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Effect of Schiff base formation on the function of the calcitriol receptor

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Effect of Schiff base formation on the function of the calcitriol receptor. The genomic action of calcitriol is mediated through the interaction of the calcitriol receptor (VDR) with vitamin D response elements (VDREs) of the target genes. We have shown that the interaction of VDRs with VDREs is inhibited by uremic toxins. We hypothesize that uremic toxins form Schiff bases with the lysine residues of the VDR DNA binding domain and inhibit the VDR interaction with the VDRE. In this study, pyridoxal 5′-phosphate was used as a probe to test Schiff base formation as the inhibitory mechanism, since it forms Schiff bases with steroid receptors. Pyridoxal 5′-phosphate inhibited the VDR binding to the VDREs and chemically modified the DNA binding domain of the VDR *in vitro*. The inhibition was reversed when pyridoxal 5′-phosphate was preincubated with lysine. Further, this chemical agent also blocked the production of chloramphenicol acetyltransferase (CAT) enzyme induced by calcitriol in cells transfected with a constructed VDRE attached to a CAT reporter gene. This finding is consistent with the hypothesis that pyridoxal 5′-phosphate could interact with the VDR and impair its DNA binding within cells. Since induction of 24-hydroxylase synthesis is a receptor mediated process, we studied the effect of pyridoxal 5′-phosphate on the synthesis of renal 24-hydroxylase in rats. When pyridoxal 5′-phosphate was infused to rats, renal 24-hydroxylase activity was suppressed, consequently, degradation of calcitriol was also reduced in these animals. Thus, chemicals capable of Schiff base formation potentially could alter the physiological function of VDR and calcitriol.

The biological action of calcitriol is a receptor-mediated process. The hormone-receptor complexes interact with vitamin D response elements (VDREs) and thus regulate the transcription of those genes. The interaction of VDRs with VDREs, however, is inhibited by uremic toxins. Consequently, end-organ resistance to calcitriol occurs in renal failure [1, 2]. The mechanism of inhibition by uremic toxins is unknown. However, since pyridoxal 5′-phosphate has been shown to form Schiff bases with steroid receptors and inhibit the receptor's interaction with DNA [3–6], we propose that compounds with reactive aldehydes or ketones in uremic plasma block VDR-VDRE interactions by an analogous Schiff base mechanism [7]. These chemical agents capable of forming a Schiff base with the VDR could influence VDR function and thereby alter calcitriol metabolism. Thus, we used pyridoxal 5′-phosphate as a probe to test this hypothesis.

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Methods

Electrophoretic mobility shift assay (EMSA) of recombinant or intestinal VDR incubated with pyridoxal 5′-phosphate

VDR binding to the osteocalcin VDRE. Preparation of recombinant human VDR. Full length recombinant human VDR and mouse retinoid X receptor α (RXRα) proteins were expressed from the vector pMalc2 (New England Biolabs). This vector produces a fusion protein of maltose binding protein followed by a cleavage site for factor Xa and the recombinant protein. The fusion proteins of VDR and RXRα are purified by affinity chromatography on an amylose column and cleaved with factor Xa as previously described [2].

Preparation of intestinal VDR. Rats were decapitated and the initial 20 cm of small intestine distal to the pylorus (duodenum and portion of jejunum) was removed and flushed with ice cold Ca/Mg-free phosphate (6.6 mM Na_2HPO_4, 250 IU/ml Trasylol and 1.5 mM KH_2PO_4)-buffered saline (CMF-PBS). The mucosal cells were scraped from the serosa, washed three times in 20 volumes of CMF-PBS and centrifuged at 200 × g for five minutes after each washing. The tissue was homogenized in a Polytron in 20 vol (wt/vol) of buffer consisting of 300 mM KCl, 200 μg/ml soybean trypsin inhibitor, 10 mM Tris-HCl (pH 7.4), 1 mM EDTA, 10 mM sodium molybdate and 5 mM dithiothreitol (KTED) [8]. Cellular protein was prepared by centrifugation at 100,000 × g for 45 minutes at 0 to 4°C.

Electrophoretic mobility shift assay (EMSA). A 36 base pair synthetic oligonucleotide encompassing the rat osteocalcin gene VDRE (base pairs −459 to −432 with CTAG overhangs [9]) was labeled by a fill-in reaction using the Klenow fragment of DNA polymerase I and [a-^{32}P]dCTP. The labeled DNA probe was purified on a Sephadex G-50 column.

Approximately 250 ng of recombinant VDR and 100 ng of RXRα were incubated for one hour with various concentrations of pyridoxal 5′-phosphate (0.5, 1.0, 2.5 and 5 mM). Thereafter, the proteins were incubated for another 15 minutes in 35 μl of mobility shift reaction buffer containing 5 mM Tris, 15 mM Hepes, 3.5 mM $MgCl_2$, 5 mM EDTA, 10% glycerol, 0.1% Tween 20, 5 mM dithiothreitol, 0.15 μg poly(dI-dC), and 100 mM KCl, pH 7.9 [2]. At the end of this incubation, approximately 30,000 cpm of radiolabeled osteocalcin VDRE probe was added in each mobility shift reaction mixture. The reaction mixtures were gently vortexed, incubated for another 10 minutes and electrophoresed on

6% nondenaturing polyacrylamide gels at 100 V for approximately three hours. The mobility shift running buffer contained 50 mM Tris, 380 mM glycine, and 2 mM EDTA (pH 8.5) [2]. The gels were dried and autoradiographed.

Schiff base reaction of pyridoxal 5'-phosphate and lysine. To test if pre-formation of a Schiff base between pyridoxal 5'-phosphate and lysine may reverse the inhibitory effect of pyridoxal 5'-phosphate on VDR binding to osteocalcin VDRE, we incubated 5 mM pyridoxal 5'-phosphate with 20 mM l-lysine (pH 7.4) at 37°C for 12 days [10]. During the preincubation, the aldehyde group of the pyridoxal 5'-phosphate is presumably in Schiff base linkage with the ε-amino group of free lysine. The mixtures were then incubated with intestinal cellular proteins (approximately 40 μg cellular protein) for electrophoretic mobility shift assay as above.

VDR binding to osteopontin

Preparation of a truncated VDR. We produced a truncated VDR (amino acids 1 to 119) that lacked the ligand binding domain. The PCR-based splicing by overlap extension technique was used to place a stop codon after amino acid 119 with a standard protocol and expressed in an *E. coli* expression plasmid vector pMalc2 (New England Biolabs). This vector produces a fusion protein of maltose binding protein followed by a cleavage site for factor Xa and the recombinant truncated VDR. The fusion proteins of VDR were purified as above. The truncated VDR includes the full zinc fingers, T and A box domains, but lacks the ligand binding domain.

A 32 bp synthetic oligonucleotide encompassing the rat osteopontin gene VDRE (bp −761 to −738 plus GATC overhangs [11]) was labeled by a fill-in reaction and used as a probe. EMSA incubations utilized approximately 10 μg of the truncated VDR. Receptors were incubated individually for one hour at 15°C with 0 or 5 mM pyridoxal 5'-phosphate. Thereafter, these receptor preparations were added to EMSA incubations as described above.

Effect of pyridoxal 5'-phosphate on calcitriol-dependent expression of a reporter gene in transfected JEG-3 cells

We used transiently transfected JEG-3 cells to study the effect of pyridoxal 5'-phosphate on VDR binding to VDRE [2]. JEG-3 cells are derived from human choriocarcinoma cells which do not contain VDR or VDRE. The human VDR cDNA was expressed from the vector pCDM. The VDR cDNA was expressed from a cytomegalovirus promoter that itself is not calcitriol responsive. The reporter plasmid was derived from pUTKAT3 [11], and contained two copies of the sequence 5'-GATCCACTAGGT-CAAGGAGGTCATGGATC ligated 5' to the basal thymidine kinase promoter driving expression of chloramphenicol acetyltransferase (CAT) (pTKD2AA). An internal control plasmid expressing human growth hormone (GH) from the basal thymidine kinase promoter (pTKGH) was utilized to control for transfection efficiency. Transfections included 4 μg of pTKD2AA, 10 ng of pCDMVDR, 1 μg of pTKGH, and 6 μg of pCDM as filler plasmid per 60 mm Petri dish. Cells were cultured ± 100 nM calcitriol for 24 hours following transfection. Pyridoxal 5'-phosphate was added for 24 hours following transfection at a final concentration of 0.5 mM. Cell lysates were analyzed for CAT activity and media for human GH as described [12]. Fold CAT induction is defined as CAT/human GH for cells cultured with calcitriol divided by CAT/human GH for cells cultured without calcitriol.

Metabolic clearance rate (MCR) of calcitriol in normal rats infused with pyridoxal phosphate

Normal Sprague-Dawley rats (200 to 230 g) fed with regular Purina rat chow containing 1.0% Ca, 0.8% P, and 4.5 IU per g vitamin D were used throughout the study. On the day of experimentation, rats were weighed and anesthetized with ether. The femoral artery and vein were cannulated with polyethylene tubing (PE) for blood sampling and fluid infusion. A PE 50 tube was inserted into the bladder for urine collection. The animals were placed in individual cages and allowed to awaken before the experiment.

Animals ($N = 6$) were infused through femoral vein for 20 hours with 20 ml normal saline solution containing 4 mg/100 g body wt pyridoxal phosphate and 0.05 μCi of radiolabeled calcitriol (1α,25 [26, 27(n)-^{3}H] dihydroxyvitamin D_3, 160 Ci/mmol. New England Nuclear). The metabolic clearance of calcitriol detailed in a previous communication [13] was determined 18 and 20 hours after the infusion. Control animals ($N = 6$) were infused with 20 ml normal saline containing radiolabeled calcitriol.

Arterial blood samples (0.5 ml) were drawn 18 and 20 hours after the fluid infusion and the plasma concentrations of radioactive calcitriol were determined [13]. The intraassay and interassay coefficients of variation for radioactive calcitriol were 6.8% and 6.7%. Since there was no difference in the calculated MCR at 18 and 20 hours, only the average values of the MCR were presented in this study.

At the end of infusion, arterial blood was obtained for the measurement of calcitriol, calcium, phosphorus, and creatinine. Urine was collected during the 20-hour infusion for the measurement of calcium, phosphorus, and creatinine. Animals were fasted during the infusion.

The MCR of calcitriol was calculated as [13]:

$$\text{MCR} = \frac{\text{Infusion rate of } ^3\text{H-calcitriol}}{\text{Mean steady state plasma concentration of } ^3\text{H-calcitriol}}$$

The production rate (PR) of calcitriol is calculated as:

$$\text{PR} = \text{MCR} \times \text{endogenous plasma concentration of calcitriol}$$

Analytical methods

Calcium was measured with an atomic absorption spectrophotometer (Model 306, Perkin Elmer, Norwalk, CT, USA). Phosphorus and creatinine were measured as described previously [14]. Plasma calcitriol was measured in duplicate according to the methods of Reinhardt et al [15] and Hollis [16]. Our interassay coefficients of variation were 7.0% for low control (20 pg/ml, $N = 12$) and 4.1% for high control (100 pg/ml, $N = 12$). The intraassay coefficients of variation were 5.4% for low control ($N = 6$) and 4.7% for high control ($N = 6$), respectively. Calcitriol recovery averaged 65%.

Renal 24-hydroxylase activity in normal rats infused with pyridoxal phosphate

Normal rats were weighed and anesthetized with ether. The veins were cannulated with polyethylene tubing (PE) for fluid infusion. A PE 50 tube was inserted into the bladder for urine

collection. The animals were placed in individual cages and injected with a bolus of 250 ng/kg calcitriol, followed by infusion for 20 hours with 20 ml saline solution containing 4 mg/100 g pyridoxal phosphate ($N = 6$) or 20 ml of saline solution ($N = 6$). At the end of infusion, animals were anesthetized with ether and bloods were drawn from the aorta. The kidneys were perfused with 10 ml of ice-cold homogenizing buffer solution and removed for measurements of 24-hydroxylase activity.

Renal 24-hydroxylase activity was measured as previously described [17, 18]. Kidneys were quickly homogenized in ice-cold oxygenated buffer solution containing 10 mM Tris-acetate (pH 7.4), 2 mM magnesium acetate, 0.19 M sucrose. A 5% weight to volume homogenate was prepared in Tris-acetate buffer solution. Two ml of the homogenate was placed in a 25 ml Erlenmeyer flask and added with one ml of ice-cold oxygenated homogenization buffer containing 25 mM sodium succinate. The reaction was initiated by the addition of 240 nmol cold $25(OH)D_3$ and 0.25 μCi 25-OH [26, 27(n)-^{3}H]D_3 (158 Ci/mmol, New England Nuclear). The homogenates were incubated at 37°C for 30 and 60 minutes. The reaction was stopped by the addition of 20 ml methanol-chloroform (2:1 vol/vol). One μg of cold $24,25(OH)_2D_3$ was added to each flask to monitor the recovery of ^{3}H-$24,25(OH)_2D_3$. The samples were extracted by the method of Bligh and Dryer as modified by Lobaugh and Drezner [19]. The chloroform extracts were reconstituted in 0.5 ml absolute ethanol. Two hundred microliters of each sample was further extracted on a C18 column and dried under nitrogen. These samples were dissolved in 500 μl of HPLC solvent containing hexane-isopropanol-methanol (88:10:2). The samples were injected into the HPLC and extracted on two Zorbax-Sil columns (0.46 × 25 cm) placed in series at a flow rate of 4 ml/min. The peak containing $24,25(OH)_2D_3$ and $25(OH)D_3$ was collected into tubes, dried and dissolved in HPLC mobile phase containing hexane-isopropanol-methanol (96:3:1) and chromatographed on a Zorbax-Sil column at a flow rate of 4 ml/min to separate $24,25(OH)_2D_3$ from $25(OH)D_3$ [18, 20]. The peak containing $24,25(OH)_2D_3$ was collected into scintillation vials for radioactivity measurements.

All data were expressed as mean ± SEM. Statistical analysis was performed using Student's t-test. A P value of < 0.05 was considered significant.

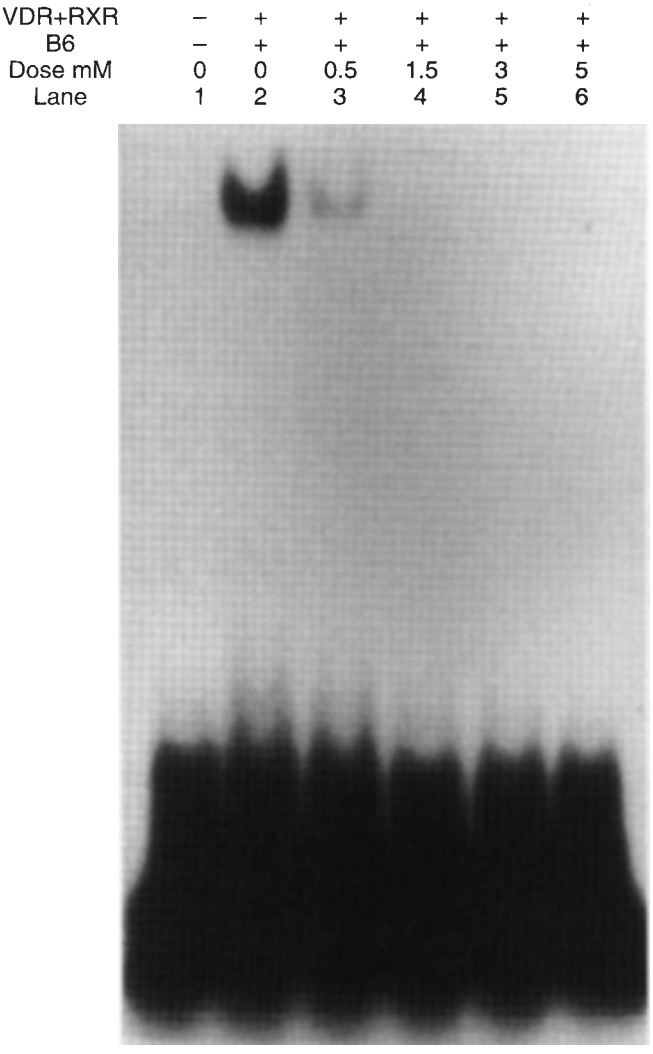

Fig. 1. *Effect of pyridoxal 5'-phosphate (B_6) on VDR-VDRE complex formation analyzed by EMSA.* Lane 1, free radiolabeled osteocalcin VDRE; lane 2, E. coli derived recombinant VDR plus RXR with VDRE; lanes 3 to 6, VDR incubated with 0.5, 1.5, 3, 5 mM pyridoxal 5'-phosphate, respectively, for one hour prior to the EMSA incubation.

Results

Electrophoretic mobility shift assay (EMSA) of recombinant or intestinal VDR incubated with pyridoxal 5'-phosphate

The effect of pyridoxal 5'-phosphate on the VDR-VDRE interaction is depicted in Figure 1. The pyridoxal 5'-phosphate inhibited the interaction of VDR with osteocalcin VDRE in a dose related manner. However, pyridoxal 5'-phosphate preincubated with lysine for 12 days no longer inhibited intestinal VDR binding to osteocalcin VDRE (Fig. 2).

The truncated VDR (119 amino acids) also had decreased binding affinity for osteopontin VDRE when it was pre-incubated with 5 mM pyridoxal 5'-phosphate for one hour (Fig. 3). This indicates that pyridoxal 5'-phosphate interacts with the VDR DNA binding domain, not the ligand binding domain.

Effect of pyridoxal 5'-phosphate on the calcitriol-dependent expression of a reporter gene in transfected JEG-3 cells

JEG-3 cells were transiently transfected with a VDR expression plasmid, a VDRE-containing CAT reporter plasmid, and an internal control plasmid expressing human GH. In the absence of transfected VDR, calcitriol did not induce CAT (average 0.99-fold, $N = 4$). However, following cotransfection with the expression vector pCDMVDR, calcitriol induced CAT activity 8.3-fold (Fig. 4, control). Addition of pyridoxal 5'-phosphate inhibited more than 20% of calcitriol-induced CAT activity (6.3-fold; Fig. 4). Furthermore, this effect of pyridoxal 5'-phosphate was on the calcitriol-induced activity, not the basal CAT activity (control, 18.0 ± 3.6 vs. pyridoxal 5'-phosphate, 11.2 ± 5.6% conversion, P = NS) or human GH expression (control, 0.63 ± 0.19 vs. pyridoxal 5'-phosphate, 0.41 ± 0.26 ng/ml, P = NS).

Metabolic clearance of calcitriol in normal rats infused with pyridoxal phosphate

Infusion of pyridoxal 5'-phosphate influenced neither calcium and phosphate excretions nor their plasma concentrations (Table 1). However, pyridoxal 5'-phosphate significantly reduced the

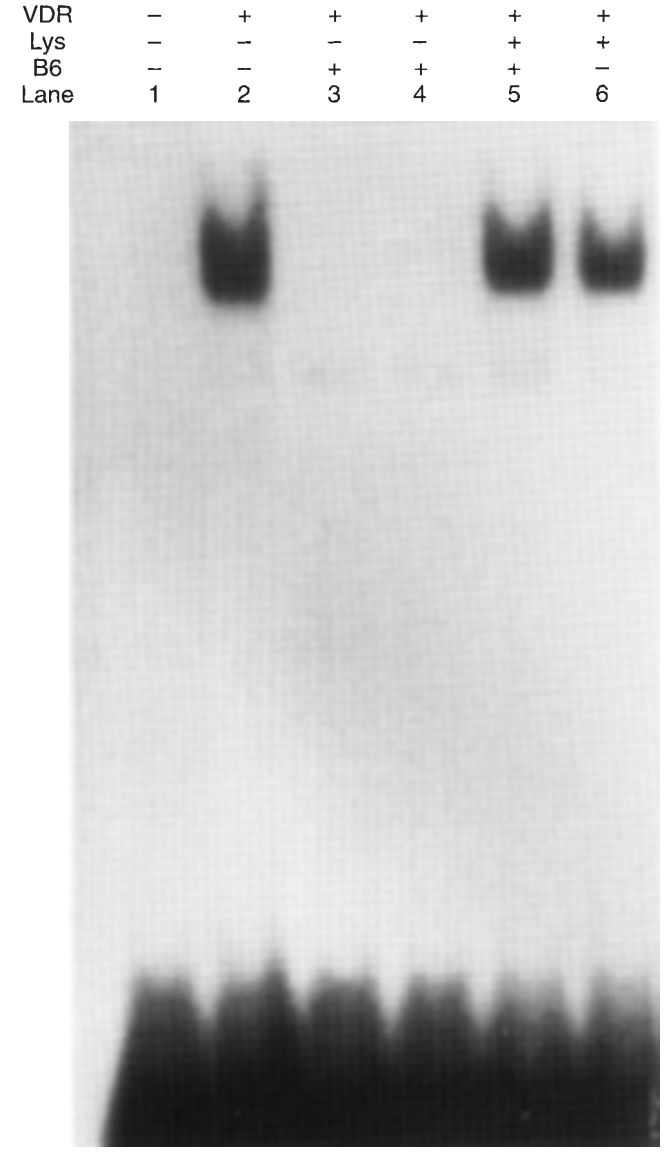

Fig. 2. *Inhibitory effect of pyridoxal 5′-phosphate on VDR-RXR-VDRE complex is reversed by l-lysine.* Lane 1, free radiolabeled osteocalcin VDRE; lane 2, intestinal VDR-VDRE complex; lane 3, addition of 5 mM pyridoxal 5′-phosphate abolished VDR-VDRE complex; lane 4, addition of 5 mM pyridoxal 5′-phosphate preincubated in water at 37°C for 12 days also abolished VDR-VDRE complex; lane 5, addition of 5 mM pyridoxal 5′-phosphate preincubated with 20 mM l-lysine at 37°C for 12 days failed to inhibit VDR binding to VDRE; lane 6, addition of 20 mM l-lysine preincubated at 37°C for 12 days did not inhibit VDR-VDRE complex formation.

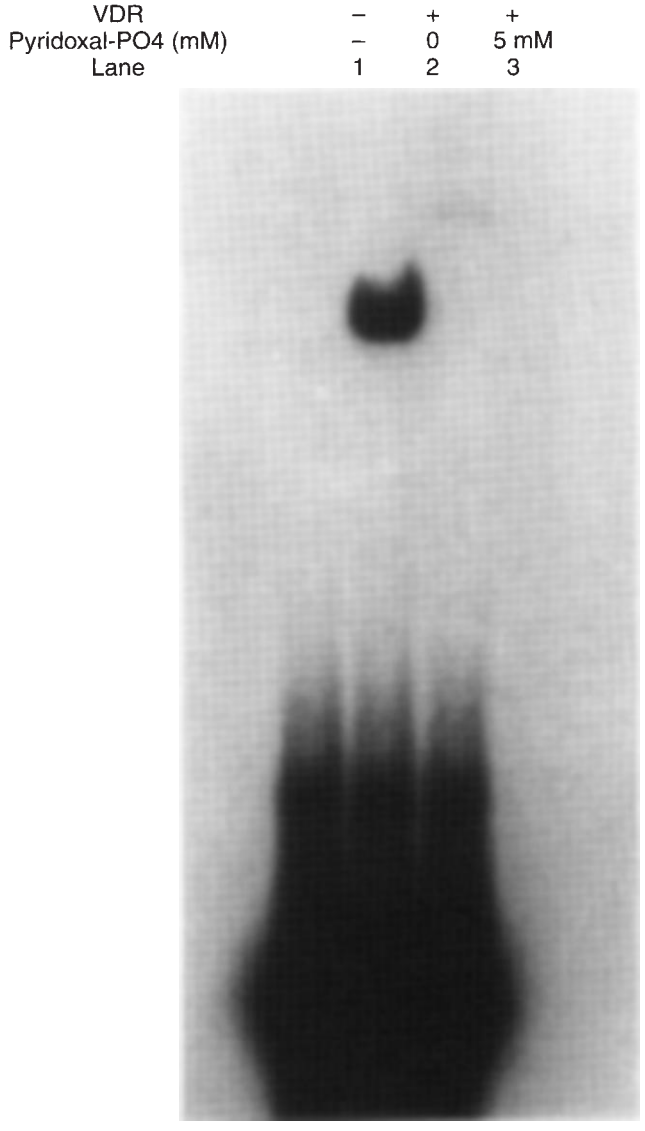

Fig. 3. *Inhibition of a truncated VDR (VDR DNA binding domain) binding to radiolabeled osteopontin VDRE by pyridoxal 5′-phosphate.* Lane 1, free radiolabeled osteopontin VDRE; lane 2, truncated VDR-VDRE complex; lane 3, truncated VDR preincubated with 5 mM pyridoxal 5′-phosphate for one hour could no longer bind to the VDRE.

metabolic clearance rate of calcitriol. Consequently, the plasma concentration of calcitriol was increased because of decreased calcitriol degradation. Production of calcitriol, however, was not affected by pyridoxal 5′-phosphate (Table 2).

Renal 24-hydroxylase activity in normal rats infused with pyridoxal 5′-phosphate

Infusion of pyridoxal 5′-phosphate significantly suppressed 24-hydroxylase activity (Fig. 5). Thus, decreased 24-hydroxylase activity could account for the decreased metabolic clearance of calcitriol, though pyridoxal 5′-phosphate could suppress other degradation enzymes as well.

Discussion

The genomic action of calcitriol is mediated through the interaction of calcitriol receptor (VDR) with vitamin D response elements (VDREs) of the target genes. The hormone-receptor complex interacts with specific DNA response elements, generally located in the 5′ flanking regions of target genes. This interaction results in production of protein that carry out the biological activity of calcitriol [21]. However, the biological activity of calcitriol is decreased in renal failure [22, 23], and one of the reasons is that uremic plasma contains factors that inhibit the interaction of hormone-receptor complex with VDRE [2]. Further, uremic toxins also alter the physiological action of calcitriol

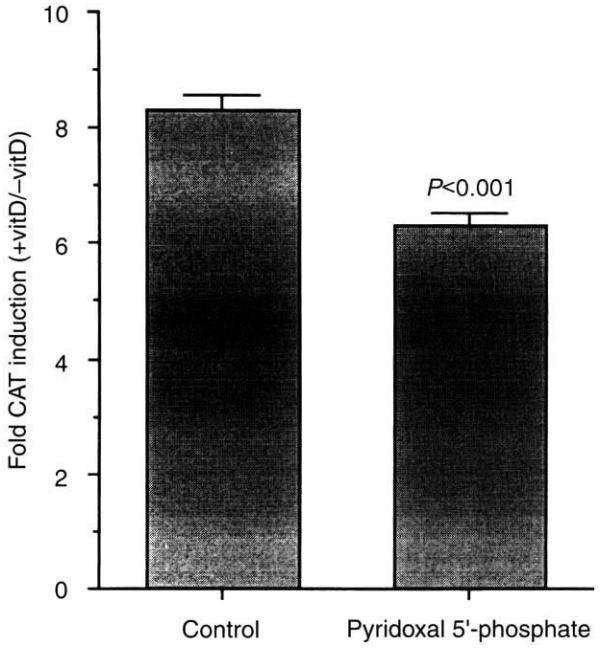

Fig. 4. *Effect of pyridoxal 5'-phosphate on calcitriol-induced expression of CAT activity on transfected JEG-3 cells.* JEG-3 cells were transfected with a VDR expression vector, a CAT reporter plasmid driven by a calcitriol responsive promoter, and a human GH-expressing reporter as an internal control. Cells were incubated with 0 (control) and 2.5 mM (experimental) pyridoxal 5'-phosphate, in each case with or without 100 nM calcitriol, for 48 hours. Fold CAT induction is defined as CAT/human GH for cells cultured without calcitriol. Results are the mean ± SE for 5 independent transfections.

within the cells, perhaps through inhibition of VDR interaction with VDREs [2].

Although the chemical structures of the uremic toxins remain unknown, we speculate that some of the chemicals have reactive aldehyde or ketone groups that form Schiff bases with lysine residues of the VDR DNA binding domain, thereby reducing VDRs binding affinity for VDREs. This supposition is based on the following reasons. (1) The DNA binding domain is sensitive to chemical modification [24], and a point mutation in the gene coding this domain impairs the VDR function [25, 26]. (2) Pyridoxal 5'-phosphate has a reactive aldehyde group and is capable of forming a Schiff base with steroid receptors and reducing steroid receptor-DNA binding [3–6]. (3) Uremic plasma contains chemical compounds with reactive aldehydes or ketones [27].

In this study, we provide evidence to support the hypothesis that Schiff base formation is responsible for the inhibitory mechanism of uremic toxins that impair VDR interaction with VDREs. We have shown that pyridoxal 5'-phosphate inhibited VDR binding to osteocalcin VDRE. We have also shown that pyridoxal 5'-phosphate inhibited the binding of a truncated VDR to the osteopontin VDRE (and osteocalcin VDRE, unpublished observation). Since the truncated VDR contains the VDR DNA binding domain and lacks the hormone binding domain, inhibition of the binding suggests that the pyridoxal 5'-phosphate alters the DNA binding domain of the VDR. Further, when pyridoxal 5'-phosphate was pre-incubated with lysine for 12 days, the pyridoxal 5'-phosphate failed to inhibit VDR binding to osteocalcin VDRE. This is consistent with the expectation that by reacting with free lysine, the pyridoxal 5'-phosphate aldehyde group would no longer be available to form a Schiff base with lysine residues in the VDR DNA binding domain.

If pyridoxal 5'-phosphate could chemically modify the VDR *in vitro*, it might be possible to detect an effect of pyridoxal 5'-phosphate on the biological action of calcitriol within cells. Thus, we introduced a VDRE-CAT reporter gene to JEG-3 cells [2] and used these transiently transfected cells to study the effect of pyridoxal 5'-phosphate on VDR activity. We found that pyridoxal 5'-phosphate inhibited the calcitriol induced CAT activity, suggesting that pyridoxal 5'-phosphate can impair the VDR-VDRE interaction within cells. Furthermore, we examined the effect of pyridoxal 5'-phosphate on the *in vivo* activity of 24-hydroxylase, a process that requires VDR interaction with a specific VDRE [28, 29]. If pyridoxal 5'-phosphate inhibits this interaction *in vivo*, it could reduce the synthesis of 24-hydroxylase. We found that infusion of pyridoxal 5'-phosphate into rats indeed significantly reduced renal 24-hydroxylase activity, suggesting that pyridoxal 5'-phosphate can chemically modify the VDR *in vivo*. The decreased activity of this calcitriol degradation enzyme activity accounted for the decreased calcitriol metabolic clearance rate in pyridoxal 5'-phosphate infused animals.

Steroid receptor superfamily members share structural homology in their DNA binding domains. These receptors have three major regions (I to III) of conserved amino acids [21]. Region I includes the DNA binding domain and contains a sequence of 66 highly conserved amino acids. Region II and III are located within the C-terminal or hormone binding domain of the receptor. The DNA binding domain has two zinc fingers that contain eight cysteine residues and two zinc molecules [30]. The cVDR DNA binding domain has seven lysine residues, three of which are highly conserved [31]. We predict that uremic toxins may also inhibit other steroid receptors binding to DNAs, perhaps through chemical modification of the receptor DNA binding domains.

In summary, we have demonstrated that pyridoxal 5'-phosphate can inhibit VDR-VDRE interaction *in vitro*. Pyridoxal 5'-phosphate also blocked calcitriol induced expression of CAT activity in transiently transfected JEG cells. When pyridoxal 5'-phosphate was infused into rats, the synthesis of 24-hydroxylase, a receptor mediated product, was reduced. Taken together, the data indicated that pyridoxal 5'-phosphate can inhibit VDR binding to VDRE, presumably through formation of Schiff base with lysine residues of the VDR DNA binding domain. Hence pyridoxal 5'-phosphate and uremic toxins could share similar properties in the regulation of the VDR and 24-hydroxylase activity. Although these substances remain unidentified (and probably are distinct from pyridoxal 5'-phosphate), they are likely to contain reactive aldehydes or ketones [27]. This group of compounds potentially could be responsible for abnormal calcitriol metabolism in renal failure by a mechanism similar to that demonstrated here for the model compound pyridoxal 5'-phosphate.

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Table 1. Plasma concentrations of creatinine, calcium and phosphate, and urinary excretion of calcium and phosphate in rats infused with pyridoxal 5'-phosphate

Groups	Body weight *g*	C_{Cr} *ml/min/100 g*	Pca *mg/dl*	Pp *mg/dl*	$U_{ca}V$ *μg/min*	U_pV *μg/min*
Control	212 ± 4.9	0.52 ± 0.02	9.80 ± 0.10	7.94 ± 0.14	0.18 ± 0.03	19.7 ± 2.68
Pyridoxal 5'-phosphate	210 ± 2.2	0.54 ± 0.03	9.83 ± 0.21	7.88 ± 0.14	0.21 ± 0.03	20.1 ± 1.59
P values	NS	NS	NS	NS	NS	NS

Abbreviations are: C_{Cr}, creatinine clearance; Pca, plasma calcium; Pp, plasma phosphorus; $U_{ca}V$, urinary calcium excretion; U_pV, urinary phosphate excretion; NS, not significant.

Table 2. Metabolic production and clearance of calcitriol in rats infused with pyridoxal 5'-phosphate

Groups	Plasma calcitriol *pg/ml*	MCR *μl/min/100 g*	PR *ng/kg/day*
Control	65.7 ± 3.26	26.7 ± 0.94	25.2 ± 1.16
Pyridoxal 5'-phosphate	87.9 ± 2.25	19.7 ± 0.64	25.0 ± 1.24
P values	<0.001	<0.001	NS

Abbreviations are: MCR, metabolic clearance rate; PR, production rate.

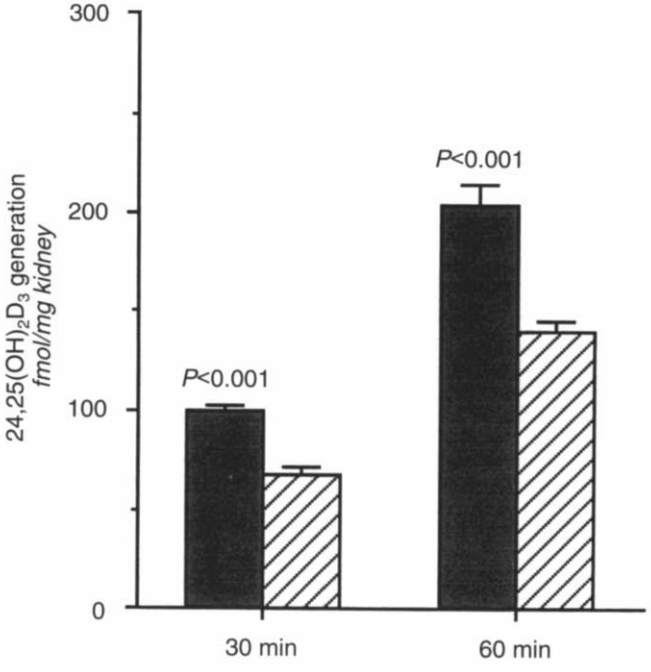

Fig. 5. *Renal 24-hydroxylase activity of rats infused with normal saline or 4 mg/100 g pyridoxal 5'-phosphate.*

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