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1,25 DIHYDROXYVITAMIN D CAUSES ATTENUATION OF CYCLIC AMP RESPONSES IN MONOCYTE-LIKE CELLS[1]

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SUMMARY. The U937 cell is a human monocyte-like line which possesses $1,25(OH)_2D$ receptors. To study $1,25(OH)_2D$ actions in these cells we have measured the cAMP produced by U937 cells during 10 minute stimulus by the ß-adrenergic agonist isoproterenol or by forskolin. cAMP produced by isoproterenol is 6 times that of forskolin. When cells are exposed to $1,25(OH)_2D$ for at least 8 hours the cAMP produced is decreased up to 55%. This attenuative effect of $1,25(OH)_2D$ is dose dependent with an EC_{50} of $10^{-10}M$. Other vitamin D metabolites are less than 1/100th as potent.

A potential role in skeletal metabolism for monocytes, which originate from the same marrow precursor cells as bone osteoclasts (1), was first appreciated when monocytes were demonstrated to cause bone resorption in vitro (2). Recognition that normal monocytes possess 1,25 dihydroxy-vitamin D ($1,25(OH)_2D$) receptors (3) then suggested that $1,25(OH)_2D$ might affect bone resorption through action on monocytes. Administration of $1,25(OH)_2D$ to an osteopetrotic child increased the in vitro monocytic bone resorption and possibly induced maturation of monocytic precursors as increased numbers of osteoblasts were seen in vivo (4).

The mechanisms of $1,25(OH)_2D$ action after an initial requirement for protein synthesis is met have remained largely unknown. Work in this labora-

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tory has shown that $1,25(OH)_2D$ affects the membrane adenylate cyclase (AC) of the osteoblast-like cell line, ROS 17/2.8, causing attenuation of AC activity when stimulated by parathyroid hormone (5). Demonstration of a similar $1,25(OH)_2D$ effect on the AC of other cell types would allow us to make some generalizations about one possible mechanism of $1,25(OH)_2D$ action.

In the present investigation we examine the monocyte-like cell line U937, known to possess a $1,25(OH)_2D$ receptor (6), for evidence of an effect on the AC. To stimulate the AC of the U937 cell we have used the ß-adrenergic agonist isoproterenol as well as forskolin, an agent active at the catalytic subunit of the AC. We have discovered that the U937 AC is highly responsive to both stimuli. With our methods we have been able to demonstrate a specific $1,25(OH)_2D$ effect in attenuating the cAMP response to these agents.

METHODS

U937 cells (gift of Dr. D Chenoweth) were grown in RPMI media with 10% fetal calf serum (FCS) and antibiotics at .5 - 2 x 10^6 cells/ml at 37 C, 95% humidity and 5% CO_2. Except when noted FCS was lowered to 2% in experimental incubations to reduce vitamin D metabolite binding to serum. Vitamin D metabolites (gift of Dr M. Uskokovic, Hoffman-LaRoche) were added in absolute ethanol. After incubations with vitamin D metabolites of up to 48 hr, cells were harvested, counted on a Coulter model ZF, pelleted and resuspended in MEM with1 mM methylisobutylxanthine at 10^7 cells/ml for 15 min. Isoproterenol, final concentration 10^{-5}M, or forskolin 15µm, were added to aliquots of 10^6 cells for stimulation of cAMP production. All points were assayed in triplicate. After 10 min the reactions were stopped by addition of isopropanol. All reactions were performed at 37 C.

cAMP produced during these stimulations was measured by RIA. Isopropanol was evaporated under N_2 gas, and cAMP redissolved in sodium acetate, pH 4.75. The sample was then acetylated, and RIA performed (7).

RESULTS

U937 cells responded to a 10 min isoproterenol stimulus with a large production of cAMP: from 100 - 400 pmole per 1 x 10^6 cells. Forskolin,

TABLE 1. $1,25(OH)_2D$ attenuation of U937 cAMP responses in multiple experiments. Cultures were exposed to 10^{-8} M $1,25(OH)_2D$ (D) or ethanol vehicle (C) for 48 hr. Stimulation of 1 x 10^6 cell aliquots by isoproterenol 10^{-5} M or forskolin 15 um for 10 min produced the cAMP shown. The final concentration of ethanol was .01%. Average %final decrement due to $1,25(OH)_2D$ effect for isoproterenol stimulation was 55%, and for forskolin stimulation was 44%. This effect has a delay of 8 hr (expt not shown); by 12 hr the cAMP produced is 70% of control, at 48 hr the cAMP was 40% of control.

cAMP pmole/10^6 cells					
Isoproterenol			Forskolin		
C	D	%decr	C	D	% decr
134 ± 10	75 ± 8	44			
221 ± 50	170 ± 30	23			
196 ± 13	119 ± 5	40	31 ± 3	16 ± 2	49
287 ± 12	109 ± 10	62	24 ± 5	14 ± 7	42
198 ± 40	73 ± 10	61			
300 ± 10	79 ± 8	74	41 ±15	26 ± 4	37
350 ± 25	106 ± 15	70	77 ± 8	41 ± 5	47
274 ± 30	160 ± 30	42			
370 ± 30	85 ± 11	77			

which acts at or near the catalytic subunit, causes a cAMP production of 30 - 80 pmoles per million cells. The variability of cAMP produced from experiment to experiment was high (Table 1) but the isoproterenol stimulation regularly produced six times the cAMP that forskolin did.

$1,25(OH)_2D$ added to cultures with 2% FCS for 48 hours incubation produced an attenuation of cAMP response to both isoproterenol and forskolin. The average decrement produced by 10^{-8} M $1,25(OH)_2D$ was 55% for isoproterenol stimulation and 44% for forskolin stimulated cultures (Table 1). This attenuative effect was dose dependent (Figure 1) with half maximal effect at 10^{-10} M. The $1,25(OH)_2D$ effect had a delay of 8 hours (experiment not shown); by 12 hours the percent decrement in cAMP produced was 30%, at 48 hr near 50%.

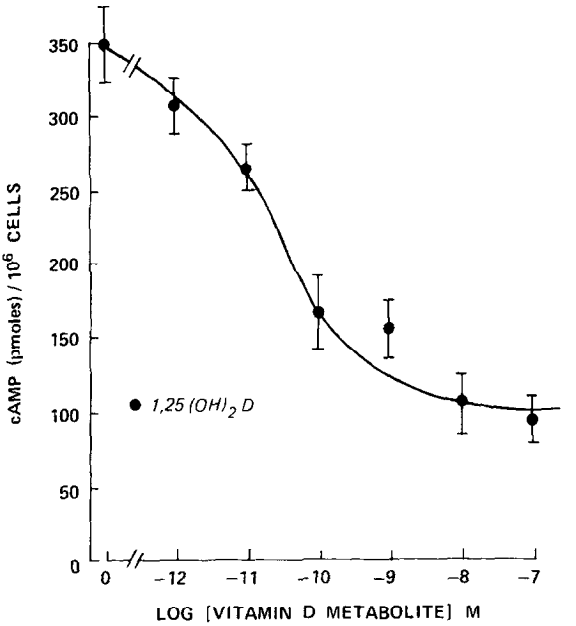

Figure 1. 1,25(OH)$_2$D dose response curve for attenuation of cAMP response in U937 cells. U937 cells were grown in 2% serum and exposed to varying concentrations of 1,25(OH)$_2$D for 48 hrs. cAMP response to 10^{-5} M isoproterenol was measured by RIA. The 1/2 maximal effect of 1,25(OH)$_2$D was at 10^{-10} M. In this experiment 24,25(OH)$_2$D and 25(OH)D were 1/100 as potent; in a replicate experiment 10^{-8} M concentrations of these metabolites were less potent than 10^{-11} M 1,25(OH)$_2$D .

24,25(OH)$_2$D and 25(OH)D were added to cultures for 48 hr at 10^{-8} M and 10^{-9}M. In one experiment 10^{-8} M concentrations of both metabolites were 1/100 as potent as 1,25(OH)$_2$D. In a replicate experment 10^{-8} M concentrations of these metabolites were less potent than 10^{-11} M 1,25(OH)$_2$D.

DISCUSSION

We are interested in 1,25(OH)$_2$D action in U937 cells for several reasons. 1,25(OH)$_2$D stimulated bone resorption by osteoclasts has been difficult to study. U937 cells are a monocytic cell line probably originating from the same marrow precursor cells as osteoclasts and they possess 1,25(OH)$_2$D receptors (6). Therefore 1,25(OH)$_2$D actions on U937 cells are a putative

model of $1,25(OH)_2D$ actions in osteoclasts. Secondly, U937 cells can be induced to mature, gaining phagocytic activity (8) and expressing receptors for the Fc region of immunoglobulin molecules (9); this is thought to be consistent with ability to participate in immune response (9). Dibutyryl cAMP is one of the most potent inducers of maturation (10) along with other agents such as phorbol esters, lipopolysaccharides and interferon (9). $1,25(OH)_2D$ itself promotes maturation of the U937 cell line (6), as it does in normal human monocytes in vitro (3). Thus, the U937 monocyte-like cell offers a means of studying $1,25(OH)_2D$ action in monocytic maturation.

Since we have evidence for an effect of $1,25(OH)_2D$ in attenuating the AC activity in osteoblast-like cells (5), we were interested in searching for a similar effect on the U937 AC system. We have shown a potent response of cAMP production to the ß-adrenergic agent isoproterenol in these cells; to our knowledge this is the first time this has been described. The U937 cAMP response to forskolin, which activates the catalytic subunit of the AC enlisting cooperation from the guanyl regulatory subunit, is also substantial. When $1,25(OH)_2D$ was included in cultures for at least 8 hours we found a dose dependent reduction in cAMP response to both agents. This effect appears to be specific for $1,25(OH)_2D$ with a delay in action of more than four hours suggesting a requirement for protein synthesis. To define these sites of $1,25(OH)_2D$ effect , further studies examining the AC complex subunits will be necessary.

The interaction of the U937 AC with maturation is, as yet, not established. However, the cAMP analog dibutyryl cAMP causes maturation (10). Since $1,25(OH)_2D$ also promotes maturation of this cell line the interaction of $1,25(OH)_2D$ with dibutyryl cAMP is of interest. The $1,25(OH)_2D$ attenuative effect on the cAMP response would appear to be at cross purposes to the implied maturational effect of increased cAMP. Perhaps the maturing action of $1,25(OH)_2D$ in these cells is through a different mechanism than

that of dibutyryl cAMP. If this is true, the attenuative effect of $1,25(OH)_2D$ on the cAMP production could serve as an internal balance to prevent an excessive degree of maturation.

Calcitonin is believed to inhibit osteoclastic bone resorption by causing an increase in intracellular cAMP. If we draw an analogy between U937 cells and osteoclasts, we might propose that by attenuating the cell's capacity to produce cAMP in response to AC agonist hormones, $1,25(OH)_2D$ could promote bone resorption by osteoclasts. At the level of the AC, $1,25(OH)_2D$ could interact with many hormones modulating a unified biological signal to control bone remodelling.

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