic versus normal epidermis. J Invest Dermatol 1987; 88: 220–222.
12. Iizuka H, Ohkawara A. Effects of glucocorticoids on the beta-adrenergic adenylate cyclase system of pig skin. J Invest Dermatol 1983; 80: 524–528.
13. Koizumi H, Yasui C, Shimizu T, Ohkawara A. Stimulation of prostaglandin-E adenylate cyclase response by hydrocortisone. Arch Dermatol Res 1989; 281: 215–217.
14. Iizuka H, Umeda K, Koizumi H, Aoyagi T, Miura Y. Epinephrine-induced cyclic AMP accumulation in the psoriatic epidermis. Acta Derm Venereol (Stockh) 1981; 61: 391–395.
15. Adachi K, Levine V, Halprin KM, Iizuka H, Yoshikawa K. Multiple forms of cyclic nucleotide phosphodiesterase in pig epidermis. Biochim Biophys Acta 1976; 429: 498–507.
16. Koizumi H, Ohkawara A. Immunoblot demonstration of protein kinase C in pig epidermis. J Dermatol 1990; 17: 24–27.
17. Koizumi H, Adachi K, Tsutsui M, Itami S, Katayama H, Halprin KM. Calcium-activated, phospholipid-dependent protein kinase in pig epidermis. J Invest Dermatol 1984; 83: 261–264.
18. Lowry OH, Rosebrough NJ, Farr AL, Randall RJ. Protein measurement with the Folin phenol reagent. J Biol Chem 1951; 193: 265–275.
19. Iizuka H, Matsuo S, Tamura T, Ohkuma N. Increased cholera toxin-, and forskolin-induced cyclic AMP accumulations in psoriatic involved versus uninvolved or normal human epidermis. J Invest Dermatol 1988; 91: 154–157.
20. Iizuka H, Kajita S, Mizumoto T, Kawaguchi H. Glucocorticoid induced modulation of the beta-adrenergic adenylate cyclase responses of epidermis: its relation to epidermal phospholipase A_2 activity. J Invest Dermatol 1986; 87: 577–581.
21. Iizuka H, Hirokawa M, Ara M, Kajita S, Watanabe M, Ohkawara A. Antipsoriatic and antimetabolic agents as stimulators of the beta-adrenergic adenylate cyclase response of epidermis. Clin Exp Dermatol 1986; 11: 238–245.
22. Marks F, Ganss M, Grimm W. Agonist- and mitogen-induced desensitization of isoproterenol-stimulated cyclic AMP formation in mouse epidermis in vivo. Biochim Biophys Acta 1981; 678: 122–131.
23. Iizuka H, Sakai H, Tamura T. Effects of the tumor promoter, phorbol 12-myristate, on the epidermal adenylate cyclase system: evidence for adenylate cyclase-regulation by protein kinase C. J Invest Dermatol 1989; 93: 387–391.
24. Dover R, Watt FM. Measurement of the rate of epidermal terminal differentiation: expression of involucrin by S-phase keratinocytes in culture and in psoriatic plaques. J Invest Dermatol 1987; 89: 349–352.
25. Morimoto S, Yoshikawa K, Hironaka T, Nahata T. Vitamin D_3 has various effects on epidermal keratinocytes proliferation and differentiation. Abstract for symposium on keratinocytes. 1988.
26. Abe J, Kondo S, Nishi Y, Kuroki T. Resistance to 1,25-dihydroxyvitamin D_3 of cultured psoriatic epidermal keratinocytes isolated from involved and uninvolved skin. J Clin Endocrinol Metabol 1989; 68: 851–854.