Effect of 1,25-Dihydroxyvitamin D₃ on Adenylate Cyclase and Protein Kinase C in Pig Epidermis

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1,25-dihydroxyvitamin D₃ (1,25(OH)₂D₃), the active form of vitamin D₃, is synthesized from provitamin D₃, 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)₂D₃ in almost all tissues, including the epidermis (2-4), suggesting that 1,25(OH)₂D₃ might have other biological functions. In keratinocytes, 1,25(OH)₂D₃ inhibits cell proliferation and stimulates terminal differentiation (2.5). Interestingly, the active forms of the vitamin D₃ derivative, including 1,25(OH)₂D₃, 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 E₂-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)₂D₃ 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 E₂-adenylate cyclase responses of the epidermis (12,13). Since the effect of 1,25(OH)₂D₃ 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 E₂ was...
Effect of 1,25(OH)₂D₃ on epidermal signal transduction

Fig. 1. 1,25(OH)₂D₃ concentration effect on β-adrenergic adenylate cyclase response. Porcine skin slices were iodoacetamidated and incubated in RPMI medium 1640 containing various concentrations of 1,25(OH)₂D₃ at 37°C in an atmosphere of 5% CO₂ 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.

Cyclic AMP and phosphodiesterase assay
Cyclic AMP content in these skin slices was measured by radio-immunassay 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 conifid 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 ultracentrifuged 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 ³²P from [γ-³²P]ATP into histone III-S. The standard reaction mixture was incubated with 50 μl containing: 1 μmol Tris-HCl pH 7.5, 250 nmol magnesium acetate; 10 μg histone III-S; 15 mmol CaCl₂; 0.5 nmol [γ-³²P]ATP (0.2-0.8 μCi/nmol); and the enzyme. One pg phosphatidylserine and 100 nM TPA were added for the stimulation of protein kinase C.

Fig. 2. 1,25(OH)₂D₃ concentration effect on prostaglandin E adenylate cyclase response. Pig skin slices were incubated in RPMI 1640 with various concentrations of 1,25(OH)₂D₃ at 37°C in an atmosphere of 5% CO₂ in air. After 24 h of incubation, the skin slices were incubated with 30 μM prostaglandin E₁ 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.

Acta Derm Venereol (Stockh) 77
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.

<table>
<thead>
<tr>
<th>Cyclic AMP (pmol/mg protein)</th>
<th>Basal</th>
<th>Epinephrine (50 μM)</th>
<th>PGE₁ + IBMX (30 μM) (1 mM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>1.8±0.5</td>
<td>6.8±1.8</td>
<td>33.8±2.7</td>
</tr>
<tr>
<td>Hydrocortisone, 100 μM</td>
<td>1.8±0.2</td>
<td>12.8±2.8*</td>
<td>46.4±6.1*</td>
</tr>
<tr>
<td>1.25(OH)₂D₃, 10 nM</td>
<td>2.1±0.4</td>
<td>5.6±1.0</td>
<td>32.2±7.3</td>
</tr>
<tr>
<td>Hydrocortisone + 1.25(OH)₂D₃</td>
<td>1.6±0.4</td>
<td>9.9±3.6</td>
<td>44.0±13.5</td>
</tr>
</tbody>
</table>

Protein concentration was measured ad medium 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)₂D₃ (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 E₂, 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)₂D₃, on β-adrenergic and prostaglandin E adenylate cyclase response. The epidermis was incubated with 1.25(OH)₂D₃ (100 pM - 100 nM) for 24 h. β-adrenergic and prostaglandin E-adenylate cyclase responses were not stimulated by 1.25(OH)₂D₃.

Porcine skin squares were incubated with various concentrations of 1.25(OH)₂D₃ 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)₂D₃ treatment had no effect on these receptor responses of epidermis. The addition of both 1.25(OH)₂D₃ 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)₂D₃ 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 Km enzymes, respectively. The incubation times for the enzyme activities were 15 (for low Km) and 40 min (for high Km). Data are expressed as mean phosphodiesterase activity (pmol/min/mg protein) ± S.E. n = 6.

<table>
<thead>
<tr>
<th>pmol activity (pmol/min/mg protein)</th>
<th>Low Km</th>
<th>High Km</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>4.4±0.2</td>
<td>45.4±5.4</td>
</tr>
<tr>
<td>Hydrocortisone, 100 μM</td>
<td>4.1±0.2</td>
<td>51.8±3.3</td>
</tr>
<tr>
<td>1.25(OH)₂D₃, 10 nM</td>
<td>4.4±0.1</td>
<td>39.4±5.3</td>
</tr>
<tr>
<td>Hydrocortisone + 1.25(OH)₂D₃</td>
<td>4.5±0.3</td>
<td>50.7±4.9</td>
</tr>
</tbody>
</table>

Fig. 3. Effects of varied 1.25(OH)₂D₃ concentrations on protein kinase C. The protein kinase C activities were assayed with the indicated concentration of 1.25(OH)₂D₃ in the presence (○○○) or absence (●●●) of 1 μg phosphatidylinerine 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)D3 or by hydrocortisone treatment (Table II).

Fig. 3 shows the effect of various concentrations of 1,25(OH)D3 on the partially purified pig epidermal protein kinase C. 1,25(OH)D3 (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)D3 to the incubation medium had no effect on the adenylate cyclase system of the epidermis (Figs. 1, 2, Table I). Neither receptor-adenyl cyclase response, nor cholera toxin-induced, or forskolin-induced cyclic AMP accumulations were modified by the 1,25(OH)D3 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)D3, 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 on psoriasis, where the defective adenylate cyclase response has been well documented (10). 1,25(OH)D3 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 D3 in psoriasis (6–9), there is evidence that 1,25(OH)D3 might be effective only for a limited population of psoriatics (25). Recently the resistance of cultured psoriatic keratinocytes to 1,25(OH)D3-inducible growth inhibition has been reported (26). Thus the mechanism of anti-psoriatic effect of 1,25(OH)D3, 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

7. Kato T, Rokujo M, Terui T, Tagami H. Successful


