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# Functional involvement of calcium in the homologous up-regulation of the 1,25-dihydroxyvitamin $D_3$ receptor in osteoblast-like cells

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In several cell types 1,25-dihydroxyvitamin $D_3$ (1,25$(OH)_2D_3$) causes up-regulation of its receptor. The present study demonstrates that in the osteoblast-like cell line UMR 106 this up-regulation is inhibited by two different calcium channel blockers (nitrendipine, verapamil). Also with chelating extracellular calcium (EGTA) and by inhibition of calcium release from intracellular stores (TMB-8) comparable results were obtained. These findings indicate that calcium is functionally involved in this cellular response to the steroid hormone 1,25$(OH)_2D_3$. Moreover, data obtained with EGTA show that the 1,25$(OH)_2D_3$ receptor level is closely regulated by the extracellular calcium concentration.

1,25-Dihydroxyvitamin $D_3$; Calcium; 1,25$(OH)_2D_3$ receptor up-regulation

## 1. INTRODUCTION

1,25-Dihydroxyvitamin $D_3$ (1,25$(OH)_2D_3$) is an important regulator of calcium homeostasis and bone metabolism. It has been shown that 1,25$(OH)_2D_3$ causes homologous up-regulation of its receptor in the osteoblast-like cell lines ROS 17/2 [1] and UMR 106 [2], and in fibroblasts and cloned kidney cells [3].

The occupied 1,25$(OH)_2D_3$ receptor is thought to act direct at the genome. However, in various cellular systems 1,25$(OH)_2D_3$ has been shown to stimulate calcium uptake independent of de novo RNA and protein synthesis [4–6], and genome independent effects on membrane potential have been reported [7]. Also, recently it has been demonstrated that 1,25$(OH)_2D_3$ causes a rapid (less than 30 s) rise of the intracellular ionized calcium concentration ($[Ca^{2+}]_i$) in isolated mouse osteoblasts [8]. The 1,25$(OH)_2D_3$-induced rise of the $[Ca^{2+}]_i$ has been shown to be inhibited by the calcium channel blockers nifedipine and verapamil, by chelating extracellular calcium with EGTA and to be reduced by blocking release from intracellular stores by 8-(diethylamino)octyl 3,4,5-trimethoxybenzoate HCl (TMB-8) [8]. In the osteoblast-like cell line ROS 17/2.8 1,25$(OH)_2D_3$ modulates dihydropyridine-sensitive L-type calcium channels [9]. Moreover, in keratinocytes and rat enterocytes 1,25$(OH)_2D_3$ increases the generation of inositol 1,4,5-trisphosphate indicating an effect on calcium release from intracellular stores [10,11]. Based on these data it is conceivable that calcium acts as an intracellular messenger in the regulation of cellular responses by 1,25$(OH)_2D_3$. However, as yet a functional role for calcium has to be demonstrated.

In the present study we have investigated whether calcium is functionally involved in 1,25$(OH)_2D_3$-induced up-regulation of its receptor in the osteoblast-like cell line UMR 106.

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## 2. MATERIALS AND METHODS

### 2.1. *Materials*

Verapamil and EGTA were obtained from Sigma, St. Louis, USA, TMB-8 from Aldrich Chemical Co., Bruxelles, Belgium. Nitrendipine was a generous gift from Dr B. Garthoff of Bayer AG, Wuppertal, FRG. [23,24-$^3$H]1,25$(OH)_2D_3$ (90 Ci/mmol) was purchased from Amersham International, Amersham, UK, and non-radioactive 1,25$(OH)_2D_3$ was generously provided by LEO Pharmaceuticals BV, Weesp, The Netherlands.

### 2.2. *Culture and treatment of the cells*

UMR 106 cells were cultured as described previously [12]. The cells were incubated for 4 h with or without 1,25$(OH)_2D_3$ in the absence or presence of nitrendipine, verapamil, EGTA, or TMB-8.

### 2.3. *Preparation of cell extracts and 1,25$(OH)_2D_3$ binding assay*

For single point assays, conditions were employed which were previously shown to provide valid estimates of total VDR content in cytosolic extracts [2]. The protein concentration was measured according to the method of Bradford [13].

### 2.4. *Data analysis*

Data presented are means ± SD of triplicate determinations of at least 2 different experiments, i.e. at least 6 replicates. Interactions between 1,25$(OH)_2D_3$ and calcium antagonists were evaluated using an analysis of variance for 2-way factorial design [14]. Other statistical analyses were done by Student's *t*-test.

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Table I

Effect of chelating extracellular calcium with EGTA and blocking calcium release from intracellular stores by TMB-8 on homologous up-regulation of VDR. Data expressed as fmol [$^3$H]1,25(OH)$_2$D$_3$ bound/mg protein.

| [1,25(OH)$_2$D$_3$] | Control | 1.5 mM EGTA | 2 mM EGTA | TMB-8 |
|---|---|---|---|---|
| 0 | 25.4 ± 1.3 | 23.5 ± 0.2 | 45.0 ± 1.6* | 19.7 ± 0.8** |
| $10^{-10}$ M | 40.6 ± 1.8* | 40.4 ± 1.6 | 45.8 ± 0.9▼▼ | 29.7 ± 1.0▼ |
| $10^{-8}$ M | 106.8 ± 3.4* | 105.9 ± 4.3 | 82.0 ± 2.1■ | 64.3 ± 1.1■ |

*$P<0.001$, **$P$, *0.05 with respect to basal VDR content (25.4 ± 1.3).* ▼$P<0.05$, ▼▼$P<0.002$ and ■$P<0.001$ calculated as the significance of interaction between 1,25(OH)$_2$D$_3$ and EGTA

## 3. RESULTS AND DISCUSSION

To our knowledge this is the first report showing a direct functional involvement of calcium as intracellular signal in a cellular response to 1,25(OH)$_2$D$_3$.

Basal 1,25(OH)$_2$D$_3$ binding was significantly reduced by the calcium channel blockers nitrendipine ($5 \times 10^{-5}$ M, $P<0.05$) and verapamil ($10^{-4}$M, $P<0.001$) [12]. With 1.5 mM EGTA no effect was observed whereas 2 mM EGTA induced an increase in VDR level (Table I). Recently it was reported that expression of murine epidermal differentiation markers is tightly regulated by restricted extracellular calcium concentrations [15]. In our study basal medium calcium concentration was 1.42 mM and in the presence of 1.5 and 2 mM EGTA 0.28 and 0.05 mM, respectively. These concentrations are in the same range as those reported to be important for the expression of epidermal differentiation markers [15]. Therefore it is likely that basal VDR level is also tightly regulated by extracellular calcium concentrations. Moreover, preliminary data obtained in our laboratory show an increase of VDR mRNA by 2 mM but not by 1.5 mM EGTA (manuscript in preparation).

As depicted in Figs. 1 and 2, VDR up-regulation by $10^{-10}$ M 1,25(OH)$_2$D$_3$ is significantly reduced by $10^{-5}$ and $5 \times 10^{-5}$ M nitrendipine (18 and 48% inhibition) and by $10^{-5}$ and $10^{-4}$ M verapamil (24 and 50% inhibition). In contrast, $10^{-8}$ M 1,25(OH)$_2$D$_3$-induced VDR up-regulation is not affected by $10^{-5}$ M nitrendipine and $10^{-5}$ M verapamil and only 30% and 40% reduction was observed with $5 \times 10^{-5}$ M nitrendipine and $10^{-4}$ M verapamil, respectively (Figs. 1 and 2). Together with the results obtained with EGTA (Table I), these data indicate that calcium is functionally involved in homologous up-regulation of VDR in UMR 106 cells. Recently we demonstrated that calcium is also involved in heterologous up-regulation of VDR [12].

A role for intracellular calcium stores in the action of 1,25(OH)$_2$D$_3$ is indicated by the data obtained with 0.1 mM TMB-8 (Table I). VDR up-regulation by $10^{-10}$ and $10^{-8}$ M 1,25(OH)$_2$D$_3$ was inhibited by 34 and 45%, respectively. This is in agreement with the reports showing effects of 1,25(OH)$_2$D$_3$ on the phosphoinositide metabolism [10,11].

Both verapamil and nitrendipine inhibited $10^{-10}$ M 1,25(OH)$_2$D$_3$-induced VDR up-regulation at a lower concentration and with a greater magnitude than the up-regulation by $10^{-8}$ M 1,25(OH)$_2$D$_3$ (Figs. 1 and 2). It is unlikely that this is due to the fact that the increase of the [$Ca^{2+}$]$_i$ by $10^{-8}$ M is more pronounced than the increase by $10^{-10}$ M 1,25(OH)$_2$D$_3$. As Lieberherr [8] reported that a maximum increase of [$Ca^{2+}$]$_i$ was

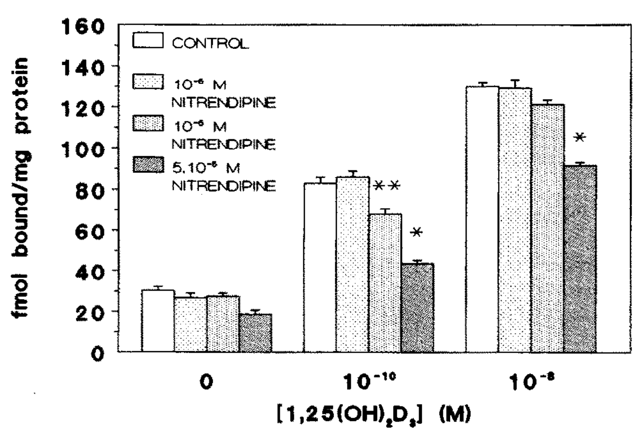


Fig. 1. Effect of nitrendipine on homologous up-regulation of VDR. $5 \times 10^{-5}$ M nitrendipine significantly ($P<0.05$) reduced basal 1,25(OH)$_2$D$_3$ binding. **$P<0.025$; *$P<0.001$ calculated as the significance of interaction between 1,25(OH)$_2$D$_3$ and nitrendipine.

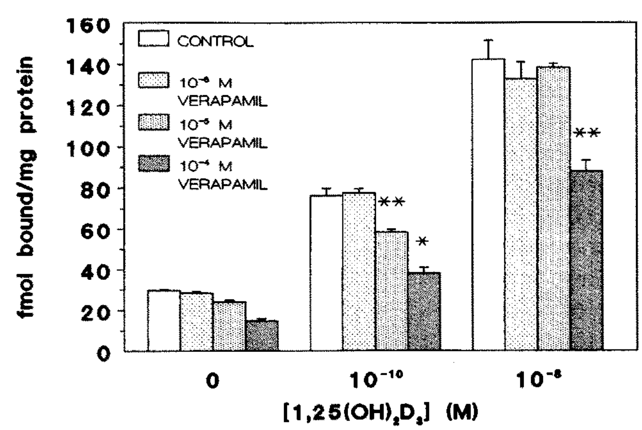


Fig. 2. Effect of verapamil on homologous up-regulation of VDR. $10^{-4}$ M verapamil significantly ($P<0.001$) reduced basal 1,25(OH)$_2$D$_3$ binding. **$P<0.01$, *$P<0.0025$ calculated as the significance of interaction between 1,25(OH)$_2$D$_3$ and verapamil.

observed at $10^{-10}$ M whereas $10^{-8}$ M, $1,25(OH)_2D_3$ has only a minor effect on the $[Ca^{2+}]_i$. Moreover, Caffrey and Farach-Carson [9] reported agonistic (0.05-5 nM) and antagonistic (> 10 nM) effects on calcium currents. Based on the current data it is tempting to suggest that $1,25(OH)_2D_3$ causes VDR up-regulation both dependent on and independent of an increase of the $[Ca^{2+}]_i$, and that the calcium dependency is more prominent at low than at high $1,25(OH)_2D_3$ concentrations.

Although Bloor et al. [16] have reported detectable levels of $1,25(OH)_2D_3$ in intestinal nuclei 5 min after administration it is, in view of the fast effect of $1,25(OH)_2D_3$ on the $[Ca^{2+}]_i$, unlikely that the effect of $1,25(OH)_2D_3$ on the $[Ca^{2+}]_i$ is exerted via the genome. Whether $1,25(OH)_2D_3$ exerts its effects on $[Ca^{2+}]_i$ via a cell surface receptor is not yet clear, although recent data point to a cell surface receptor for $1,25(OH)_2D_3$ [11] and a steroid receptor has been identified on the surface of *Xenopus* oocytes [17].

Taken together, the current study demonstrates (1) a role for the extracellular calcium concentration in the regulation of the VDR level, and (2) a functional messenger role for intracellular calcium in the action of $1,25(OH)_2D_3$ in osteoblast-like cells and thereby provides new insights in the mechanism of action of this steroid hormone.

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