Expression of 25(OH)D₃ 24-hydroxylase in distal nephron: coordinate regulation by 1,25(OH)₂D₃ and cAMP or PTH

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Yang, Wen, Peter A. Friedman, Rajiv Kumar, John L. Omdahl, Brian K. May, Mei-Ling Siu-Caldera, G. Satyanarayana Reddy, and Sylvia Christakos. Expression of 25(OH)D₃ 24-hydroxylase in distal nephron: coordinate regulation by 1,25(OH)₂D₃ and cAMP or PTH. Am. J. Physiol. 276 (Endocrinol. Metab. 39): E793–E805, 1999.—Previous studies using microdissected nephron segments reported that the exclusive site of renal 25-hydroxyvitamin D₃ 24-hydroxylase (24OHase) activity is the renal proximal convoluted tubule (PCT). We now report the presence of 24OHase mRNA, protein, and activity in cells that are devoid of markers of proximal tubules but express characteristics highly specific for the distal tubule. 24OHase mRNA was undetectable in vehicle-treated mouse distal convoluted tubule (DCT) cells but was markedly induced when DCT cells were treated with 1,25 dihydroxyvitamin D₃ [1,25(OH)₂D₃]. 24OHase protein and activity were also identified in DCT cells by Western blot analysis and HPLC, respectively. 8-Bromo-cAMP (1 mM) or parathyroid hormone [PTH-(1–34); 10 nM] was found to potentiate the effect of 1,25(OH)₂D₃ on 24OHase mRNA. The stimulatory effect of cAMP or PTH on 24OHase expression in DCT cells suggests differential regulation of 24OHase expression in the PCT and DCT. In the presence of cAMP and 1,25(OH)₂D₃, a four- to sixfold induction in vitamin D receptor (VDR) mRNA was observed. VDR protein, as determined by Western blot analysis, was also enhanced in the presence of cAMP. Transient transfection analysis in DCT cells with rat 24OHase promoter deletion constructs demonstrated that cAMP enhanced 1,25(OH)₂D₃-induced 24OHase transcription but this enhancement was not mediated by cAMP response elements (CREs) in the 24OHase promoter. We conclude that 1) although the PCT is the major site of localization of 24OHase, 24OHase mRNA and activity can also be localized in the distal nephron; 2) both PTH and cAMP modulate the induction of 24OHase expression by 1,25(OH)₂D₃ in DCT cells in a manner different from that reported in the PCT; and 3) in DCT cells, upregulation of VDR levels by cAMP, and not an effect on CREs in the 24OHase promoter, is one mechanism involved in the cAMP-mediated modulation of 24OHase transcription.

vitamin D regulation; parathyroid hormone

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21, 28), previous studies using microdissected nephron segments reported that the exclusive site of renal 1α-hydroxylase (9, 33) and 240Hase (33) activities is in the renal proximal tubule (PCT). However, recent studies using antibodies against 240Hase by Kumar et al. (38) showed that epitopes for 240Hase are present in both proximal and distal tubules of the human kidney. In addition, 240Hase protein was identified in both proximal and distal tubules of rat kidney using quantitative immuno electron microbiologic analysis (32). 240Hase mRNA, although predominantly in the PCT, was also recently observed under certain conditions in microdissected cortical collecting ducts (CCD) of the rat distal nephron (29). 240Hase mRNA in the CCD was reported to be induced in intact rats fed low dietary calcium, a condition that resulted in a striking suppression of 240Hase mRNA in the PCT (29). These findings strongly suggest that the proximal tubules are not the sole site of localization of 240Hase in the kidney.

Although the major site of localization of 240Hase is the proximal tubule, in this study we report that 240Hase can also be localized in the distal nephron, consistent with recent immunolocalization studies (32, 38) and recent studies localizing 240Hase mRNA using microdissected nephron segments (29). Because 1,25(OH)2D3 and PTH are major regulators of vitamin D metabolism (18, 27), we examined the effect of 1,25(OH)2D3 and PTH or cAMP (a second messenger of PTH action) on 240Hase expression as well as on the mechanisms involved in the coordinate regulation by 1,25(OH)2D3 and cAMP or PTH of 240Hase transcription. Our findings suggest differential regulation of the 240Hase enzyme in the PCT and DCT and therefore different biological effects of PTH and cAMP on 1,25(OH)2D3 action in these different nephron segments. It is also evident from this study that in the distal nephron, upregulation of VDR levels by cAMP, and not an effect on CREs in the 240Hase promoter, is one mechanism by which cAMP modulates 1,25(OH)2D3-induced transcription of 240Hase.

**MATERIALS AND METHODS**

**Materials.** [14C]chloramphenicol (50 mCi/mmol) and [32P]deoxycytidine triphosphate (3,000 Ci/mmol, 370 MBq/ml) were obtained from DuPont-New England Nuclear (Boston, MA). RadPrime DNA labeling system and all restriction enzymes were purchased from Gibco BRL-Life Technologies (Gaithersburg, MD). Biotrans nylon membranes were obtained from ICN Biochemicals (Costa Mesa, CA). Oligo (dT) cellulose was purchased from Boehringer Mannheim (Indianapolis, IN). 8-Bromo-cAMP, acetyl coenzyme A, phenol, for manide, and guanidinium isothiocyanate were purchased from International Biotechnologies (New Haven, CT). Rat anti-vitamin D receptor antibody was from Affinity BioReagents (Neshanic Station, NJ). This antibody has been reported to cross-react with avian and mammalian VDR (43). Polyclonal antibody against rat 240Hase was generated and characterized in the laboratory of Dr. R. Kumar (38). Mouse 240Hase has been reported to share 94.7% amino acid identity with rat 240Hase (31). Thus the rat antibody cross-reacts with mitochondrial protein prepared from mouse cells. Polyclonal antibody against rat thiazide-sensitive NaCl co transporter was provided by S. Hebert of Vanderbilt University School of Medicine (Nashville, TN). The specificity of this antibody has been characterized previously (45). Immunocytochemical studies have indicated that the rat NaCl cotransporter antibody cross-reacts with mouse kidney and specifically labels the apical membrane of the mouse distal convoluted tubule and early collecting duct (personal communication, Dr. S. Hebert). Chemically synthesized 1,25(OH)2D3 was provided by Dr. M. Uskokovic of Hoffmann-La Roche (Nutley, NJ). 1,25(OH)2[23,24-N-3H]vitamin D3 (95 Ci/mmol) and 25(OH)[26(27)-methyl-3H]vitamin D3 (21 Ci/mmol) were purchased from Amersham Life Science (Arlington Heights, IL).

**Cell culture.** The preparation, culture conditions, and characterization of immortalized mouse DCT cells have been described previously (22, 44). These cells, which are derived from both DCT and cortical thick ascending limbs of Henle's loop, are devoid of markers of proximal tubules (such as alkaline phosphatase and Na+-glucose cotransport) but express characteristics of DCT cells, including thiazide-inhibitable but bumetanide-inhibitable sodium transport. DCT cells were maintained in Dulbecco's modified Eagle's medium plus Ham's F12 nutrient mixture (DMEM-F12, Gibco BRL-Life Technologies) supplemented with 5% heat inactivated fetal bovine serum (FBS) (Gemini, Calabasas, CA) in a humidified atmosphere of 95% O2-5% CO2 at 37°C. Cells were grown to 60% confluence, and 24 h before the start of experiments, medium was changed to serum-free DMEM-F12 medium. Cells were treated with the vehicle or the compounds noted at the concentrations and times indicated.

Studies were also done using primary cultures derived from either mouse DCT or PCT prepared with a double-antibody separation procedure as previously described (44). Primary cultures of these cells have been previously characterized and shown to exhibit a phenotype specific to their site of origin in the nephron (44). Cells were initially plated at a density of 3 x 10^5 cells/cm2 in 100-mm tissue culture plates in Opti-Mem (Gibco BRL-Life Technologies) and allowed to attach and grow for 4-5 days at 37°C in a humidified atmosphere of 95% O2-5% CO2. After 5 days, media were changed. At 7-8 days in culture, cells were treated with vehicle or 1,25(OH)2D3.

**RNA isolation and Northern blot hybridization analysis.** Total RNA was prepared from DCT cells by the guanidinium thiocyanate-phenol chloroform method described by Chomczynski and Sacchi (13). Polyadenylated [poly(A)+] mRNA was prepared by two cycles of oligo (deoxythymidine)-cellulose chromatography. Northern blot analysis was performed as previously described (58). Labeled probes were prepared according to the random prime method (19) using the Random Primers DNA Labeling System (Gibco BRL-Life Technologies). The blots were hybridized to specific 32P-labeled cDNA probes for 16 h at 42°C, washed, and subjected to autoradiography as previously described (58). To detect any problems with transfer or differences in loading, blots were probed with 32P-labeled β-actin cDNA and/or 32P-labeled 18S rRNA cDNA. All autoradiograms were analyzed by densitometric scanning using the Dual-Wavelength Flying Spot Scanner (Shimadzu Scientific Instruments, Princeton, N J ). The relative optical densities obtained using the test probes were divided by the relative optical density obtained after probing with the control probe to normalize for sample variation.

**Measurement of 240Hase activity.** DCT cells were subcultured into 9.6-cm2 wells and maintained in 2 ml DMEM-F12 medium supplemented with 5% FBS. Twenty-four hours
before the experiments, the incubation media was changed to DMEM-F12 medium containing 2% charcoal-dextran stripped FBS to ensure vitamin D-free conditions. The experiments were initiated under induced conditions by treatment with unlabeled 1α,25(OH)2D3 (10−7 M in 0.1% ethanol) or 1,25(OH)2D3 + 1 mM 8-bromo-cAMP and under basal conditions by treatment with vehicle alone (0.1% ethanol). At the end of 24 h, the cells were rinsed with serum-free media and 0.05 µCi [26,27-methyl-3H]25(OH)D3 (1 nM) was added to each well containing 2 ml serum-free DMEM-F12 medium with 0.2% BSA. The incubations were terminated at 1 h with 1 ml methanol. HPLC was performed as described by Siu-Caldera et al. (53). Lipids from both cells and media were extracted using the procedure described by Reddy and Tserng (46). All samples were spiked with 1 µg unlabeled 25(OH)D3 before lipid extraction to assess the recovery of tritiated metabolites. Before HPLC analysis, 1 µg of unlabeled 24(R)25-dihydroxyvitamin D3 [24(R),25(OH)D3] and 23(S)25-dihydroxy-24-oxo-vitamin D3 [24(S),25(OH)2-24-oxo-D3] standards were added to each lipid extract sample. HPLC analysis was performed with a Waters System Controller (model 600E) equipped with a photodiode array detector (model PDA 990) to monitor ultraviolet-absorbing material at 265 nm (Waters, Milford, MA). The samples were analyzed using a Zorbax-SIL column (4.6 mm x 25 cm; DuPont, Wilmington-DE) eluted with isopropanol-hexane (3:97, vol/vol) at a flow rate of 2 ml/min. One-minute fractions were collected, and the radioactivity in each vial was measured in a scintillation counter (Beta Trac TM Analytic, Elk Grove Village, IL) after the addition of 4 ml Scintilene (Fisher Scientific, Pittsburgh, PA). The identity of individual radioactive peaks of both 24(R),25(OH)D3 and 23(S),25(OH)2-24-oxo-D3 from the first HPLC system were further verified by comigration with their respective synthetic standards on a second HPLC system.

Western blot analysis of VDR, 24OHase, and NaCl transporter proteins. For Western blot analysis of VDR, DCT cells were processed for preparation of chromatin as previously described (59). Aliquots of the KCl-extracted chromatin preparation were assayed for protein concentration by the method of Bradford (8), and 20 µg of protein from each sample were used for Western blot analysis. For Western blot analysis of NaCl transporter, DCT cell membrane protein for the NaCl transporter or membrane protein for the NaCl transporter or KCl-extracted chromatin preparation for VDR) was used for the preparation of Western blot analysis of the NaCl transporter. Membrane protein was prepared by homogenization of harvested DCT cell in 0.32 M sucrose, 5 mM Tris·HCl (pH 7.5), and 2 mM EDTA and centrifugation at 3,000 g for 10 min. The resulting supernatant was removed and centrifuged at 100,000 g at 4°C for 30 min. The pellet was resuspended in buffer containing 5 mM Tris·HCl (pH 7.5) and 2 mM EDTA. Protein was measured by the method of Bradford (8), and 30 µg of protein were used for Western analysis. DCT cell protein (mitochondrial protein for 240Hase and membrane protein for the NaCl transporter or KCl-extracted chromatin preparation for VDR) was used for electrophoresis either on a 12% SDS polyacrylamide gel for VDR or on 9% SDS gel for 240Hase and NaCl transporter. After electrophoresis, proteins were transferred electrophoretically to a nitrocellulose membrane (Hybond-ECL; Amer-sham) that was incubated with antibody [anti-VDR monoclonal antibody 9A7, 1:2,000 dilution; anti-rat 240Hase polyclonal antibody (38), 1:400 dilution; or anti-NaCl transporter protein, 1:1,000 dilution] in Tris-buffered saline, pH 7.5 (TBS) for 12 h at 4°C. After washing with TBS, the membrane was incubated with secondary antibody [goat anti-rabbit IgG conjugated to horseradish peroxidase (HRP) was used, and for the polyclonal antibodies goat anti-rabbit IgG conjugated to HRP (Sigma was used)] for 1 h at room temperature. After washing with TBS, the antigen-antibody complex was detected using the electrochemiluminescent Western blotting detection system (Amersham) according to the manufacturer’s protocol.

Complementary DNA probes, 240Hase-chloramphenicol acetyltransferase constructs, cell transfection, and assay of chloramphenicol acetyltransferase activity. The 3.2-kb rat 240Hase cDNA was obtained by EcoR I digestion and was a generous gift from K. Okuda (Hiroshima University School of Dentistry, Hiroshima, Japan) (42). The rat 240Hase cDNA has previously been shown to hybridize with mRNA prepared from mouse kidney (55). A 1.7-kb rat VDR cDNA was obtained by digestion of plBl76 with EcoR I (43). Hybridization of the rat VDR cDNA with mRNA prepared from mouse cell lines has previously been reported (36). The 18S rRNA CDNA was obtained from R. Guntaka (University of Missouri, Columbia, MO), and the 2.1-kb chicken β-actin cDNA was obtained from M. W. Kirchner (17).

For transfection studies, constructs of a chimeric gene in which the rat 240Hase promoter (−1,367/+74) was linked to the chloramphenicol acetyltransferase (CAT) gene and deletion mutant constructs (−671/+74 and −291/+74) as previously described (34) were used. A −160/+3 fragment was isolated using the rat 240Hase promoter sequence −1,367/+74 as a template. Two 20-bp primers corresponding to region −161/−141 of the 240Hase promoter and to −3/+17 of its complementary strand were synthesized and used for amplification by PCR. The size of the amplified product, −161/+3 of the rat 240Hase promoter, was confirmed by agarose gel electrophoresis. This product was purified by the “crush and soak” method described by Maxam and Gilbert (41). After phosphorylation of the PCR product with T4 polynucleotide kinase in the presence of 50 mM Tris·HCl, pH 8.0, 10 mM MgCl2, 15 mM dithiotreitol (DIT), and 0.33 mM ATP, the PCR fragment was ligated into the Sma I site of phCAT (which is derived from pSV2CAT by deleting the simian virus 40 promoter; phCAT was a gift from M. Tocci, Merck, Sharpe and Dohme). The orientation of the inserts was determined by DNA sequencing. Within −161/+3 of the 240Hase promoter is the proximal VDRE as well as a putative cAMP response element (CRE) (TGACTCCA) (35). To mutate the putative CRE, −132/−125 was replaced by a random sequence GACCTAGG by PCR as described above using a sequence corresponding to region −160/−97 with the putative CRE replaced by the random sequence and a 20-bp sequence corresponding to region +3/−17 of the complementary strand as primers. The mutation construct was sequenced and the base substitution was confirmed. To construct a proximal rat 240Hase VDRE-thymidyl kinase (tk) CAT reporter plasmid, two complementary oligonucleotides containing the proximal VDRE of the rat 240Hase promoter (5′-CTAGAGGCCCCC GGCGCCTCACCCTGCGATCGAT CGTCTCT-3′ and 5′-CTAAGGACATGCTGCAGGTAGTG GAGGGCCG GCCGCTC-3′) were synthesized with Xba I half-site overhangs at their 5′ end. One hundred micromolar oligonucleotide were mixed with 7 µl medium salt buffer (1× medium salt buffer; 100 mM Tris·HCl, 100 mM magnesium acetate, 500 mM NaCl, 10 mM DTT, pH 7.5; Boehringer Mannheim), and the final volume was adjusted to 70 µl with H2O. The two complementary oligonucleotides providing the 5′ overhang of the Xba I half-site were allowed to anneal by heating at 100°C for 5 min and cooling to room temperature. After separation of the annealed double-stranded DNA from oligonucleotides by agarose gel electrophoresis, the annealed product was excised from the gel and purified (41). The
purified DNA fragment was phosphorylated by T4 polynucleotide kinase as described above. Phosphorylated double-stranded DNA fragments were ligated into the Xba I site of the tk promoter CAT reporter gene construct by T4 DNA ligase in the presence of 50 mM Tris-HCl (pH 7.6), 10 mM MgCl2, 1 mM ATP, 1 mM DTT, and 5% polyethylene glycol-8,000. The number of inserts was checked by Pst I digestion, and the orientation was confirmed by DNA sequencing. The VDRE multimers were inserted 105 bp upstream of the tk transcription start site. The pBLCAT2 contains the tk promoter inserted upstream of the reporter CAT gene (from J. W. Pike, University of Cincinnati, Cincinnati, OH).

For transfections, cells were plated at a density of $1 \times 10^{6}$ cells/100 mm plate in serum-free media 24 h before transfection. DCT cells were cotransfected with reporter plasmid (8 µg) and the β-galactosidase expression vector pCH110 (4 µg; from Pharmacia, Piscataway, NJ), an internal control for transfection efficiency, using the calcium phosphate DNA precipitation method (5). Cells were transfected for 16 h, shocked for 1 min with 10% dimethyl sulfoxide-PBS, washed with PBS, and treated for 24 h with vehicle (0.1% ethanol) or test compound at the concentrations indicated. After 24-h incubation, cells were harvested and cell extracts were prepared by freeze (−80 °C)-thaw (37 °C) three times, 5 min each. The CAT assay was performed at constant β-galactosidase activity following standard protocols (5, 23). To verify that regions in the promoter other than the 24OHase VDRE were not involved in the cAMP enhancement, additional studies were done using mutant 24OHase promoter luciferase constructs with mutations introduced into the proximal VDRE (M1) or in the proximal and distal VDREs (M4). The M1 and M4 mutations have been described previously (34). Luciferase activity, performed at constant β-galactosidase activity, was determined using a luciferase assay system (Promega). Results were quantitated as relative light units using a luminometer.

Statistical analysis. Data were tested for significance by Student’s t-test for two-group comparison or analysis of variance for multiple-group comparison. Differences <0.05 were assumed to be significant.

RESULTS

Expression of 24OHase mRNA, protein, and activity in DCT cells. Because of the discrepancy between immunolocalization and PCR studies, which showed that epitopes and mRNA for 24OHase are present in both proximal and distal tubules (29, 32, 38), and previous studies that reported that the exclusive site of 24OHase activity is in the proximal tubule (33), we tested the recently available DCT cell line (22, 44) (which is devoid of markers of proximal tubules but which expresses characteristics consistent with the distal tubule) for the presence of 24OHase mRNA, protein, and activity. With the use of Northern blot analysis, 24OHase mRNA was observed in DCT cells after 1.25(OH)2D3 treatment (10−7 M, 24 h); however, basal levels were undetectable. Although 8-bromo-cAMP (1 mM) or 12-O-tetradecanoylphorbol 13-acetate (TPA; 100 mM) alone had no effect on 24OHase mRNA expression, cAMP or TPA enhanced 1.25(OH)2D3-dependent upregulation of 24OHase mRNA by 10- and 6-fold, respectively (Fig. 1). To confirm that the mouse DCT indeed contains 24OHase mRNA, Northern blot analysis was carried out using primary cultures derived specifically from the distal nephron (cortical thick ascending limb plus DCT) or from proximal tubules. Previous studies characterizing the cells derived from the distal nephron in primary culture have indicated, similar to the immortalized cells, that they are devoid of markers of proximal tubules (for example alkaline phosphatase and Na＋-glucose cotransporter) but express characteristics of the distal tubule (44). Basal levels of 24OHase mRNA were undetectable in primary cultures derived from both the PCT and DCT. However, 1.25(OH)2D3 treatment induced the expression of 24OHase mRNA derived from both nephron segments. Primary cultures derived from the PCT exhibited fivefold higher levels of 24OHase mRNA than cells derived from the DCT (Fig. 2). Besides 24OHase mRNA, 24OHase protein was also identified in DCT cells by Western blot analysis using a polyclonal antibody against rat 24OHase. Western blot analysis also indicated the presence of the highly specific distal tubule marker, thiazide-sensitive NaCl transporter, in DCT cells (Fig. 3). In addition, as determined by HPLC, DCT cells were found to metabolize [3H]25(OH)D3 to polar metabolites produced by 24OHase. After pretreatment of DCT cells with 1.25(OH)2D3 (10−7 M, 24 h), the two major metabolites detected at 1 h comigrated with authentic 24(R),25(OH)2D3 and 23(S),25(OH)24-oxo-D3 (Fig. 4, Table 1), both of which are products of the 24OHase enzyme (1, 6).

Effect of cAMP on the induction of 24OHase mRNA by 1.25(OH)2D3 and on VDR mRNA in DCT cells. In previous in vivo studies in rats fed a diet low in calcium, which results in an increase in circulating levels of the PTH and 1.25(OH)2D3, 24OHase mRNA was reported to be expressed in the distal nephron but strikingly suppressed in the PCT (29). To obtain a better un-
standing of the regulation of 24O Hudsonase mRNA in the distal nephron, we analyzed the time course of 24O Hudsonase mRNA expression in vitro in DCT cells in the presence of 1,25(OH)2D3 or 1,25(OH)2D3 + cAMP, a second messenger of PTH action. Because it had been reported that regulation of VDR in the PCT plays an important role in the reciprocal regulation of 24O Hudsonase mRNA in these different nephron segments (29), we also examined the regulation of VDR mRNA in DCT cells.

DCT cells were treated with 10−7 M 1,25(OH)2D3 or 10−7 M 1,25(OH)2D3 + 1 mM 8-bromo-cAMP. Results of Northern blot analysis are shown in Fig. 5. Marked induction in 24O Hudsonase mRNA by 1,25(OH)2D3 alone was observed at 12 h, although quantitatively minor induction was observed after longer autoradiographic exposure as early as 6 h. The maximal response was observed at 48 h. However, VDR mRNA levels remained unchanged during this period (Fig. 5, A and B). In the presence of both 1,25(OH)2D3 and cAMP, 24O Hudsonase mRNA was induced as early as 3 h, reached a plateau from 6 h to 24 h, and then decreased at 48 h (Fig. 5, C and D). Thus cAMP enhanced the rapidity of 24O Hudsonase mRNA expression induced by 1,25(OH)2D3 alone. HPLC analysis indicated that pretreatment of DCT cells with 10−7 M 1,25(OH)2D3 + 1 mM 8-bromo-cAMP for 24 h also resulted in an induction in the production of metabolites of [3H]25(OH)D3 produced by 24O Hudsonase over the levels observed in the presence of 1,25(OH)2D3 alone [1,25(OH)2D3 + cAMP/1,25(OH)2D3 = 1.8-fold for the production of 23(S),25(OH)2-24-oxo-D3 and 2.4-fold for
Table 1. 3H-labeled metabolites of 25(OH)D3 produced by DCT cells incubated with [3H]25(OH)D3 after pretreatment with 1,25(OH)2D3 or 1,25(OH)2D3 + 8-bromo cAMP

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Radioactive Counts (cpm x 10^3)</th>
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<tr>
<td></td>
<td>Unmetabolized substrate</td>
</tr>
<tr>
<td>Control</td>
<td>26</td>
</tr>
<tr>
<td>1,25(OH)2D3</td>
<td>22</td>
</tr>
<tr>
<td>1,25(OH)2D3 + cAMP</td>
<td>14</td>
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Radioactive counts for unmetabolized substrate and for metabolites 23,25(OH)2-oxo-D3 and 24(R)25(OH)2D3 represent counts per minute in fractions 9–15, 21–24, and 24–38, respectively (see Fig. 4). Similar results were observed in 2 additional experiments for control and 1,25 dihydroxyvitamin D3 [1,25(OH)2D3] pretreated cells (10^-7 M, 24 h) and duplicate experiment in presence of 1,25(OH)2D3 (10^-7 M) and 8-bromo cAMP (1 mM) (pretreatment for 24 h) yielded similar results.

In the presence of cAMP, the first significant induction in VDR mRNA was at 3 h. VDR mRNA levels reached a plateau after 6 h. The effect of cAMP on VDR mRNA expression suggests that cAMP may mediate the enhanced induction of 24Ohase mRNA by 1,25(OH)2D3 through upregulation of VDR levels.

The induction of 24Oase mRNA by 1,25(OH)2D3 was dose dependent, and cAMP potentiated the effect of 1,25(OH)2D3 (Fig. 6). At 10^-8 M 1,25(OH)2D3, 24Oase mRNA was clearly observed in the cells treated with 1,25(OH)2D3 + cAMP but was detectable in the cells treated with 1,25(OH)2D3 alone only after longer autoradiographic exposure. Increasing the concentration of 1,25(OH)2D3 from 10^-9 M to 10^-6 M did not significantly affect the induction of VDR mRNA by cAMP (Fig. 6).

Further Northern analyses performed using poly(A)^+ RNA from DCT cells treated for 24 h in the presence of 1 mM 8-bromo-cAMP alone indicated that cAMP alone was able to induce VDR mRNA 5.5 ± 1-fold over basal levels (data not shown), equivalent to the induction observed with 1,25(OH)2D3 + cAMP (Fig. 6B). These findings suggest that the effect of cAMP on VDR mRNA expression was due to an increase in VDR protein levels.

In 2 additional experiments for control and 1,25(OH)2D3 pretreated cells (10^-7 M, 24 h) and duplicate experiment in presence of 1,25(OH)2D3 (10^-7 M) and 8-bromo-cAMP (1 mM) (pretreatment for 24 h) yielded similar results.

Fig. 5. Time-dependent effect of 1,25(OH)2D3 or 1,25(OH)2D3 + cAMP on levels of vitamin D receptor (VDR) mRNA and 24OHase mRNA in DCT cells. A: Northern analysis was performed using 8 μg poly(A)^+ RNA per lane from DCT cells that had been treated with 10^-7 M 1,25(OH)2D3 or vehicle control and harvested at various times after 1,25(OH)2D3 administration. The filter was hybridized with 32P-labeled rat VDR; then blots were stripped and rehybridized with 32P-rat 24Ohase and mouse β-actin cDNAs sequentially. Data from 3 independent experiments (mean ± SE) are expressed as percentage of maximal response. Quantitation of VDR mRNA included both transcripts. Data were normalized on basis of results obtained on rehybridization with β-actin cDNA. C: Northern analysis was performed using 8 μg poly (A)^+ RNA per lane from mouse DCT cells that had been treated with 1,25(OH)2D3 (10^-7 M) + 8-bromo-cAMP (1 mM) and harvested at various times after treatment. The filter was hybridized as described above for experiments done in absence of cAMP. D: quantification of results obtained by Northern blot analysis. Data from 3 independent experiments (mean ± SE) are expressed as percentage of maximal response. Quantitation of VDR mRNA and normalization of data were done as described above.
expression is independent of the 1,25(OH)2D3 concentration. In summary, cAMP not only accelerated 24OHase mRNA expression induced by 1,25(OH)2D3, but it also shifted the dose-response curve to the left so that 1,25(OH)2D3 was effective at lower concentrations.

Similar to the effect of 8-bromo-cAMP, when DCT cells were treated with 1,25(OH)2D3 alone (10^{-7} M) or 1,25(OH)2D3 in the presence of PTH (10 nM, 1—34) for 12 h, PTH upregulated VDR mRNA by 3.3-fold and also potentiated 1,25(OH)2D3 induction of 24OHase mRNA expression by 6.4-fold (results are the mean of two separate experiments; data not shown).

**Effect of cAMP and 1,25(OH)2D3 on VDR protein levels.** It was suggested from the time course of VDR mRNA expression in the presence of 8-bromo-cAMP and 1,25(OH)2D3 that cAMP may mediate the enhanced induction of 24OHase mRNA by upregulating VDR levels. Thus the effect of 1,25(OH)2D3 and 8-bromo-cAMP on the levels of VDR protein was examined. After DCT cells were treated with vehicle, 1,25(OH)2D3, or 1,25(OH)2D3 + cAMP for 24 h, the cells were harvested and chromatin-associated proteins were isolated and analyzed for VDR by Western blot analysis. Interestingly, 1,25(OH)2D3 enhanced the level of VDR protein (Fig. 7), although 1,25(OH)2D3 had no effect on VDR mRNA (Fig. 5, A and B), suggesting that 1,25(OH)2D3-induced upregulation of VDR protein is mediated by a posttranscriptional mechanism. cAMP further enhanced the expression of 1,25(OH)2D3-induced VDR (Fig. 7). Densitometric analysis of the VDR protein band obtained from five separate determinations indicated a 6.8 ± 1.3-fold induction of VDR in the presence of 1,25 (OH)2D3 and a 12.5 ± 2.0-fold induction in the presence of 1,25 (OH)2D3 + cAMP (P < 0.01). These results indicate that the enhancement of 1,25(OH)2D3-induced 24OHase mRNA expression by cAMP is due, at least in part, to the upregulation of VDR.

![Fig. 6. Dose-dependent effect of 1,25(OH)2D3 in presence or absence of cAMP on levels of VDR mRNA and 24OHase mRNA in DCT cells. A: Northern analysis was performed using 8 µg poly(A)^+ RNA per lane from mouse DCT cells that had been treated for 24 h with indicated concentrations of 1,25(OH)2D3 in presence or absence of 8-bromo-cAMP (1 mM). Filter was hybridized with 32P-labeled rat VDR cDNA; then blots were stripped and rehybridized with 32P-rat 24OHase and mouse β-actin cDNAs sequentially. B: quantification of results obtained by Northern blot analysis. Data from 3 independent experiments (mean ± SE) are expressed as percentage of maximal response. Quantitation of VDR mRNA included both transcripts. Data were normalized on basis of results obtained on rehybridization with β-actin cDNA. In presence of 8-bromo-cAMP, VDR and 24OHase mRNAs were significantly induced at all concentrations of 1,25(OH)2D3 [P < 0.05 compared with treatment with 1,25(OH)2D3 alone]. It should be noted that 8-bromo-cAMP (1 mM) alone, in absence of 1,25(OH)2D3, was able to induce VDR mRNA 5.5 ± 1-fold (not shown), equivalent to induction observed with 1,25(OH)2D3 + cAMP.](https://example.com/figure6)

![Fig. 7. Effect of 1,25(OH)2D3 and cAMP on VDR protein content. DCT cells were treated with vehicle 0.1% ethanol (−D), 10^{-7} M 1,25(OH)2D3 (−D), or 1,25(OH)2D3 + 1 mM 8-bromo-cAMP (cAMP + D) for 24 h. Nuclear associated proteins were prepared as described in MATERIALS AND METHODS. Western analysis was performed using 30 µg protein and probed with polyclonal antibody against rat VDR. Molecular size markers are indicated at right. VDR is visualized as a 50-kDa immunoreactive band. Nature of highest molecular mass protein cross-reacting with VDR antibody (which has been observed by others (36)) is not known.](https://example.com/figure7)
stimulated significantly by 1,25(OH)\textsubscript{2}D\textsubscript{3} (10\textsuperscript{-7} M, 24 h). All the constructs responded to a threefold potentiation of the 1,25(OH)\textsubscript{2}D\textsubscript{3} effect in the presence of 8-bromo-cAMP (1 mM). However, cAMP (1 mM 8-bromo-cAMP) or PTH (10 nM) alone was found to have no effect on the transcription of the rat 24OHase gene (CAT activity was not significantly different from basal CAT activity (not shown)). The effect of cAMP on 1,25(OH)\textsubscript{2}D\textsubscript{3}-induced transcription using −671/+74 phCAT is shown in Fig. 8, A and B. These results demonstrate that modulation of the 1,25(OH)\textsubscript{2}D\textsubscript{3} induction of 24OHase mRNA by cAMP is at the transcriptional level. Similarly, PTH was observed to potentiate the dose-dependent activation of transcription of the 24OHase gene by 1,25(OH)\textsubscript{2}D\textsubscript{3} (Fig. 8C).

Towler and Rodan (57) have reported that cAMP upregulated the transcription of osteocalcin, a vitamin D-dependent gene, through a novel CRE. Whether the 24OHase promoter contains a functional CRE, which mediates cAMP action on 24OHase expression, was not known. We examined the rat 24OHase promoter for consensus CREs. Three putative CREs were found by computational analysis (Fig. 9A; Refs. 57, 62). To evaluate the functional activity of each CRE, a series of deletion mutant fragments of the 5′-flanking region of rat 24OHase gene were linked to a CAT reporter gene, and data are expressed as percentage of maximal response. Cells treated with 1,25(OH)\textsubscript{2}D\textsubscript{3} + cAMP had increased CAT activity at all doses of 1,25(OH)\textsubscript{2}D\textsubscript{3} (P < 0.01). Cells treated with 10\textsuperscript{-7} M 1,25(OH)\textsubscript{2}D\textsubscript{3} exhibited 36±2-fold induction of CAT activity over control. CAT assay was performed, and data are expressed as percentage of maximal response. Cells treated with 1,25(OH)\textsubscript{2}D\textsubscript{3} in presence or absence of PTH (10 nM) for 24 h. CAT assay was performed, and data are expressed as percentage of maximal response. Note for CAT assays done using −671/+74 24OHase promoter construct and transfection in DCT cells, basal levels were undetectable.

Fig. 8. Effects of cAMP or parathyroid hormone (PTH) on 1,25(OH)\textsubscript{2}D\textsubscript{3}-induced transcription on rat 24OHase promoter chloramphenicol acetyltransferase (CAT) construct −671/+74. A: DCT cells were treated with CAT construct of rat 24OHase promoter −671/+74 (which contains both vitamin D response elements (VDREs) at −258/−244 and −150/−136), using calcium phosphate DNA precipitation method described in MATERIALS AND METHODS. Transfected cells were treated with vehicle (0.1% ethanol; −D) or 10\textsuperscript{-7} M 1,25(OH)\textsubscript{2}D\textsubscript{3} in presence (cAMP + D) or absence (+D) of 1 mM 8-bromo-cAMP for 24 h. CAT assay was performed and β-galactosidase activity was used for normalization. B: DCT cells were transfected with −671/+74 phCAT. Transfected cells were treated with indicated concentrations of 1,25(OH)\textsubscript{2}D\textsubscript{3} in presence (○) or absence (●) of 1 mM 8-bromo-cAMP for 24 h. CAT assay was performed, and data from 3 independent experiments (mean ± SE) are expressed as percentage of maximal response. Cells treated with 1,25(OH)\textsubscript{2}D\textsubscript{3} + cAMP had increased CAT activity at all doses of 1,25(OH)\textsubscript{2}D\textsubscript{3} (P < 0.01). Cells treated with 10\textsuperscript{-7} M 1,25(OH)\textsubscript{2}D\textsubscript{3} exhibited 36±2-fold induction of CAT activity over control. C: DCT cells were transfected with −671/+74 phCAT. Transfected cells were treated with indicated concentrations of 1,25(OH)\textsubscript{2}D\textsubscript{3} in presence (△) or absence (●) of PTH (10 nM) for 24 h. CAT assay was performed, and data are expressed as percentage of maximal response.
-160/+3 phCAT do not include the distal and middle CREs, respectively, and the proximal putative CRE sequence was mutated in the construct -160m/+3 phCAT (Fig. 9B). The synthesized constructs were transfected into DCT cells and treated with 1,25(OH)2D3 (10⁻¹⁰⁻¹⁰⁻⁷ M) in the presence or absence of cAMP (1 mM), and transcriptional activity was assessed using the CAT assay.

Using -160/+3 phCAT (only one putative CRE) and -160m/+3 phCAT (the one putative CRE mutated), at all concentrations of 1,25(OH)2D3, treatment with 1,25(OH)2D3 + cAMP exhibited a three- to fourfold higher CAT activity than 1,25(OH)2D3 alone (Fig. 10, A and B). Both constructs retained the cAMP effect, similar to what was observed using -160/74+74 phCAT (not shown), -671/74+74 phCAT (Fig. 8), and -291/74+74 phCAT (not shown), resulting in a potentiation of 1,25(OH)₂D₃-induced transcriptional activity by a similar degree (three- to fourfold). These results suggest that the putative CREs are not primarily involved in the enhancement of 1,25(OH)₂D₃-induced 24OHase mRNA expression by cAMP.

To rule out the possibility that other sequences in the rat 24OHase gene may mediate the cAMP effect, we prepared a VDRE/tkCAT construct by introducing multiple copies of the proximal VDRE (−1367/−174) of the rat 24OHase promoter into the CAT plasmid. Figure 10C shows that this construct responded to 1,25(OH)₂D₃ and cAMP, indicating that the VDRE of the rat 24OHase gene, in the absence of other sequences in the 24OHase promoter, is able to confer cAMP enhancement. In addition, when a construct (−186/+74) was used with the −150/−136 VDRE mutated (M1) as well as an additional construct (−298/+74) with the proximal as well as the distal VDRE mutated (M4; see Ref. 34), no increase in transcription greater than 1.6-fold over basal levels was observed in the presence of 1,25(OH)₂D₃ alone (10⁻⁸ M), 8-bromo-cAMP alone (1 mM), or 1,25(OH)₂D₃ + cAMP [M1: 1,25(OH)₂D₃ (1.6 ± 0.2-fold), cAMP (1.6 ± 0.3-fold), 1,25(OH)₂D₃ + cAMP (1.4 ± 0.2-fold over basal); M4: 1,25(OH)₂D₃ (1.5 ± 0.1-fold), cAMP (1.3 ± 0.1-fold), 1,25(OH)₂D₃ + cAMP (1.4 ± 0.1-fold over basal)]. The results of these experiments provide additional evidence suggesting that regions in the 24OHase promoter other than the VDRE are not primarily involved in the marked enhancement of 1,25(OH)₂D₃-induced 24OHase transcription by cAMP.

**DISCUSSION**

Although 24OHase is primarily localized in the proximal tubule, recent immunocytochemical studies (32, 38) as well as studies localizing 24OHase mRNA using microdissected nephron segments (29) and the findings we report in this study strongly suggest that the proximal tubule is not the exclusive site of localization of 24OHase in the kidney. It is possible in previous studies, because of the difficulty of microdissecting DCT, which is of very short length and comprises only a small fraction of the tubular structure of the renal cortex, that low levels of 24OHase activity in the distal tubule went undetected. In addition, low levels of 24OHase activity may only be detected in the microdissected distal nephron under conditions in which it may be maximally induced at this site, for example under low dietary calcium conditions, as was suggested by studies of 24OHase mRNA in discrete nephron segments (29).
and PTH in DCT cells is in contrast to the effect in cells of 24OHase mRNA by 1,25(OH)2D3. The effect of cAMP potentiated rather than inhibited the induction of 24OHase by 1,25(OH)2D3 (8-bromo-cAMP for 24 h. CAT assays were performed, and results are expressed as percentage of maximal response (mean ± SE). Cells transfected with 8 µg VDRE-tkCAT. Cells transfected with VDRE-tkCAT construct -160/-3, or rat 24OHase promoter CAT construct (−151/−137) thymidine kinase (tk) CAT construct. A: DCT cells were transfected with rat 24OHase promoter CAT construct for 24 h. CAT assays were performed, and results are expressed as percentage of maximal response (mean of duplicate experiments). Cells treated with 10−7 M 1,25(OH)2D3 exhibited average 4.8-fold induction in CAT activity. B: DCT cells were transfected with rat 24OHase promoter CAT construct (−160/-3) and treated with indicated concentrations of 1,25(OH)2D3 in presence (●) or absence (○) of 1 mM 8-bromo-cAMP for 24 h. CAT assays were performed, and results are expressed as percentage of maximal response (mean of duplicate experiments). Cells transfected with (−160/-3) and treated with 10−7 M 1,25(OH)2D3 exhibited an average 4.2-fold induction in CAT activity. C: 3 copies of proximal VDRE (−151/−137) were linked to tkCAT reporter gene construct. MDCt cells were transfected with 8 µg VDRE-tkCAT construct (−151/−137) thymidine kinase (tk) CAT construct. Cells transfected with VDRE-tkCAT construct and treated with 10−7 M 1,25(OH)2D3 exhibited a 9.3 ± 2-fold induction in CAT activity. Transfected cells were treated with indicated concentrations of 1,25(OH)2D3 in presence (●) or absence (○) of 1 mM 8-bromo-cAMP for 24 h. CAT assay was performed and data from 3 independent experiments (mean ± SE) are expressed as percentage of maximal response. Cells treated with 1,25(OH)2D3 and cAMP had increased CAT activity at all doses of 1,25(OH)2D3 (P < 0.01).

In our studies in DCT cells, we found that PTH or cAMP potentiated rather than inhibited the induction of 24OHase mRNA by 1,25(OH)2D3. The effect of cAMP and PTH in DCT cells is in contrast to the effect in cells that have characteristics of the proximal tubule (40), suggesting differential regulation of 24OHase in different sites of the nephron. Previous studies of PTH effects using kidney homogenates (52, 56), primary renal cultures from total kidney (24, 26), or renal slices (4) would not reflect differences in hormonal regulation of 24OHase between the proximal and distal tubule particularly because markedly lower levels of 24OHase protein and mRNA have been reported to be present in the distal nephron compared with the levels in the proximal tubule (29, 32). The biological significance of the specific action of PTH in vitamin D metabolism in the DCT may be related to the interaction between 1,25(OH)2D3 and PTH on calcium transport in the DCT. The major effect of 1,25(OH)2D3 at concentrations of 10−9 and 10−10 M is to shorten the time course of PTH-dependent Ca2+ uptake (21). 1,25(OH)2D3 alone was reported to have no effect on distal tubular Ca2+ uptake (21). Assuming a biological function of 24OHase is to control the intracellular level of 1,25(OH)2D3, it is reasonable to hypothesize that 1,25(OH)2D3 and PTH may work cooperatively first to enhance calcium uptake at the distal tubule and then to catabolize 1,25(OH)2D3 at higher concentrations of the hormone (>10−9 M; Fig. 6), preventing a hypercalcemic effect by resulting in a reduction of cellular 1,25(OH)2D3 and therefore a suppression of the enhanced calcium uptake. Consistent with this hypothesis, higher concentrations of 1,25(OH)2D3 were found to have decreased effects on calcium uptake (21). Taken together, these data suggest that the different biological functions of PTH in the DCT and the PCT result in discrete interactions between PTH and 1,25(OH)2D3 on 24OHase in the different nephron segments.

Although a cAMP response region was reported in the promoter of the vitamin D-responsive osteocalcin gene (57), and cAMP response regions have been identified in the promoters that direct the expression of enzymes important in steroidogenesis [steroid 21-chain cleavage enzyme (16)], we did not find that the cAMP modulation of 1,25(OH)2D3 induction of 24OHase expression in DCT cells was mediated via CREs in the 24OHase promoter. It is possible that CREs in the 24OHase promoter may be important for the inhibitory effect of PTH in the proximal tubule and that the mechanisms involved in the regulation of 24OHase by cAMP may be cell type specific. Further studies using cell lines derived from the proximal tubule would be needed to examine the molecular mechanisms involved in a negative regulation of 24OHase by PTH. Thus far, however, very few studies have been done related to the regulation of 24OHase as well as VDR expression using cell lines derived from kidney tubules. Besides our study using the distal tubular cell line, previous studies have been done using two cell lines of proximal tubular origin, the opossum kidney cell line OK (30) and the monkey kidney cell line JTC-12 (40). Using JTC-12 cells, a 30% reduction in 1,25(OH)2D3-induced 24OHase activity was observed in the presence of PTH (40). The effect of PTH or cAMP on 24OHase expression was not
studied in OK cells (30). However, Northern analysis of mRNA from OK cells or JTC-12 cells treated with 1,25(OH)\textsubscript{2}D\textsubscript{3} indicated only very weak hybridization with the rat VDR and 24OHase cDNAs (Yang and Christakos, unpublished observation). In more recent studies by Reinholz and DeLuca (49) using the AOK-B50 cell line (a subset of LLC-PK\textsubscript{1} porcine renal epithelial cell line that stably expresses opossum receptors for PTH), PTH inhibited 24OHase mRNA in a dose-dependent manner. The authors suggest that the AOK-B50 cell line is the first cell line in which regulation of 24OHase mRNA by PTH resembles in vivo regulation in the proximal tubule. Thus, this cell line should be useful in future studies characterizing the molecular mechanisms involved in the regulation of 24OHase by PTH in the proximal tubule, which may involve, in part, transcriptional regulation via CREs in the 24OHase promoter.

In our study using DCT cells derived from the distal tubule, we found that regulation of VDR levels by cAMP, and not an effect on CREs in the 24OHase promoter, is one mechanism by which cAMP and PTH may modulate 1,25(OH)\textsubscript{2}D\textsubscript{3}-induced transcription of 24OHase. It has been reported that VDR abundance is one of the major factors determining the level of response to 1,25(OH)\textsubscript{2}D\textsubscript{3} and that homologous regulation of VDR, as well as regulation of VDR by signal transduction pathways, may play an important role in modulating target cell responsiveness to 1,25(OH)\textsubscript{2}D\textsubscript{3} (36). In our study, we found, similar to reports in other cell lines and in transformed yeast cells (2, 37, 51, 60), that 1,25(OH)\textsubscript{2}D\textsubscript{3} treatment resulted in an upregulation of VDR protein levels, but VDR mRNA was not affected by 1,25(OH)\textsubscript{2}D\textsubscript{3}, suggesting that induction of VDR protein by 1,25(OH)\textsubscript{2}D\textsubscript{3} is due to altered stability of the occupied receptor. 8-Bromo-cAMP treatment, however, resulted in an increase in VDR mRNA, as well as an enhancement of 1,25(OH)\textsubscript{2}D\textsubscript{3} induction of VDR protein levels and a potentiation of the effect of 1,25(OH)\textsubscript{2}D\textsubscript{3} on 24OHase mRNA and transcription. In previous studies, elevation of intracellular cAMP levels in NIH-3T3 mouse fibroblasts (36), mouse osteoblasts (MC3T3-E1 cells) (35), or in rat osteosarcoma cells (UMR106–01 cells) (35) was also reported to result in an induction in VDR mRNA and protein. Similar to our studies, the change in VDR by agents that raise intracellular cAMP in NIH-3T3 cells (36) and in UMR cells (3, 35) corresponded to an enhanced functional response (3, 35, 36). However, it should be noted that opposite findings concerning the effect of activation of protein kinase A (PKA) on VDR have been reported by others. In rat osteosarcoma cells (ROS17/2.8), which exhibit a more osteoblastic phenotype than MC3T3-E1 or UMR106 cells, PTH or forskolin has been shown to downregulate VDR (48), suggesting that cell type, proliferation state, and stage of differentiation may affect the interaction between 1,25(OH)\textsubscript{2}D\textsubscript{3} and the PKA pathway. Cell type specificity of VDR regulation under conditions that result in an elevation of PTH was also noted in vivo studies reported by Iida et al. (29) using microdissected rat nephron segments. In PCTs, VDR mRNA was found to be markedly downregulated to barely detectable levels under conditions of low dietary calcium, resulting in a marked inhibition of 24OHase mRNA. However, in the distal nephron under low dietary calcium conditions, VDR mRNA was not downregulated and 24OHase mRNA was found to be induced. Although the exact mechanism of regulation of VDR mRNA in the microdissected nephron segments was not clearly defined, the authors suggested that intracellular signaling caused by PTH in response to hypocalcemia may be an important determining factor involved. It will be of interest in future studies to examine the promoter of the VDR gene for CREs and to determine the role of coactivators, such as CRE-binding protein, and the role of phosphorylation of VDR and other transcriptional coactivators that may be involved as part of the mechanism underlying the interaction between 1,25(OH)\textsubscript{2}D\textsubscript{3} and the PKA signaling pathway. The role of cell type-specific factors that may be involved in the downregulation of VDR mRNA in the PCT but not in the DCT also needs to be considered. In addition, the interaction between 1,25(OH)\textsubscript{2}D\textsubscript{3} and the PKA pathway may not only be cell type specific but may also be gene specific and may involve effects not only on VDR levels but also on the promoter of certain target genes in specific cell types.

It should be noted that besides modulation via the cAMP-dependent PKA signal pathway, evidence also exists for protein kinase C involvement in the regulation of renal 24OHase (12, 25, 26, 39). The phorbol ester TPA has been reported to increase the production of 24,25(OH)\textsubscript{2}D\textsubscript{3} and to decrease the production of 1,25(OH)\textsubscript{2}D\textsubscript{3} (12, 25, 39). It has been suggested that PKC and PKA act through independent mechanisms to alter the production of 1,25(OH)\textsubscript{2}D\textsubscript{3} (25). In our studies in DCT cells we found, similar to the report of Chen et al. (12) using primary cultures of rat kidney cells, that TPA enhanced the 1,25(OH)\textsubscript{2}D\textsubscript{3}-induced increase in 24OHase mRNA (Fig. 1). TPA increased the rapidity of the response to 1,25(OH)\textsubscript{2}D\textsubscript{3}, and dose-response studies indicated a shift to the left in the presence of TPA. Unlike studies with 8-bromo-cAMP or PTH, TPA resulted in a significant decrease in VDR mRNA at 1, 3, and 6 h after treatment, and Western blot analysis did not indicate an upregulation of VDR in the presence of TPA (Yang and Christakos, unpublished observations). These findings suggest that the effect of TPA on 24OHase is not mediated by an effect on new receptor synthesis but rather may be due to effects on regulatory regions in the 24OHase promoter and/or an effect on DNA binding to transcription factors.

In summary, although the PCT is the major site of localization of 24OHase, our findings provide evidence for the first time that 24OHase mRNA, protein, and activity can be localized in the distal nephron. In DCT cells both cAMP and PTH modulate the 1,25(OH)\textsubscript{2}D\textsubscript{3} induced expression of 24OHase in a manner different from that reported in the PCT, suggesting different roles for PKA activation in the DCT and PCT. We suggest that 24OHase in the DCT may play a role in modulating 1,25(OH)\textsubscript{2}D\textsubscript{3} action on calcium transport by
controlling cellular 1,25(OH)2D3 levels. In DCT cells, regulation of VDR levels by cAMP, and not an effect on CREs in the 24OHase promoter, is one mechanism by which cAMP modulates 1,25(OH)2D3-induced transcription of 24OHase.

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