Negative Regulation of the Human Atrial Natriuretic Peptide Gene by 1,25-Dihydroxyvitamin D₃*

(Qian Li$ and David G. Gardner§
From the Metabolic Research Unit and the Department of Medicine, University of California, San Francisco, California 94143

We have examined the effect of 1,25-dihydroxyvitamin D₃ on the promoter activity of the atrial natriuretic peptide (ANP) gene in cultured neonatal rat atrial myocytes. In acute transfection studies, 1,25-dihydroxyvitamin D₃ inhibited the expression of a human ANP (hANP) promoter-driven chloramphenicol acetyltransferase reporter (−1150 hANP CAT) in a dose-dependent fashion (10⁻¹⁰⁻¹⁰⁻⁸⁵m). When an expression vector for the vitamin D receptor (VDR) (pSVL-VDR) was introduced together with the reporter plasmid, there was a significant ligand-dependent amplification of the vitamin D receptor-dependent inhibition. Deletion analysis of the 5′-flanking sequence localized the suppressible promoter sequence to within 104 base pairs of the transcription start site of the hANP gene.

Thyroid hormone, glucocorticoid, estrogen, and retinoid acid receptor were incapable of mimicking the VDR-dependent inhibition. Retinoid X receptor, on the other hand, effected a significant reduction in hANP promoter activity which was at least additive with that produced by the liganded VDR. The VDR-dependent inhibition displayed promoter selectivity. Both the SV40 promoter and a conventional vitamin D response element linked to a truncated SV40 promoter were activated by the liganded vitamin D receptor, whereas the Rous sarcoma virus promoter was unaffected. On the other hand, the cardiac-specific troponin T promoter was suppressed in a fashion similar to ANP. These findings imply a potentially important role for vitamin D₃ in the regulation of gene transcription in myocardial cells.

The secosteroid hormone 1,25-dihydroxyvitamin D₃ (1,25-(OH)₂D₃) interacts with specific nuclear receptors in classic target tissues such as intestine, kidney, and bone to regulate gene transcription and modulate calcium homeostasis (DeLuca, 1992; Enomoto et al., 1992; Stumpf et al., 1979). Transcriptional control has been linked to specific cis-acting regulatory vitamin D response elements (termed VDREs) normally located in close proximity to the target gene's promoter (Noda et al., 1990; Okazaki et al., 1991; Sone et al., 1991). Although its functional role is less well defined in other tissues, low levels of vitamin D receptor have also been identified in lung (Walters et al., 1987a), testis (Osmundsen et al., 1989), as well as in tissues of the immune (Minghetti and Norman, 1988) and cardiovascular systems (Mitsushashi et al., 1991; Walters et al., 1986).

1,25-(OH)₂D₃ has been shown to increase calcium uptake in isolated adult rat ventricular myocytes, presumably through association with high affinity receptors (Walters et al., 1987b). 1,25-(OH)₂D₃ has also been shown to regulate myocardial contractile function and vitamin D deficiency leads to significant alterations in myocardial morphology (Weishaar et al., 1990). Little, however, is known regarding the ability of this hormone to regulate myocardial gene expression.

Atrial natriuretic peptide (ANP) is a hormone which is synthesized primarily in the cardiac atria. Expression of the ANP gene is known to be controlled by a variety of mechanical and biochemical stimuli including a number of hormones (e.g. glucocorticoids and thyroid hormones) which act predominantly in the nucleus (Gardner et al., 1987, 1988). Recent histochemical studies have co-localized [³H]1,25-(OH)₂D₃ and immunoreactive ANP within cells of the rat right atrium, a prominent site of ANP biosynthesis (Bidmon et al., 1991). This finding raises the intriguing possibility that the ANP gene might represent a physiologically relevant target for regulation by this hormone. In the present study, we have examined the effect of 1,25-(OH)₂D₃ on ANP promoter activity in cultured neonatal rat atrial myocytes. Our findings demonstrate that the liganded VDR suppresses ANP gene activity and imply a potentially important role for this hormone in the regulation of gene transcription in myocardial cells.

EXPERIMENTAL PROCEDURES

Materials—Restriction enzymes were purchased from Boehringer Mannheim. [α-³²P]CTP and [³H]acetyl coenzyme A were purchased from Du Pont NEN, and 1,25-(OH)₂D₃ from Biomol Research Laboratory (Plymouth Meeting, PA). Other reagents were purchased from standard commercial suppliers.

Plasmid Constructions—Fusion plasmids containing varying lengths of the 5′-flanking sequence from the human ANP gene linked to baculovirus promoters and acetyltransferase coding sequence have been described previously (La Pointe et al., 1988; Wu et al., 1991), as has the construction of the mutant A1 hANP CAT MUT (Kovacic-Milivojevic and Gardner, 1992). The latter construct contains a nonconservative transversion mutation at each of seven positions included in a consensus AP-1 binding site (positions 241 to 235 with respect to the transcription start site) in −410 hANP CAT. For construction of −104 hANP LUC, a BamHII-HindIII fragment, including the relevant promoter sequence, was excised from −104 hANP CAT and subcloned into compatible restriction sites in pFOX LUC, a vector in which luciferase coding sequence has been positioned downstream from a polylinker which includes the aforementioned restriction sites. The construction

* This work was supported by National Institutes of Health Grant RO1 HL-35753 and by a grant-in-aid from the American Heart Association (California Affiliate).§ Supported by an Established Investigator Award from the American Heart Association. To whom correspondence should be addressed: Metabolic Research Unit, Box 0540, University of California, San Francisco, San Francisco, CA 94143. Tel.: 415-476-2729; Fax: 415-476-1660.

1 The abbreviations used are: 1,25(OH)₂D₃, 1,25-dihydroxyvitamin D₃; ANP, atrial natriuretic peptide; hANP, human ANP; VDR, vitamin D receptor; VDRE, vitamin D response element; CAT, chloramphenicol acetyltransferase; RXR, retinoid X receptor; RSV, Rous sarcoma virus; TRE, 12-O-tetradecanoylphorbol-13-acetate response element; LUC, luciferase; TnT, troponin T.

2 M. German, personal communication.
of lamin CAT (La Pointe et al., 1988; Wu et al., 1991), RSVCAT (Gorman et al., 1982a), pSV2CAT (Gorman et al., 1982b), and DR-3-CAT (Umesono et al., 1991) have been described. The full-length cDNA for the human VDR was generously provided by Dr. A. Baker (Baker et al., 1988). A 5.0-kilobase EcoRI fragment containing the entire coding sequence for the receptor was subcloned into pSVL. This latter vector allows expression of receptor coding sequence from the SV40 promoter. The expression vectors for the human thyroid hormone receptor (Thompson and Evans, 1989), human estrogen receptor (Green et al., 1992), human retinoid X receptor (Kliewer et al., 1992), and human retinoic acid receptor (Giguere et al., 1987) have been described. The expression vector for the human glucocorticoid receptor, provided by Dr. B. West, contains a full-length human glucocorticoid receptor cDNA positioned downstream from an SV40 enhancer-RSV promoter. The human cjun expression vector (RSV-cjun) was provided by Drs. R. Tjian and V. Baichwal (Turner and Tjian, 1989).

Cell Preparation—Atrial myocytes were isolated from the upper one-third of 1-day-old neonatal rat hearts by alternate cycles of trypsin digestion and mechanical disruption as previously described (La Pointe et al., 1988; Wu et al., 1991). Following isolation, cells were subjected to electroporation (see below) before plating. Transfected cells were cultured in Dulbecco's modified Eagle's medium-H-21 containing 10% fetal calf serum for 24 h before switching to serum-free media (Bauer et al., 1976). Specific chemical additives or agonists were diluted 1:1000 from stock solutions into serum-free media; similar concentrations of ligand-free vehicle were without effect in these cultures. Transfection efficiency, assessed from measuring β-galactosidase activity, varied by less than 15% within a given experiment.

Cardiac fibroblasts were prepared as described previously (Wu et al., 1991). After 1–2 weeks in culture, fibroblast cells were removed from the culture plates with trypsin and electroporated as described below.

DNA Transfection and CAT Assay—Transient transfection of the atrial myocytes was achieved by electroporation with 250 V at 250 microfarads as detailed previously (Kovacic-Milivojevic and Gardner, 1992). After transfection, cells were plated in six-well dishes at a density of 105 cells/well. Media was changed at 24 h, and the appropriate agonist(s) added to the fresh culture media. Cells were harvested and lysed in 250 μl Tris, 0.1% Triton X-100 60–72 h after transfection. Protein concentration of each cell extract was measured using the Coomassie protein reagent (Pierce Chemicals Co.). Fifty to 100 μg of the cellular protein were used to assay CAT activity for each sample. Assays were carried out as described by Neumann et al. (1987). Where possible, the CAT activity for each hANP construct was expressed as a percentage of that determined for the −1150 hANP CAT construct (arbitrarily assigned as 100%) in the same experiment.

Statistical Analysis—Unless stated otherwise, statistical differences were evaluated by one-way analysis of variance with the Newman-Keuls test for significance or by the unpaired Student's t test.

RESULTS

To determine the effects of liganded VDR on the transcriptional activity of the atrial natriuretic peptide gene, atrial myocytes were transfected with chimeric constructs linking 1150 base pairs of human ANP gene 5′-flanking sequence to a bacterial CAT reporter. After 24 h, varying concentrations of 1,25-(OH)2D3 were added to the media. The incubation was continued and cells were harvested 48 h later for measurement of reporter activity. As shown in Fig. 1A, treatment of cells expressing the −1150 hANP CAT construct with 1,25-(OH)2D3 resulted in a small reduction in reporter activity. When pSVL-VDR was introduced into these cells, there was a significant amplification of the vitamin D-dependent reduction in reporter activity. Both the sensitivity of the hANP promoter to the ligand and the maximal level of inhibition were increased in those cultures overexpressing the vitamin D receptor. Varying the amounts of co-transfected receptor revealed ligand-dependent inhibition with as little as 1 μg of pSVL-VDR in the transfection and a maximal effect with 5 μg of the receptor plasmid (Fig. 1B). Increasing levels of pSVL-VDR to 30 μg resulted in a substantial decrease in the measured reporter activity in the presence or absence of the ligand suggesting a nonspecific effect on transcription. All subsequent transfections were carried out with 5 μg of pSVL-VDR.

In an attempt to localize the site of VDR action on the hANP gene promoter, we employed a similar co-transfection strategy with a series of 5′-deletion mutants containing varying lengths of 5′-flanking sequence upstream from the same CAT reporter. As shown in Fig. 2, the roughly 50% inhibition of hANP promoter activity seen in the presence of VDR plus ligand was preserved as the deletions were extended to −104 relative to the transcription start site. In the case of −104 hANP CAT, liganded VDR was no longer capable of inhibiting promoter activity in a statistically significant fashion. However, it is noteworthy that even in this case the relatively low level of receptor expression appeared to fall modestly in the vitamin D-treated, VDR-transfected cells. To evaluate this latter finding further, we transferred the −104 promoter fragment to a more sensitive luciferase reporter system (−104 hANP LUC). As expected, this setting the reporter function was suppressed by 42 ± 17% (p < 0.05; n = 3) in the presence of the liganded vitamin D receptor. This latter finding, in addition to providing further localization of the inhibitory locus, argues against a possible contribution from the reporter plasmid itself in effecting the VDR-dependent inhibition.

Previous studies on the osteocalcin gene suggested that VDR controls the transcriptional activity of that gene by virtue of its ability to interfere with the activity of transcription factor AP-1.

**Fig. 1. Effect of 1,25-(OH)2D3 on hANP gene transcription.** A, dose-dependent inhibition of ANP gene promoter by vitamin D3. Freshly isolated atrial cells were transfected with −1150 hANP CAT with (hatched bars) or without (solid bars) the VDR expression vector. Varying concentrations of vitamin D3 were added to the culture media 48 h prior to cell harvesting for CAT assay. B, dose response for VDR. Atrial cells were transfected with −1150 hANP CAT in the absence (CTRL) or presence of varying concentrations of VDR expression vector. Twenty-four hours after transfection, the cells were treated with 10 nM vitamin D3 (hatched bars) or vehicle (solid bars) for 48 h prior to preparation of cytosolic extracts for CAT assay. All values are expressed as a percentage of the CAT activity in control cells (non-vitamin D3-treated or VDR-transfected). Values represent mean ± S.D. from three to five different experiments. In each case the hatched bar group was statistically different, when compared to the solid bar group, by two-way analysis of variance.
The VDR belongs to a larger family of nuclear receptors, including those for the thyroid, glucocorticoid, estrogen, and retinoic acid receptors, which possess similar structural and functional properties (Evans, 1988). Since several of these receptors are actually present in normal cardiac tissue, it was important to document the specificity of the VDR-mediated effect. We addressed this question using an approach similar to that described above, that is, by transfecting atrial myocytes with expression vectors capable of overproducing one of a number of steroid or thyroid hormone receptors and determining the effect of this maneuver on the activity of a co-transfected hANP promoter. As shown in Table I, the thyroid, glucocorticoid, estrogen, or retinoic acid receptor did not effect a significant decrease in reporter activity. In fact, in each case there was a modest increase in reporter activity in the presence of either the liganded or the unliganded receptor. However, when RXR, a related member of the retinoic acid receptor family, was tested, a reduction in reporter activity, which approached that seen with the VDR, was observed. The combination of RXR and VDR together proved to be at least additive in effect, reducing expression of the reporter to approximately 15% of the control levels. These data indicate that while the inhibitory properties of the liganded VDR are present in and/or amplified by at least one other member of the steroid/thyroid receptor family, they are by no means universally shared by all members of the group, implying specificity in the inhibition.

Next, we investigated the promoter specificity of the VDR-dependent inhibition. As shown in Fig. 4, co-transfection with a liganded VDR had little or no effect on expression of either the RSV promoter or a promoter-less lamin CAT construct in atrial myocytes. A construct linking 268 base pairs of the cardiac troponin T promoter to CAT (-268 cTNT CAT) (Mar et al., 1988) responded in a fashion similar to the hANP gene promoter with a fall in reporter activity. However, both an SV40 promoter-driven construct (pSV2 CAT), as well as a construct linking one copy of a putative vitamin D response element (AGGTCA agg AGGTCA (Umesono et al., 1991)) to an enhancerless SV40 early promoter-CAT reporter (DR-3-CAT), responded to overexpression of liganded VDR with a significant increase in reporter activity. Thus, the response of individual promoters to liganded VDR in these cells is highly idiosyncratic and implies that the vitamin D-dependent inhibition of the hANP gene promoter does not reflect nonspecific toxicity at the level of the cardiac myocyte, nor does it represent a global suppression of all RNA polymerase II-dependent transcription in these cells.

Because our atrial myocyte cultures are uniformly contaminated with non-myocardial elements (e.g. fibroblasts), it remained possible that the observed VDR-dependent inhibition of ANP gene transcription could have resulted indirectly from disruption of some key paracrine interaction between the atrial myocytes and cardiac mesenchymal cells in these cultures. This is a reasonable concern since vitamin D receptors have been described in the heart (Walters et al., 1986; Weishaar et al., 1990) and a number of mesenchymally derived growth factors (e.g. fibroblast growth factors) and cicosanoids (e.g. prostaglandin-$\delta_2$) clearly possess the ability to activate ANP gene expression in myocardial cells (Gardner and Schultz, 1990; Parker et al., 1990). Furthermore, morphological abnormalities in the myocardium, which are induced by vitamin D deficiency, have been shown to involve the interstitial compartment where fibroblasts predominate (Weishaar et al., 1990). To address this question we performed a mixing experiment. We independently transfected cardiac mesenchymal cells with the VDR expression plasmid and atrial myocytes with -1150 hANP CAT, mixed the cells together, and carried them in co-culture in the presence of 1,25-(OH)$_2$D$_3$ for 48 h. Reporter gene expression was then compared with that achieved following co-transfection of both plasmids into atrial myocytes alone. As shown in Fig. 5, transfection of VDR into cardiac mesenchymal cells followed by co-culture with atrial myocytes failed to reproduce the effect seen when both plasmids were introduced into the myocardial cells. This implies that overexpression of VDR must take place within the myocyte itself for maximal inhibition to be realized and argues against direct involvement of non-myocardial elements in the effect.

Finally, because vitamin D is known to have important effects on cellular calcium dynamics in a number of tissues, in-
Studies from many different laboratories have provided convincing evidence that vitamin D has the properties characteristic of a circulating hormone. The identification of high affinity nuclear receptors for 1,25-(OH)_{2}D_{3}, the relevant bioactive metabolite of vitamin D_{3}, in target tissues such as intestine and bone raised the possibility that it might function in a manner analogous to the steroid hormones within the cell. Subsequent cloning of the vitamin D_{3} receptor confirmed that it was, in fact, a member of this broad class of nuclear transcription factors (Baker et al., 1988). Specific structural features supported a further assignment to a subgroup of receptors which included those for thyroid hormone, estrogen, and retinoic acid. In its role as a transcription factor, the VDR has been linked to the regulation of a number of genes. These include those for osteocalcin (Kerner et al., 1989; Demay et al., 1990), osteopontin (Noda et al., 1990), and calbindin (Gill and Christakos, 1993) where it acts as a positive regulatory factor, and type I collagen (Owen et al., 1990), parathyroid hormone (Okazaki et al., 1988, 1991) and the helix-loop-helix protein Id (Kawaguchi et al., 1992), where it has been shown to inhibit transcriptional activity. In most examples where positive regulatory activity has been identified, the VDR effect has been linked to the putative

including the heart, we sought to determine whether the inhibitory properties displayed here were in any way calcium dependent. We approached this question by either supplementing (i.e. exogenous calcium chloride) or sequestering (i.e. EGTA treatment) calcium in the medium. The suppression of ANP gene promoter activity was not altered by the addition or reduction (i.e. EGTA treatment) of calcium to the culture medium (Table II). This would argue that the VDR does not depend upon secondary effects on calcium exchange across the sarcolemmal membrane to achieve full inhibitory activity.

**DISCUSSION**

Studies from many different laboratories have provided convincing evidence that vitamin D has the properties characteristic of a circulating hormone. The identification of high affinity nuclear receptors for 1,25-(OH)_{2}D_{3}, the relevant bioactive metabolite of vitamin D_{3}, in target tissues such as intestine and bone raised the possibility that it might function in a manner analogous to the steroid hormones within the cell. Subsequent cloning of the vitamin D_{3} receptor confirmed that it was, in fact, a member of this broad class of nuclear transcription factors (Baker et al., 1988). Specific structural features supported a further assignment to a subgroup of receptors which included those for thyroid hormone, estrogen, and retinoic acid. In its role as a transcription factor, the VDR has been linked to the regulation of a number of genes. These include those for osteocalcin (Kerner et al., 1989; Demay et al., 1990), osteopontin (Noda et al., 1990), and calbindin (Gill and Christakos, 1993) where it acts as a positive regulatory factor, and type I collagen (Owen et al., 1990), parathyroid hormone (Okazaki et al., 1988, 1991) and the helix-loop-helix protein Id (Kawaguchi et al., 1992), where it has been shown to inhibit transcriptional activity. In most examples where positive regulatory activity has been identified, the VDR effect has been linked to the putative

- Figure 3. Effect of liganded VDR on c-jun-activated hANP gene promoter activity. A, atrial cells were transfected with -1150 hANP CAT and varying combinations of the c-jun (1 pg) or VDR (5 pg) expression vector and collected for assay 72 h later. Where indicated, 1,25-(OH)_{2}D_{3} (10^{-8} M) was included for the final 48 h of incubation. B, Inhibition of the ANP promoter by 1,25-(OH)_{2}D_{3} is independent of the AP-1 site. The wild type -1150 hANP CAT or a variant (−410 hANP CAT) was transfected into atrial cells in the presence or absence of 5 pg of VDR and cultured for 72 h before CAT measurement. Those cells treated with 1,25-(OH)_{2}D_{3} were exposed to the ligand for the last 48 h of the incubation. All values are normalized to the CAT activity measured in cells transfected with −1150 hANP CAT alone. The data represent results from three to six separate experiments and are expressed as the mean ± S.D. *p < 0.05 relative to the relevant control.

**TABLE I**

<table>
<thead>
<tr>
<th>Ligand</th>
<th>Exp. 1</th>
<th>Exp. 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>100.0</td>
<td>100.0</td>
</tr>
<tr>
<td>TR</td>
<td>154.0 ± 14.0</td>
<td>156.0 ± 4.0</td>
</tr>
<tr>
<td>ER</td>
<td>163.0 ± 14.0</td>
<td>222.0 ± 44.0</td>
</tr>
<tr>
<td>GR</td>
<td>178.0 ± 35.0</td>
<td>202.0 ± 19.0</td>
</tr>
<tr>
<td>RA</td>
<td>119.0 ± 7.0</td>
<td>116.0 ± 7.0</td>
</tr>
</tbody>
</table>

- Table 1. Effects of nuclear hormone receptors on hANP gene transcription

- Figure 4. Effect of vitamin D_{3} on the expression of other gene promoters in atrial cells. pSV2 CAT, DR-3 CAT, lamin CAT, and RSV CAT, or −268 cTNT CAT were introduced into atrial cells with or without co-transfection of 5 µg of the VDR expression vector. Vitamin D_{3} treatment and subsequent analyses were carried out as described in Fig. 3. All values are normalized to the CAT activity in control cells (CTRL) for each individual group. The data represent results from three to four separate experiments and are expressed as the means ± S.D. *p < 0.05 versus CTRL.
occupancy of a cis-acting element, termed the VDRE, by the receptor. The mechanism underlying negative regulatory activity is only partially understood. In the case of the osteocalcin gene, as mentioned above, the VDR, through its association with the osteocalcin receptor, competes for promoter occupancy of a cis-acting element, termed the VDRE, by the ubiquitous transcription factor which normally acts to reduce expression of the osteocalcin gene (Owen et al., 1990). This competition arises because the putative cis-acting binding sites for the VDR and AP-1 are to some extent overlapping. In the case of the hANP gene there is no VDRE-like sequence in the vicinity of the consensus AP-1 binding site, suggesting limited opportunity for direct physical interaction of these transcription factors. Thus, while liganded VDR was capable of suppressing jun-dependent hANP gene transcription, the persistent inhibition seen with -410 hANP CAT MUT, which is mutated across the AP-1 binding site, suggests that the inhibition is mediated by a regulatory element(s) which lies outside this core sequence.

We have localized the minimal suppressible promoter sequence in the hANP gene to within 104 base pairs of the transcription start (i.e. CAP site). We have scanned the DNA sequence between -194 and +18 in the hANP gene for regions of homology to the consensus VDRE (in either orientation) described by Kerner et al. (1989) and Demay et al. (1990) and found none, though several half-sites were found further upstream between -1500 and -2500 relative to the CAP site. Furthermore, we have carried out gel mobility shift studies with in vitro translated VDR and a radiolabeled restriction fragment which covers this region of the gene. Under conditions which permitted identification of interactions between the VDR and a conventional VDRE, we found no evidence for association of the VDR with the proximal (-104 to +18) hANP gene fragment (data not shown) when employed alone or in the presence of HeLa cell extract or RXR (to provide the accessory factor which appears to be required for optimal VDR-VDRE association (Liao et al., 1990)). There are a number of explanations which could account for our findings. The inhibition of hANP gene transcription could be indirect and mediated by a gene product which is itself regulated by such elements. Alternatively, the inhibition could be mediated through interaction of VDR with other regulatory transcription factors or key components of the basal transcriptional machinery in a fashion which does not require direct binding to a cognate VDRE (i.e. protein-protein interaction). Vitamin D-dependent modulation of non VDR-mediated protein-DNA interactions in the "TATA" box region of the osteocalcin promoter have recently been described (Bortell et al., 1992).

The inhibitory activity of the liganded VDR was not specific for the ANP gene promoter in these cells. Neither was it generalizable to all polymerase II-dependent transcription. The most striking effects were found with the ANP promoter and with the cardiac troponin T promoter constructs, while no effect or a modest increase in promoter activity was seen with the RSV, SV40, and DR-3 constructs. The latter construct, which links a structural homologue of the VDRE upstream of the SV40 promoter, has been shown to respond positively to liganded VDR in other systems (Umesono et al., 1991). ANP and troponin T are both expressed in the myocytes of the heart. These findings raise the intriguing possibility that the inhibitory properties of the liganded VDR may be targeted toward genes which are selectively expressed within cardiac myocytes. The effect clearly displayed specificity for the VDR. Overexpression of the receptors for the glucocorticoids, thyroid hormones, estrogens, and retinoic acid effected a modest increase, rather than a decrease, in hANP CAT reporter activity. RXR, on the other hand, proved to be exceptional within this group in that it not only inhibited hANP promoter activity on its own but amplified the VDR-dependent inhibition as well. The RXR protein is exceptional in other ways. Like other members of the thyroid hormone receptor family, RXR is capable of binding as a homodimer to hormone response elements composed of half-sites (5'AGGTCA3', a sequence which is also recognized by other members of this receptor family) organized in tandem array (Lee et al., 1993). In addition, RXR appears to function as an "accessory" protein, heterodimerizing with other members of the extended receptor family (including the VDR, TR, and RAR) and increasing binding affinity of these complexes for the hormone response element (Leid et al., 1992). This increase in binding affinity is accompanied by an increase in functional activity (Klewe et al., 1992; Yu et al., 1991; Zhang et al., 1992) which, in our system, could account for the observed amplification of the VDR effect.

The present study adds to a growing body of evidence suggesting that the cardiovascular system represents an important target for vitamin D activity. A number of studies have identified significant effects of 1,25-(OH)2D3 on contractile function, growth properties and proto-oncogene expression in cardiovascular tissues (Mitsuhashi et al., 1991; Weishaar and Simpson, 1987). In addition, vascular endothelial cells have been shown to possess a 1α-hydroxylase activity capable of converting vitamin D precursors to the bioactive form of the vitamin.
Vitamin D Suppresses ANP Gene Transcription

4939

hormone (Merke et al., 1989). This raises the intriguing possibility that an important paracrine relationship exists between the endothelium and neighboring vascular smooth muscle and/or myocardial tissues. Simpson et al. showed that vitamin D deficiency in the rat results in an increase in cardiac contractility, blood pressure, and peripheral vascular resistance (Weishaar et al., 1990; Weishaar and Simpson, 1987). The changes in blood pressure and peripheral vascular resistance were reversed when the animals were repleted with sufficient calcium to render them normocalcemic; however, the contractile abnormalities persisted, implying a direct effect of vitamin D deficiency on the myocardial cells. This lends support to our findings (Fig. 5) that the liganded VDR regulates the hANP gene promoter through a mechanism which is not directly dependent on levels of calcium in the extracellular compartment.

1,25-(OH)2D3 has been shown to possess potent anti-proliferative activity in a number of different systems (Frampton et al., 1983; Hosomi et al., 1983). Because they are terminally differentiated cells, cardiac myocytes hypertrophy rather than divide in response to proliferative stimuli like mechanical overload, vasoactive peptides and growth factors (for review, see Bugnasky et al., 1991). Cardiac hypertrophy, at least in the rodent, has been shown to activate the immediate early gene (i.e. c-jun, c-fos, c-myc) response as well as an embryonic repertoire of myocardial genes encoding structural and secretory proteins, among them ANP. It is tempting to speculate that 1,25-(OH)2D3 derived from local production in the endothelium of the heart or coronary vessels, could play an important role in modulating or dampening the transcriptional component of the hypertrophic response. Such a mechanism might serve to control the rate and extent of hypertrophy and thereby prevent uncontrolled, and potentially counter-productive, growth in the myocardium.

Acknowledgments—We are grateful to Karl Nakamura for technical assistance and to Drs. Branica Kovacic-Milivojevic and Ralf Ribeiro for critical evaluation of the manuscript. We also express our gratitude to the laboratories of Drs. Ron Evans, Andrew Baker, Peter Kushner, Brian West, Michael German, and Robert Tjian who made plasmids available to us for these studies.

REFERENCES