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# HETEROLOGOUS UP-REGULATION OF THE 1,25-DIHYDROXYVITAMIN $D_3$ RECEPTOR BY PARATHYROID HORMONE (PTH) AND PTH-LIKE PEPTIDE IN OSTEOBLAST-LIKE CELLS

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1,25-Dihydroxyvitamin $D_3$ (1,25-$(OH)_2D_3$) receptor content in cultured osteogenic sarcoma cells (UMR-106) was found to be increased after treatment with both bovine and human PTH and human PTH-like peptide (hPLP). The dose dependent increase of receptors was preceded by a dose dependent stimulation of cAMP production. This suggests a role for cAMP as mediator of the PTH- and hPLP-induced 1,25-$(OH)_2D_3$ receptor up-regulation. Furthermore, evidence was obtained that new mRNA and de novo receptor synthesis is involved in this heterologous 1,25-$(OH)_2D_3$ receptor up-regulation. 

Parathyroid hormone (PTH) and 1,25-dihydroxyvitamin $D_3$ (1,25-$(OH)_2D_3$ are important regulators of calcium and bone metabolism. From both in vivo and in vitro studies evidence was obtained indicating an interaction between PTH and 1,25-$(OH)_2D_3$ on their major targets (1-4). In bone, receptors for both calcitrophic hormones are only found in osteoblasts (5). Recently, we and others reported that exposure of osteoblast-like cells to 1,25-$(OH)_2D_3$ decreased their cAMP response to PTH (6). This phenomenon has been referred as heterologous desensitization. At present it is not known whether PTH in return is also capable to influence 1,25-$(OH)_2D_3$ action in osteoblasts.

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In the rat osteogenic sarcoma cell line UMR-106 we have recently shown that 1,25-$(OH)_2D_3$ elicits a homologous up-regulation of its receptor (7). UMR-106 cells are also responsive to PTH by showing a cAMP response (8). Therefore, we used these cells to analyze the effects of both human and bovine PTH on 1,25-$(OH)_2D_3$ receptor level. Furthermore, we examined the effect of human PTH-like peptide (hPLP), a hormone involved in the pathogenesis of humoral hypercalcemia of malignancy (9,10). In the current study we have observed that both PTH and hPLP up-regulates the 1,25-$(OH)_2D_3$ receptor.

## MATERIALS AND METHODS

The rat osteogenic sarcoma cell line UMR-106 was supplied by Dr. M.P.M. Herrmann-Erlee (Laboratory of Cell Biology, University of Leiden, Leiden, The Netherlands). [23,24-$^3$H]1,25-$(OH)_2D_3$ (102 Ci/mmol) was obtained from Amersham and nonradioactive 1,25-$(OH)_2D_3$ was generously provided by LEO Pharmaceuticals. Cycloheximide, actinomycin D, bovine and human PTH(1-34) (bPTH(1-34), hPTH(1-34)) were from Sigma, while synthetic human PTH-like peptide(1-34) (hPLP(1-34)) was a generous gift of Dr. C.W.G.M. Löwik (Clinical Investigation Unit, University Hospital Leiden, The Netherlands). Fetal calf serum (FCS), MEM medium, penicillin, streptomycin and glutamine were purchased from Flow Laboratories.

**Cell culture conditions:** UMR-106 cells were seeded at $0.6 \times 10^5$ cells/cm$^2$ and cultured in MEM supplemented with 2 mM glutamine, 100 U/ml penicillin, 100 ug/ml streptomycin and 10% FCS. After an additional 16 h preincubation in MEM with 2 % charcoal-treated FCS, the confluent cultures were incubated in serum-free MEM with or without the compounds indicated. The incubations were carried out for 0-6 h at 37° C with the culture flasks placed on a slightly angled slowly rotating plate. At the end of the incubation the cells were rinsed with ice-cold Hanks medium, trypsinized and,after inactivation of trypsine, centrifuged.

**Preparation of cell extracts and [$^3$H]1,25-$(OH)_2D_3$ binding assay:** For single point assays, conditions were employed which were previously shown to provide valid estimates of total receptor content in cytosolic extracts (7). The cell pellet was extracted on ice in a hypertonic buffer consisting of 300 mM KCl, 10 mM Tris-HCl (pH 7.4), 1 mM EDTA, 5 mM dithiothreitol, 10 mM sodium molybdate and 0.1 % Triton X-100. High-speed supernatants were obtained and 200 ul aliquots were incubated at 0° C overnight with 0.5 nM [$^3$H]1,25-$(OH)_2D_3$ in the presence or absence of a 200-fold molar excess of unlabeled hormone. Receptor-bound 1,25-$(OH)_2D_3$ was separated from unbound sterol by charcoal adsorption

(11). The protein concentration was measured according to the method of Bradford (12).

**Cyclic AMP assay:** The cells were incubated for 3 min (maximal effect) at 37° C in Hanks-Hepes buffer with 0.5 % BSA and supplemented with the peptides to be tested (8). cAMP was measured by the protein binding assay of Lust et al (13). The DNA content was determined fluorimetrically by the method of Johnson-Wint (14).

## RESULTS

In Fig. 1 the time-course of the effect of $10^{-8}$ M bPTH(1-34) on the cellular level of 1,25-$(OH)_2D_3$ receptors is shown. The increase in 1,25-$(OH)_2D_3$ binding following treatment with PTH was found to reach a maximum at 4 h. In vehicle-treated cultures no change in cellular 1,25-$(OH)_2D_3$ receptor level was found. Scatchard analysis after 4 h incubation with PTH showed no significant change in the apparent dissociation constant (≃20 pM) of the 1,25-$(OH)_2D_3$ receptor (results not shown).

To determine whether the observed increase in receptors reflected changes in translation and/or transcription, we examined the ability

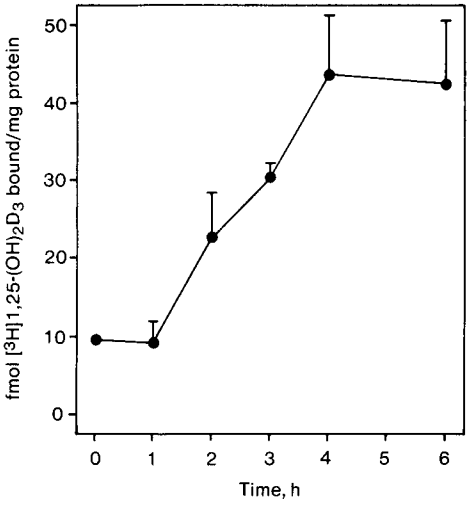


**Fig. 1:** Time course of the PTH-induced 1,25-$(OH)_2D_3$ receptor up-regulation. At the start of the experiment the medium was changed from MEM with 2 % charcoal-treated FCS to serum-free MEM without or with $10^{-8}$ M bovine PTH(1-34). At the times indicated 1,25-$(OH)_2D_3$ receptor content was assayed in cytosolic extracts as described in "Materials and Methods". Values are the means ± SD of 2 experiments.

**Table I:** Effect of cycloheximide and actinomycin D on the ability of bPTH(1-34) to up-regulate the $1,25\text{-}(OH)_2D_3$ receptor level in UMR-106 cells

| TREATMENT | Vehicle | bPTH(1-34) |
|---|---|---|
| Vehicle | 10.6 ± 0.8 | 35.9 ± 8.0 |
| Cycloheximide | 5.9 ± 0.4 | 6.8 ± 1.7 |
| Actinomycin D | 6.8 ± 0.5 | 8.8 ± 0.9 |

After the medium was changed from MEM with 2 % charcoal-treated FCS to serum-free MEM, UMR-106 cells were treated for 4 h with vehicle or bPTH(1-34) in the absence or presence of cycloheximide (10 ug/ml) or actinomycin D (1 ug/ml). $1,25\text{-}(OH)_2D_3$ receptor concentration was determined in cytosolic extracts as described in "Materials and Methods". Results are expressed in fmol [$^3$H]$1,25\text{-}(OH)_2D_3$ bound per mg protein. Values are the means ± SD of triplicate determinations.

of cycloheximide and actinomycin D, respectively, to block the increase in receptors induced by bPTH(1-34). As shown in Table I, the cells simultaneously treated with bPTH(1-34) and cycloheximide or actinomycin D fail to exhibit the up-regulation of the receptor found in the absence of these inhibitors.

The effect of various concentrations of bovine and human PTH(1-34) on $1,25\text{-}(OH)_2D_3$ receptor up-regulation are depicted in Fig. 2. Although we observed a similar $ED_{50}$ for both peptides (approximately $10^{-9}$ M), the maximum achieved in the presence of bPTH(1-34) seems somewhat higher. The maximum response to hPLP is comparable to that obtained with the PTH fragments. However, the dose response curve is shifted to the left with an $ED_{50}$ of about $10^{-10}$ M.

Both PTH fragments and hPLP also produced a dose-dependent stimulation of the cAMP production in UMR-106 cells (Fig. 3). However, compared to the effect on $1,25\text{-}(OH)_2D_3$ receptor up-regulation approximately 10 times higher concentrations of the peptides were required to elicit a half maximum effect.

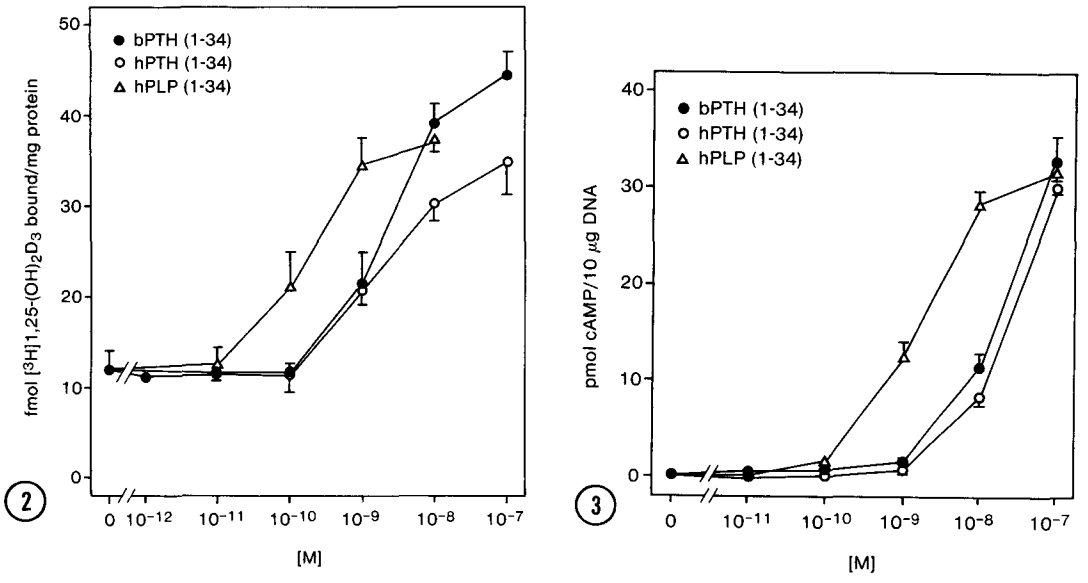


**Fig. 2:** Effect of bPTH, hPTH and hPLP on $1,25\text{-}(OH)_2D_3$ receptor concentration. After the medium was changed from MEM with 2 % FCS to serum-free MEM the UMR-106 cells were incubated for 4 h with various concentrations of bPTH, hPTH and hPLP. $1,25\text{-}(OH)_2D_3$ receptor content was assayed in cytosolic extracts as described in "Materials and Methods" Values are the means ± SD of 2 experiments.

**Fig. 3:** Effect of bPTH, hPTH and hPLP on cAMP accumulation. cAMP production in response to various concentrations bPTH, hPTH and hPLP was determined as described in "Materials and Methods". Values are the means ± of 2 experiments.

## DISCUSSION

To our knowledge this is the first report describing a dose-dependent heterologous up-regulation of the $1,25\text{-}(OH)_2D_3$ receptor by PTH fragments and hPLP in an osteoblast-like cell line. Based on the current results this represents an increase in the number of functional receptors since Scatchard analysis data indicated a single class of binding sites with equivalent affinity contants for $1,25\text{-}(OH)_2D_3$ in vehicle and PTH-treated cultures. Furthermore, the inhibition of the PTH-stimulated receptor up-regulation by actinomycin D support the hypothesis that this process is dependent upon new RNA synthesis.

Although there seems to exist some difference between the maximum effects achieved in the presence of the peptides tested, the lower

$ED_{50}$ for hPLP as compared to human and bovine PTH is remarkable. For the cAMP responses a similar phenomenon was observed. Other investigators also obtained evidence that hPLP is more potent than PTH in stimulating cAMP formation and also in generating a biological response (plasminogen activator activity) in clonal osteogenic sarcoma cells (9,10).

As shown in Figs. 2 and 3 the dose-dependent increase of 1,25-$(OH)_2D_3$ receptors by PTH and hPLP parallels the dose-dependent stimulation of cAMP generation. This suggests an involvement of cAMP as a second messenger in the observed up-regulation of binding sites for 1,25-$(OH)_2D_3$. The fact that low concentrations of PTH and hPLP induce an increment of 1,25-$(OH)_2D_3$ receptors, but do not stimulate cAMP generation, do not directly implicate the involvement of another second messenger. For instance other studies also showed that small increases in cAMP production, not detectable by the methods used, can be sufficient to fully activate cellular response units (15). The difference in the $ED_{50}$ for induction of receptor up-regulation and stimulation of cAMP production is not unique for the biological response examined in the present study. Recently, a comparable difference in de $ED_{50}$ between stimulation of ornithine decarboxylase activity and cAMP production in UMR-106 cells was described (8).

The maximum 1,25-$(OH)_2D_3$ receptor up-regulation by PTH and hPLP is lower than the homologous up-regulation induced by 1,25-$(OH)_2D_3$ itself (7). It remains to be established whether this difference reflects the recent finding that homologous up-regulation of the 1,25-$(OH)_2D_3$ receptor not only results from an increase in receptor synthesis, but also from a prolongation of receptor half-life (16).

The physiological significance of our findings that PTH and hPLP exhibit up-regulation of the 1,25-$(OH)_2D_3$ receptor is not yet clear. However, preliminary observations in our laboratory indicate a higher 1,25-$(OH)_2D_3$ inducible 24-hydroxylase activity after pretreatment of

the cultures with PTH. We interpret this finding to indicate that the PTH-induced increase in 1,25-$(OH)_2D_3$ receptors apparently results in a greater biological response of the cell to 1,25-$(OH)_2D_3$. Whether cAMP is indeed the second messenger involved in the PTH- and hPLP-induced 1,25-$(OH)_2D_3$ receptor up-regulation or that other intracellular messengers are involved remains to be established. Our recent observation that the cAMP analog dibutyryl cAMP is capable of mimicking the described effect on 1,25-$(OH)_2D_3$ receptor level (results not shown) does support a role for cAMP . From a physiological point of view it is interesting that 1,25-$(OH)_2D_3$ attenuates the PTH-stimulated cAMP response in osteoblasts (6), because this could be a potential mechanism in counteracting the heterologous up-regulation of 1,25-$(OH)_2D_3$ receptors by PTH.

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