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THE EFFECT OF 1,25-DIHYDROXYVITAMIN D3 TREATMENT ON CALCITONIN AND CALCITONIN GENE-RELATED PEPTIDE mRNA LEVELS IN CULTURED HUMAN THYROID C-CELLS

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We utilized the TT cell, a human C-cell line derived from a medullary thyroid carcinoma, to study the effects of 1,25-dihydroxyvitamin D3 on cell growth and expression of the calcitonin gene. The growth rate of cells treated for 8 days with 1,25-dihydroxyvitamin D3 did not differ significantly from control or 24,25-dihydroxyvitamin D3 treated cells. Total RNA was isolated, and calcitonin and calcitonin gene-related peptide (CGRP) levels were measured by hybridization. 1,25 D3 lowered calcitonin and CGRP mRNA levels in a time- and dose- dependent fashion; 24,25 D3 had no effect. Northern blots revealed a decrease in the mature mRNA as well as the common precursor forms, indicating a transcriptional effect of 1,25 D3.
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The calcitonin gene codes the production of two unique peptide hormones, calcitonin and calcitonin gene-related peptide (CGRP) (1,2). Peptide production is controlled by a tissue-specific mechanism involving the mutually exclusive RNA processing of a common precursor RNA to produce either calcitonin mRNA in the thyroid C-cell, or CGRP mRNA in tissues of neural origin (1). The TT cell line, a human C-cell line derived from medullary thyroid carcinoma (3), produces both mRNAs, and therefore provides a model system in which to study factors which regulate calcitonin gene transcription and alternative RNA processing (4). Several substances including: phorbol esters, cAMP analogues, and glucocorticoid hormones have been shown to cause transcriptional enhancement of the calcitonin gene (4-7). Glucocorticoids have additional effects on the alternative RNA processing, causing an elevation in calcitonin mRNA levels with a simultaneous decrease in CGRP mRNA (4).

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Abbreviations: CGRP=calcitonin gene-related peptide; 1,25 D3=1,25 - dihydroxyvitamin D3; 24,25 D3=24,25 dihydroxyvitamin D3.

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Previous work in intact animals has shown an effect of vitamin D and its active metabolite 1,25 D3 to reduce thyroidal C-cell calcitonin content (8). It has not been determined whether this effect is mediated by cellular depletion of calcitonin caused by the vitamin D-mediated increase in serum calcium (9), or by a direct vitamin D effect. To answer this question, we have studied the effect of vitamin D analogs on calcitonin gene expression in a model of the human C-cell. The results presented here indicate a direct effect of 1,25 D3 to lower both calcitonin and CGRP mRNA levels, probably by inhibiting transcription of the calcitonin gene.

MATERIALS AND METHODS

Cell Culture Methodology. The TT cells were maintained in RPMI 640 medium supplemented with 10% fetal calf serum (Gibco Laboratories, Grand Island, NY), using previously described techniques (10). Cell counts were determined with the aid of a Coulter Counter (model ZF), as previously described (10). Vitamin D metabolites (1,25-dihydroxyvitamin D3 and 24,25-dihydroxyvitamin D3, Hoffman LaRoche) were added to the medium as 25 mM stocks prepared in ethanol. Control cells received an equal volume of ethanol.

RNA Isolation and Hybridization. Total cellular RNA was isolated by the guanidine isothiocyanate procedure (5). The TT cells were plated at an initial density of ~3 x 106 cells/100 mm dish. The cells were allowed to attach for 24 hours prior to treatment. Following treatment, total RNA was isolated and hybridization was performed as previously described (4).

RESULTS AND DISCUSSION

Treatment of other cell lines with vitamin D analogs has previously demonstrated an inhibition of cell growth as a result of toxicity or induction of cell differentiation (11). In contrast, neither 1,25 D3 (10^{-7}M) nor 24,25 D3 (10^{-7}M) had an effect on cell growth in the TT cell line when examined over an 8 day period with fresh medium and treatment changes every two days (data not shown). The lack of effect of these vitamin D analogs differs from the growth inhibitory effects of phorbol esters (6) and dexamethasone (4) in this same cell line.

The steady-state levels of both calcitonin and CGRP mRNA were determined in TT cells treated with 1,25 D3, and compared to values obtained for 24,25 D3-treated and untreated controls. Figure 1 clearly demonstrates a parallel decrease in both calcitonin and CGRP steady-state mRNA levels in TT cells treated with 10^{-7} M 1,25 D3 over a six day time course. This effect was reversible upon removal of treatment. The calcitonin and CGRP mRNA levels in 24,25 D3-treated cells were not lowered to the same extent as 1,25 D3

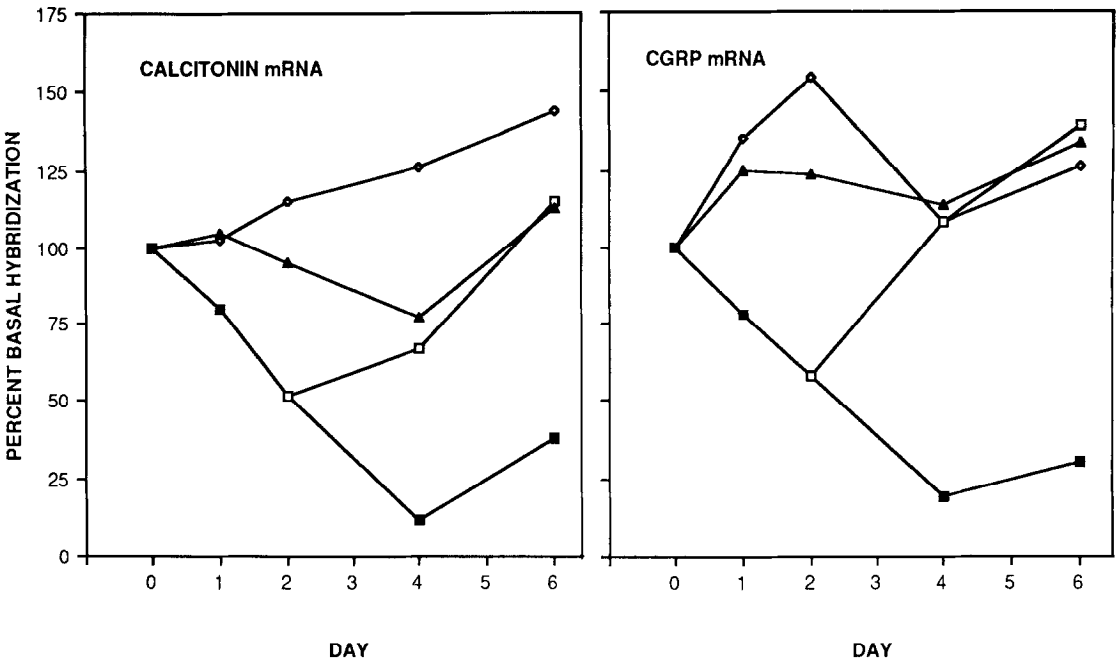

Figure 1. The effect of 1,25 D3 and 24,25 D3 treatment on calcitonin and CGRP mRNA levels. TT cells were plated in 35mm dishes at an initial density of ~200,000 cells/dish. Treatment groups were as follows: control (◇), 1,25 D3, 10^{-7}M (■), 24,25 D3, 10^{-7}M (▲), or withdrawal after 48h of 1,25 D3 (□). Medium was changed at 48 hour intervals. RNA measurements were performed by dot hybridization of the pooled total RNA isolated from five dishes at each time point. Hybridization values are expressed relative to the day-zero control.

during the time course at the same dosage. As previously reported we observed a growth-related increase in both mRNA levels in control cells (4). It is curious that calcitonin and CGRP mRNA levels appear to rebound at day six. We have observed this phenomenon more than once and are uncertain whether it represents an artifact, is cell growth related, or perhaps represents vitamin D receptor down regulation. Figure 2 shows a dose-dependent inhibitory effect of 1,25 D3 on calcitonin and CGRP mRNA levels with a half maximal effect at ~10^{-9} M 1,25 D3 for calcitonin and ~10^{-8} for GCRP. Whether these values reflect true differences in dose response will require further examination.

Northern blot hybridization confirmed the dot blot studies by showing 1,25 D3 to specifically lower appropriately sized calcitonin and CGRP mRNA as well as precursor forms (Figure 3). Despite the fact that the autoradiograph in Figure 3 has been deliberately overexposed in order to examine the precursor RNA bands, the inhibitory effect of 1,25 D3 on calcitonin and CGRP mRNA is still clearly evident. Densitometric analysis of autoradiographs exposed for shorter times reveals a five-fold reduction in both mRNA levels

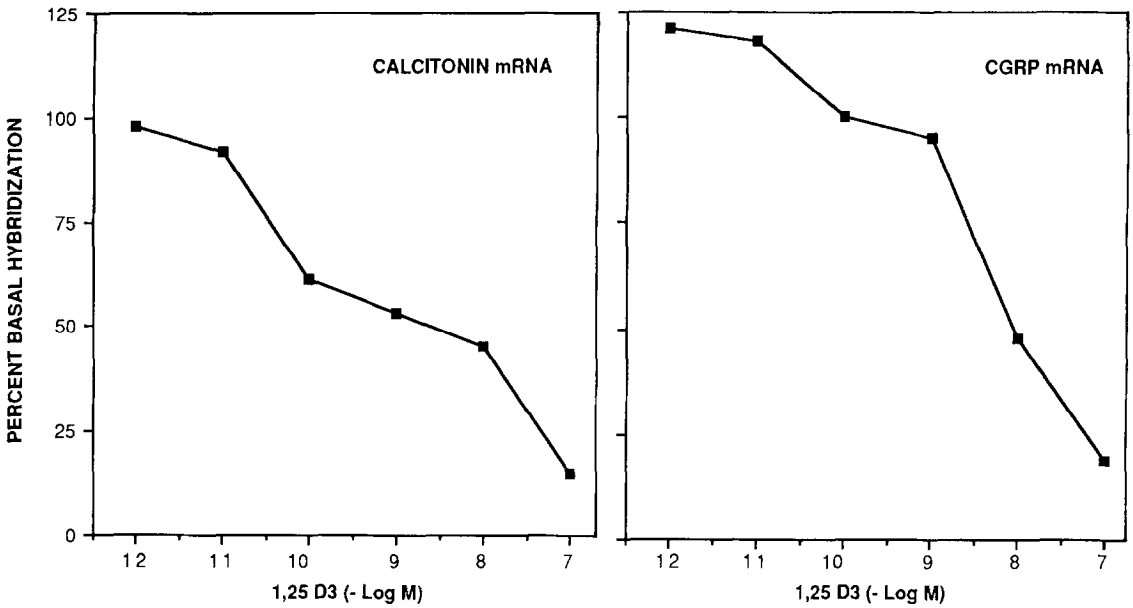

Figure 2. The effect of 1,25 D3 dosage on calcitonin and CGRP mRNA levels. TT cells were plated as described in Fig. 2. Cells were treated with the indicated dosage of 1,25 D3 for four days with a single medium change at two days. Calcitonin and CGRP mRNA levels were determined by dot hybridization of the pooled total RNA isolated from five dishes at each dosage. Hybridization values are expressed relative to untreated controls.

following a four day treatment with 10^{-7} M 1,25 D3. From Figure 3 it is clear that the precursor RNA levels are depressed to the same extent as the mature mRNA bands in the 1,25 D3-treated samples.

The results presented in this paper demonstrate that the previously described effect of 1,25 D3 to lower glandular calcitonin content is caused by a specific effect of 1,25 D3 on the production of calcitonin mRNA. Although the effect in the intact animal may involve additional mechanisms, such as a vitamin D effect to increase serum calcium levels thereby causing depletion of cellular calcitonin (9), it is clear from these studies that the 1,25 D3 effect could be explained by an effect on calcitonin mRNA.

The decrease we have seen in the steady state levels of calcitonin and CGRP mRNA can be explained by an inhibition of calcitonin gene transcription, or by enhanced degradation of the two mRNA components. The parallel decrease of both mature and precursor RNAs at a rate reflecting their previously reported half-lives (4) suggests that 1,25 D3 affects calcitonin gene transcription, rather than RNA stability. This conclusion is supported by examples of both negative and positive transcriptional regulation by 1,25 D3 (12,13). To our knowledge, 1,25 D3 induced mRNA destabilization has not been reported.

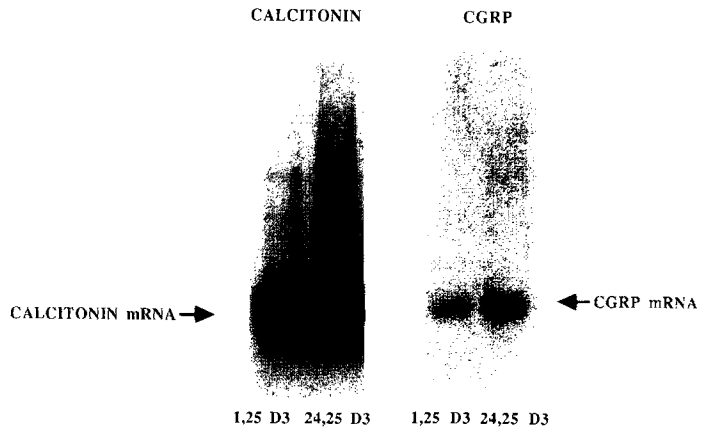

Figure 3. Northern analysis of calcitonin and CGRP mRNA in 1,25 D3 treated TT cells. Total RNA was isolated from TT cells treated with 10^{-7} M 1,25 D3 and 24,25 D3 for four days. Northern hybridizations were performed using 15 µg of total RNA per lane.

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