Inhibitory Effect of Uremic Solutions on Protein-DNA-Complex Formation of the Vitamin D Receptor and Other Members of the Nuclear Receptor Superfamily

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Abstract Chronic renal failure is often associated with a resistance to the biologically active form of vitamin D_3, the nuclear hormone 1α,25-dihydroxyvitamin D_3 (VD). The actions of VD are mediated by the vitamin D receptor (VDR), a ligand-dependent transcription factor that binds as a dimeric complex with the retinoid X receptor (RXR) to specific DNA binding sites in the promoter regions of primary VD responding genes, referred to as VD response elements (VDREs). It could be shown in this study that uremic solutions derived from ultrafiltrate from hemodialysis patients and dialysate from peritoneal dialysis patients had an inhibitory effect on the complex formation and ligand inducibility of VDR-RXR heterodimers on different VDRE types. This inhibition was attributed to the formation of Schiff bases between "reactive aldehydes" and lysine residues of the DNA binding domain (DBD) of the VDR, but point mutagenesis data of different lysine residues in this study could not confirm this idea. However, the inhibitory effect of uremic solutions could also be observed for the complex formation of other homo- or heterodimer forming nuclear receptors, whereas an as a monomer binding nuclear receptor did not appear to be affected. These results indicate that VDR is a target of substances in uremic solutions in vitro, but also to some extent other nuclear receptors (i.e., other endocrine signaling systems) may be affected by renal failure. J. Cell Biochem. 74:386–394, 1999. © 1999 Wiley-Liss, Inc.

Key words: vitamin D_3; vitamin D receptor; nuclear hormone signaling; protein-DNA interaction; renal failure

Vitamin D_3 is a hormonal precursor that is either taken up systemically through diet or synthesized in the skin from 7-dehydrocholesterol. Enzymatically controlled hydroxylation reactions at carbon atoms 25 and 1 (C-25 and C-1) that are mainly performed in the liver and the kidney respectively, convert vitamin D_3 into the biologically active form, the nuclear hormone 1α,25-dihydroxyvitamin D_3 (VD). VD has pleiotropic physiological effects [Walters, 1992]: its classical function is the regulation of calcium homeostasis by affecting its classical target tissues such as intestine, bone, kidney and parathyroid glands [Reichel and Norman, 1989]. Moreover, VD has been shown to inhibit cell growth and to induce differentiation in several normal and malignant cell types; recently, VD has also been shown to induce apoptosis (programmed cell death) in human breast cancer and leukemic cell lines [Welsh et al., 1995]. In vivo and in vitro studies have demonstrated the promising therapeutic potential of VD [Jones and Calverley, 1993; Pols et al., 1994]. The actions of the nuclear hormone are mediated by its nuclear receptor, the transcription factor vitamin D receptor (VDR), a member of the nuclear receptor superfamily [Mangelsdorf et al., 1995]. An essential prerequisite for mediating transactivation by a nuclear hormone is the

Abbreviations used: DBD, DNA binding domain; DR, direct repeat; IP, inverted palindrome; LBD, ligand binding domain; RXR, retinoid X receptor; RZR, retinoid Z receptor; T_3, 3,5,3′-triiodothyronine; T_3R, T_3 receptor; T_3RE, T_3 response element; VD, 1α,25-dihydroxyvitamin D_3; VDR, VD receptor; VDRE, VD response element.

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positioning of the respective ligand-activated nuclear receptor in close proximity to the basal transcriptional machinery. This is initially achieved through specific binding of the nuclear receptor to DNA binding sites, referred to as hormone response elements, located in the regulatory regions of primary hormone responding genes. This makes the complex formation between the nuclear receptor and its response element a key step in nuclear hormone signaling [Carlberg and Polly, 1998].

Hormone response elements are composed of hexameric core binding motifs [Glass, 1994]. Simple VDREs are formed by two hexameric core binding sites of the consensus sequence RGKTSA (R = A or G, K = G or T, S = C or G) that are found to be arranged as direct repeats (DRs) and inverted palindromes (IPs) [Carlberg, 1995]. The main partner receptor for the VDR is the retinoid X receptor (RXR), which is the nuclear receptor for 9-*cis*-retinoic acid [Carlberg, 1996b]. VDR-RXR heterodimers bind preferentially to DR3-type VDREs or to IP9-type VDREs, but also DR4-type VDREs are known [Carlberg, 1996a].

The nuclear receptor superfamily can be subclassified into those receptors that (1) form homodimers, (2) form heterodimers with RXR, and (3) bind DNA as a monomer. All nuclear receptors are structurally related; all contain a highly conserved DNA binding domain (DBD) of 66–70 amino acids, which is formed by two zinc finger structures and a moderately conserved C-terminal ligand binding domain (LBD) of approximately 250 amino acids, composed of 12 α-helices [Moras and Gronemeyer, 1998]. The DBD and the LBD are separated by a hinge region that allows free rotation of the two domains relative to each other. The structural relationship between the members of this transcription factor family suggests similar function in DNA binding, transactivation and contact to other nuclear proteins [Glass, 1994]. However, each nuclear receptor also demonstrates individual properties that are characteristic and not shared by its respective relatives.

In chronic renal failure abnormal VD metabolism and action plays a key role in the development of secondary hyperparathyroidism and renal osteodystrophy [Slatopolsky and Brown, 1997]. Decreased renal mass and phosphate retention are the main causes for a decreased renal production of VD. Furthermore, the responsiveness of target tissues to VD is decreased, so that some patients with secondary hyperparathyroidism become resistant to classical VD therapy [Akizawa et al., 1993; Fukagawa et al., 1995, 1997]. Their parathyroid glands escape the suppressive effects of VD on parathyroid hormone synthesis and secretion, so they have to be removed by surgery. This "VD resistance" may be attributable to a reduced expression of the VDR in the parathyroid glands [Korkor, 1987; Merke et al., 1987; Brown et al., 1989; Fukuda et al., 1993], but it also appears to occur at the level of VDR function. Uremic ultrafiltrates were shown to reveal an inhibitory effect on the binding of the intestinal VDR to the DR3-type VDREs of the rat osteopontin and rat osteocalcin gene [Hsu and Patel, 1995]. Consequently, "reactive aldehydes" from the uremic serum have been suggested to inhibit the complex formation of VDR on VDREs via the formation of Schiff bases with lysine residues in the DNA binding domain of the VDR [Patel et al., 1996].

In this study, the influence of human uremic ultrafiltrate from chronic hemodialysis patients and dialysate from peritoneal dialysis patients on the complex formation of VDR-RXR heterodimers on different VDRE types was analyzed. Moreover, RXR homodimers, thyroid hormone receptor (T_3R) homodimers, T_3R-RXR heterodimers, and monomers of the orphan nuclear receptor RZR were included in the study.

MATERIALS AND METHODS

Compounds and Uremic Solutions

VD was kindly provided by C. Mørk Hansen (LEO Pharmaceutical Products, Denmark) and dissolved in 2-propanol at a stock of 4 mM and diluted in ethanol. Pyridoxal phosphate was dissolved in H_2O at a stock of 20 mM. For control, pyridoxal phosphate was incubated with 40 mM L-lysine 10 days at 37°C in order to saturate aldehyde groups [Higgins and Bunn, 1981]. Uremic solutions were derived from five patients with chronic renal failure. Three ultrafiltrates were recovered at the beginning of chronic hemodialysis sessions (creatinine: 2.5/0.7/1.3 mg/dl; urea: 42.8/19.3/21.4 mg/dl) and two dialysates were recovered after 24 h of peritoneal dialysis (creatinine: 7.9/4.0 mg/dl; urea: 171.2/107 mg/dl). The solutions were standardized on the basis of the concentration of critical ions (Na^+, K^+, Ca^{2+}, Cl^-, PO_4^{2-}), creatinine, urea, and pH. The solutions were 5× concentrated by vacuum centrifugation and

used in a 1× concentration in the gel shift experiments.

DNA Constructs

The cDNA of human VDR, human RXRα, chicken $T_3R\alpha$ and human RZRα were subcloned into the expression plasmid pSG5 (Stratagene) [Sap et al., 1986; Carlberg et al., 1993, 1994]. The VDR construct was used as template for a linear polymerase chain reaction (PCR) reaction using native *Pfu* DNA polymerase (Stratagene) with a profile of 1 min at 94°C, 1 min at 55°C and 11 min at 68°C for 16 cycles. The following primer pairs were used for the K45A, K91A, K123A point mutations (K = lysine, A = alanine):

K45A+ TATGACCTGTGAAGGCTGC**GCA**GGCTTCTTCA and
K45A− TGAAGAAGCC**TGC**GCAGCCTTCACAGGTCATA,
K91A+ ATCGGCATGATC**GCG**GAGTTCATTCTG and
K91A− CAGAATGAACTC**CGC**CATCATGCCGAT,
K123A+ CAGTCTGCGGCCC**GCG**CTGTCTGAGGAG and
K123A− CTCCTCAGACAG**CGC**GGGCCGCAGACTG

Methylated parental DNA was then digested selectively with *Dpn*I and supercompetent *Epicurian coli* XL-1 (Stratagene) were transformed with nondigested, PCR-generated plasmid DNA. The respective point mutations were confirmed by sequencing. Response elements were formed by annealing complementary oligonucleotides carrying core sequences as given in Figures 1, 2, and 5 and flanking *Xba*I sites that permit radioactive labeling by a fill-in reaction using [α-^{32}P]dCTP and the Klenow fragment of DNA polymerase I (Promega, Madison, WI).

Gel Shift Assays

Linearized DNA from the pSG5-based constructs of VDR_{wt}, VDR point mutants, RXR, T_3R, and RZR were transcribed with T_7 RNA polymerase and translated using rabbit reticulocyte lysate as recommended by the supplier (Promega). For the formation of heterodimers, VDR (or VDR mutant or T_3R) and RXR protein were mixed (2.5 µl of each) in a total volume of 20 µl binding buffer (10 mM Hepes [pH 7.9], 1 mM DTT, 0.2 µg/µl poly[d(I-C)] and 5% glycerol), for homodimers or monomers only 5 µl of each T_3R, RXR, or RZR were taken. In the presence of 5 µM VD (or ethanol as control), incubation was then continued with additives (1× uremic solution, 5 mM pyridoxal phosphate or 5 mM pyridoxal-phosphate preincubated with 10 mM lysine) for 1 h at room temperature. The buffer was adjusted to the indicated salt concentrations by the addition of respective amounts of 1 M KCl. Approximately 1 ng of [α-^{32}P]-labeled probe (50,000 cpm) was added to the receptor-ligand mixture, and incubation was continued for 20 min. Protein-DNA complexes were resolved on a 5% nondenaturing polyacrylamide gel (at room temperature) in 0.5× TBE (45 mM Tris, 45 mM boric acid, 1 mM EDTA, pH 8.3). The gels were dried and exposed to a Fuji MP2040S imager screen overnight. The ratio of free probe to protein-probe complexes was quantified on a Fuji FLA2000 reader using Image Gauge software (Raytest). Each condition was analyzed, at least, in triplicate.

RESULTS

For an investigation of the influence of serum components from uremic patients on the complex formation of VDR-RXR heterodimers on VDREs, gel shift assays were performed using in vitro translated VDR and RXR and different types of VDREs: the DR3-type VDREs of the mouse osteopontin gene [Noda et al., 1990] (Fig. 1) and of the rat ANF gene [Kahlen and Carlberg, 1996] (Fig. 2A), the DR4-type VDRE of the rat Pit-1 gene [Rhodes et al., 1993] (Fig. 2B), and the IP9-type VDRE of the human calbindin D_{9k} gene [Schräder et al., 1995] (Fig. 2C). All assays were carried out at a physiological salt concentration of 150 mM monovalent cations in the absence or presence of a saturating concentration of VD (5 µM). In this model system, the effect of uremic solutions (ultrafiltrates from chronic hemodialysis and dialysates from peritoneal dialysis) on VDR-RXR heterodimer complex formation on the four different VDREs was studied. Pyridoxal phosphate, which is known to inhibit an assembly of protein and DNA via formation of Schiff bases, served as a positive control. Specific VDR-RXR heterodimer complexes were quantified in reference to free probes. The results were normalized to complex formation of untreated, nonliganded VDR-RXR heterodimers of the respective VDREs. Figure 1A shows a representative gel and Figure 1B the quantification of three independent experiments. The representative ultrafiltrates derived from chronic hemodialysis were found to reduce VDR-VDRE complex formation by 40–70% of control, whereas two dialysates derived from peritoneal dialysis reduced the amount of complex formation only

by 25–40%. By contrast, in the presence of pyridoxal phosphate hardly any complex formation could be observed. However, pretreatment of pyridoxal phosphate with L-lysine reversed most of the inhibitory effect of pyridoxal phosphate (Fig. 1B). The same results were also essentially observed with the three other VDREs referred to the unliganded complex. All gel shift reactions appeared to be ligand-dependent with a 2.3- to 3.4-fold increase of complex formation through addition of VD (depending on VDRE-type). Interestingly, in the presence of uremic solutions, ligand inducibility of complex formation on the two DR3-type VDREs and the DR4-type VDRE was significantly reduced, but not on the IP9-type VDRE.

As uremic solutions had an apparent negative effect on VDR-RXR heterodimer formation on different VDRE types, the question was raised, whether the order of treatment is critical. In the experiments shown in Figures 1 and 2, uremic solutions have been added to the forming VDR-RXR heterodimers before the VDREs were added. In the experiment shown

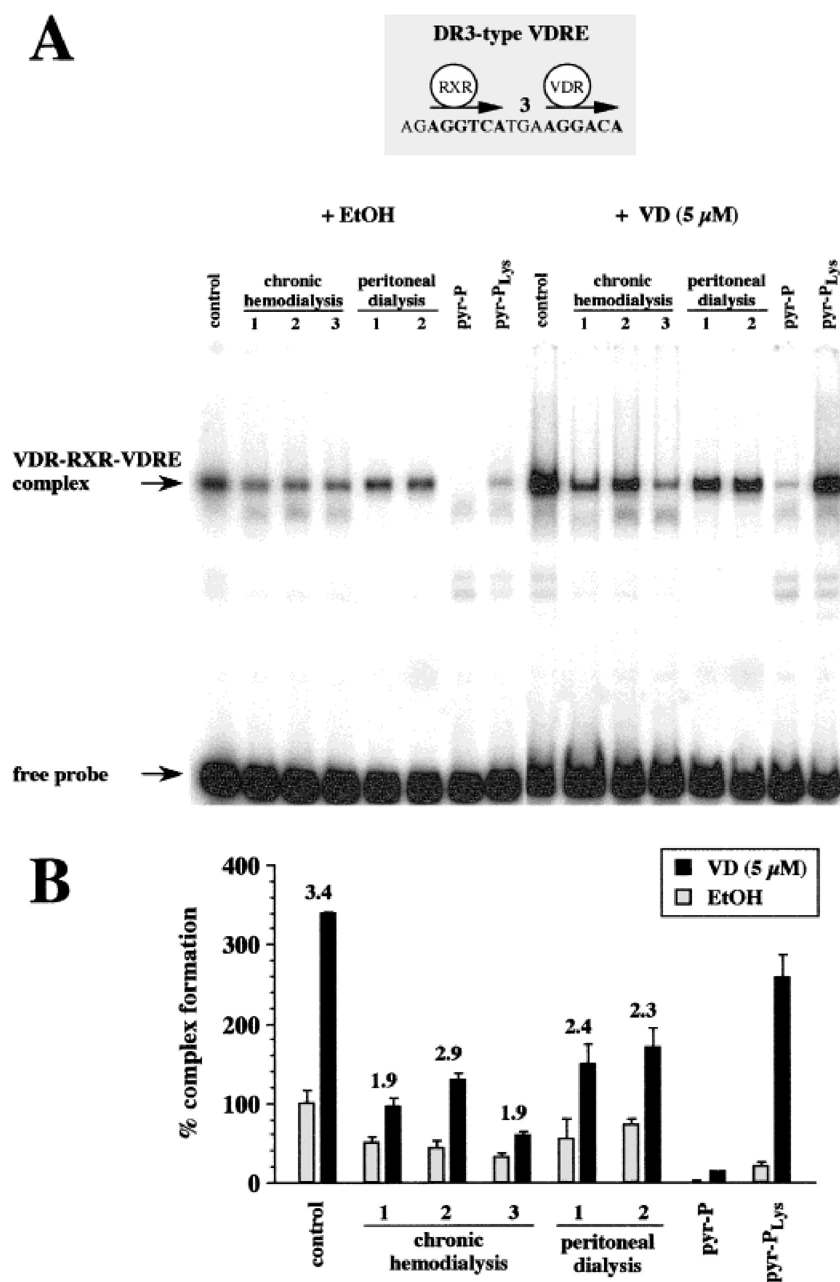
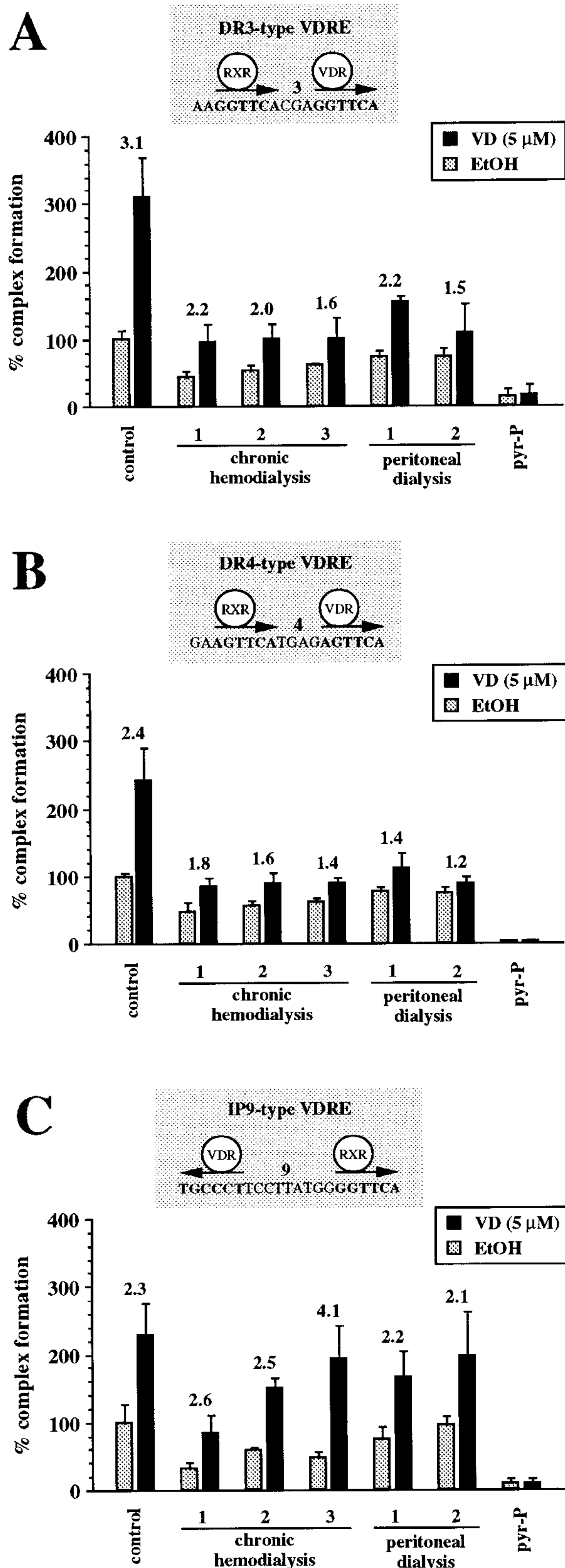


Fig. 1. Uremic solutions reduce the VDR-RXR complex formation on a DR3-type VDRE. Gel-shift experiments were performed using in vitro translated human VDR_{wt} and RXR_{wt} and the DR3-type VD response element of the rat ANF gene. A model representing the protein-DNA complex is shown schematically above the gel. The concentration of monovalent ions was kept constant at 150 mM. VDR-RXR heterodimers were pre-incubated with H_2O (control), uremic solutions (three ultrafiltrates derived from chronic hemodialysis and two dialysates from peritoneal dialysis), pyridoxal phosphate (pyr-P), or pyridoxal phosphate preincubated with lysine (pyr-P_{Lys}) for 1 h. In parallel, VDR-RXR heterodimers were incubated either in the absence (5% EtOH) or presence of ligand (5 μM VD). The radiolabeled response element probe (50,000 cpm) was subsequently added and the reaction was further incubated for another 20 min. The VDR-RXR-VDRE complex was separated from the free probe on a 5% polyacrylamide gel. The gels were dried and exposed to a Fuji MP20405 imager screen. **A:** A representative gel. The relative amount of VDR-RXR-VDRE complex formation was quantified using a Fuji FLA-2000 reader and Image Gauge software. **B:** Relative complex formation is presented in reference to the control in the absence of ligand. Columns represent triplicates and bars indicate standard deviation. Values above the columns indicate fold-induction of complex formation by ligand.



in Figure 3, one of the ultrafiltrates derived from chronic hemodialysis was added after VDR-RXR heterodimers have already been formed on the rat ANF DR3-type VDRE. Interestingly, in the absence of ligand, the inhibitory effect of the uremic solution on the VDR-RXR heterodimer-formation on the VDRE was found to be similar to that of the comparable experiment shown in Figure 1B (reduction to approximately 40% of control). However in this case, ligand inducibility remains restored (3.1-fold vs 3.4-fold). Moreover, a treatment with pyridoxal phosphate showed no significant effect with this order of treatment.

In order to test the hypothesis that uremic solutions act through the formation of Schiff bases with lysine residues [Patel et al., 1996], three possible critical lysines from the P-box of the DBD (VDR_{K45A}), the T-box of the hinge region (VDR_{K91A}) and the link between the hinge region and the LBD (VDR_{K123A}) were mutated into alanines. The complex formation of VDR_{wt} with RXR on the rat ANF DR3-type VDRE was then compared with that of the three point mutated VDRs (Fig. 4). The K45A and K123A mutations modulate the absolute amount of complex formation (Kahlen and Carlberg, 1997; M. Quack and C. Carlberg, unpublished results), but that was not the topic of the present investigation. Therefore, the control reactions of each VDR were individually normalized to 100%. Interestingly, the use of this analysis demonstrated that the inhibitory effect of uremic solutions on VDR complex formation was found to be equal for VDR_{wt} and the three lysine mutants (reduction by 40% compared with control). By contrast, the inhibitory effect of pyridoxal phosphate differed significantly between VDR_{wt} and the mutants, which is indicated by the determined ratio. The ratio of the inhibitory effect of pyridoxal phosphate and that of the

Fig. 2. Comparison of the inhibitory effect of uremic solutions on VDR-RXR complex formation on different types of VDREs. Gel-shift experiments were performed using in vitro translated human VDR_{wt} and RXR_{wt} and the DR3-type VDRE of the mouse osteopontin gene **(A)**, the DR4-type VDRE of the rat Pit-1 gene **(B)** or the IP9-type VDRE of the human calbindin D_{9k} gene **(C)**. A model representing the protein-DNA complex is schematically shown above each diagram. Experiments were performed and quantified as indicated in Figure 1. Relative complex formation is presented in reference to the control in the absence of ligand. Columns represent triplicates and bars indicate standard deviation. Values above the columns indicate fold-induction of complex formation by ligand.

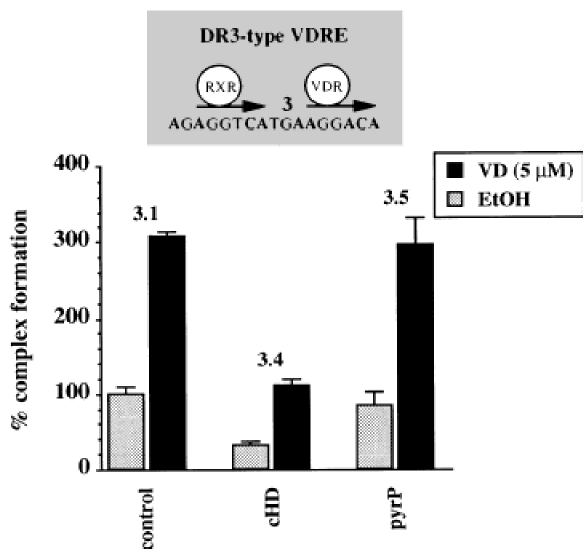


Fig. 3. Effect of uremic solutions is independent of the order of complex formation of VDR-RXR heterodimers on a DR3-type VDRE. Gel-shift experiments were performed using in vitro translated human VDR_{wt} and RXR_{wt} and the DR3-type VD response element of the rat ANF gene. A model representing the protein-DNA complex is schematically shown above the diagram. VDR-RXR heterodimers were first incubated with the radiolabelled response element probe (30,000 cpm) for 20 min. In parallel, VDR-RXR heterodimers were incubated either in the absence (5% EtOH) or presence of ligand (5 μM VD). Second, the protein-DNA complexes were incubated with H_2O (control), ultrafiltrate derived from chronic hemodialysis (cHD) or pyridoxal phosphate (pyr-P) for another 20 min. Experiments were further performed and quantified as indicated in Figure 1. Relative complex formation is presented in reference to the control in the absence of ligand. Columns represent triplicates and bars indicate standard deviation. Values above the columns indicate fold-induction of complex formation by ligand.

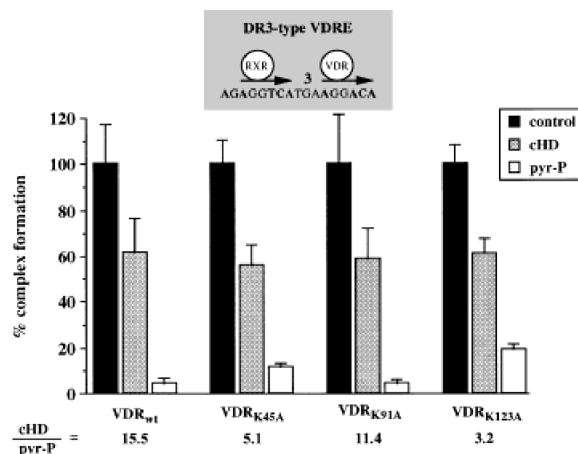


Fig. 4. Lysine-mutants of the VDR differentiate the inhibitory effect of uremic solutions and pyridoxal phosphate on VDR-RXR complex formation on DNA. Gel shift experiments were performed using in vitro translated human VDR_{wt}, lysine mutants of the VDR (VDR_{K45A}, VDR_{K91A}, VDR_{K123A}) and RXR_{wt} and the DR3-type VD response element of the rat ANF gene. A model representing the protein-DNA complex is shown schematically above the diagram. VDR-RXR heterodimers were pre-incubated with H_2O (control), ultrafiltrate derived from chronic hemodialysis (cHD) or pyridoxal phosphate (pyr-P) for 1 h. Experiments were further performed and quantified as indicated in Figure 1. It should be noted, that the point mutations themselves modulate complex formation of the VDR-RXR heterodimers on DNA. Therefore, relative complex formation is presented in reference to the respective control that has been normalized to 100%. Columns represent triplicates and bars indicate standard deviation. The ratios of the relative complex formation in the presence of cHD and pyr-P are given below the diagram.

uremic solution was found to be 15.5 with VDR_{wt}, but only 11.4 with VDR_{K91A}, 5.1 with VDR_{K45A} and 3.2 with VDR_{K123A}.

Uremic solutions may also affect the complex formation of other nuclear receptors. Therefore, the effect of uremic solutions were also tested on RXR homodimers on a DR1-type response element, on T_3R homodimers on the DR4-type T_3RE of Moloney murine leukemia virus (MMLV) [Sap et al., 1989] and on the IP6-type T_3RE of the mouse myelin basic protein gene [Farsetti et al., 1992], T_3R-RXR heterodimers on the same two types of T_3REs and on RZR monomers on a cryptic DR3-type VDRE of the rat bone sialo protein gene [Schräder et al., 1996] (Fig. 5). Interestingly, the inhibitory effect of uremic solutions on the complex formation of T_3R-RXR heterodimers on both T_3RE types and on T_3R homodimers on the IP6-type T_3RE is comparable to that of VDR-RXR heterodimers on different VDRE types (reduced by 40–60% compared with control). In contrast, the complex formation of T_3R homodimers on the DR4-type T_3RE was only reduced by 20% by the addition of uremic solutions and the binding of RZR monomers to their binding site was not affected.

DISCUSSION

The complex formation of the VDR with RXR on VDREs in the promoter regions of primary VD responding genes is the central step in nuclear VD signaling. It was shown in this study that the VDR complex formation on different types of VDREs can be reduced by uremic solutions from chronic hemodialysis patients or dialysate from peritoneal dialysis patients, respectively. This observation extends previous investigations on DR3-type VDREs [Patel et al., 1995] and would predict that the inducibility of probably all primary VD responding genes is reduced under the influence of uremic solu-

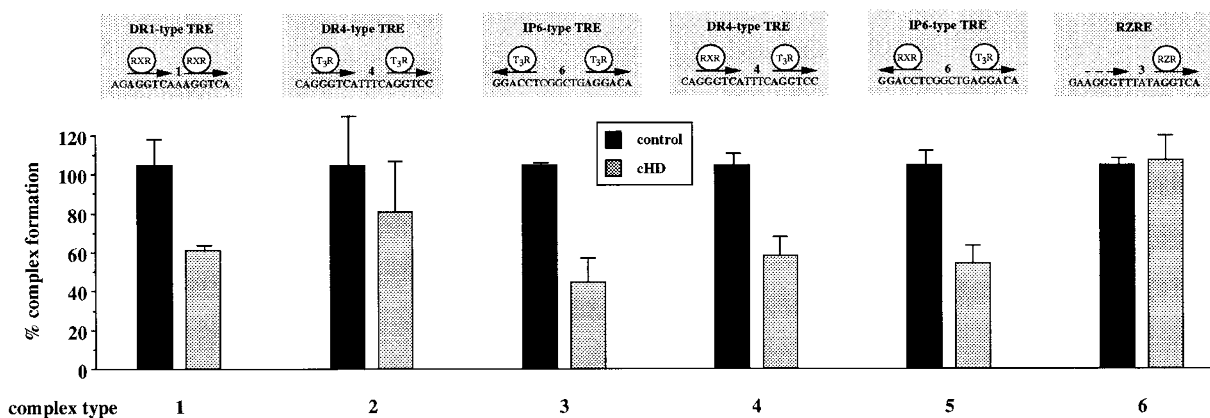


Fig. 5. Differential effect of uremic solutions on the complex formation of nuclear receptors on DNA. Gel shift experiments were performed with the following in vitro translated nuclear receptors and the respective response elements: RXR homodimers on a DR1-type response element (1), T_3R homodimers on the DR4-type response element of MMLV (2), T_3R homodimers on the IP6-type response element of the mouse myelin basic protein gene (3), T_3R-RXR heterodimers on the DR4-type response element of MMLV (4), T_3R-RXR heterodimers on the IP6-type response element of the mouse myelin basic protein gene (5) and RZR monomers on the cryptic DR3-type response element of the rat bone sialoprotein gene (6). Models representing each protein-DNA complex are schematically shown above of the diagram. Experiments were performed and quantified as indicated in Figure 1. Relative complex formation is presented in reference to the control in the absence of ligand. Columns represent triplicates and bars indicate standard deviation.

tions. Moreover, this study described a “fine-tuned” response of different types of VDREs to the influence of uremic solutions; that is, the complex formation on all VDRE types appears to be affected, but the VD inducibility of IP9-type VDREs remains active when the uremic solution is added to the heterodimeric complex at the moment of its formation. However, the inducibility of DR3-type VDREs is resistant to uremic solutions only if they are added after complex formation.

The described effects of uremic solutions on the complex formation between VDR-RXR heterodimers and VDREs could be a molecular explanation for decreases in VD responsive processes in target tissues and the resistance to VD actions observed in combination with chronic renal failure. However, the hypothesis that lysine residues of the DBD of the VDR are blocked by reactive aldehydes or ketones from these uremic solutions [Patel et al., 1995; 1996] is not supported by the results from this study. The positive control for reactive aldehydes, pyridoxal phosphate, could be confirmed as being able to block complex formation of VDR-RXR heterodimers via the interference with lysine residues of the DBD and the hinge region, but the point mutation of these critical lysine residues was found to have no consequence on the inhibitory effect of the uremic solutions. Moreover, the lysine residue of the P-box of the DBD (K45 in the VDR) is highly conserved within the nuclear receptor superfamily [Kahlen and Carlberg, 1997]; therefore, in accordance with the hypothesized mechanism, all members of the transcription factor family should be influenced by uremic solutions. This study could demonstrate that in addition to VD signaling, uremic solutions can also affect RXR and thyroid hormone signaling. RXR is the heterodimeric partner to most known nuclear receptors, so that it can be assumed that many nuclear hormone signalling pathways are modulated by uremic solutions. However, the amplitude of the effect did not appear to be comparable to the complex formation of all nuclear receptors. In addition, the orphan nuclear receptor RZR, which binds DNA preferentially as a monomer, was found to be unaffected.

The data presented in this article do not exclude the possibility that the protein-DNA interaction between the nuclear receptor and its respective core binding motif within the response element is the major target site, although lysine residues appear not to play a central role. However, as most nuclear receptors only show sufficient affinity for a stable contact with DNA, when present as a dimeric complex with a partner receptor, additional target sites could theoretically be the dimerization interfaces of the nuclear receptors that are located within the DBD, the hinge region and the

LBD. Primarily, in the case of monomer RZR receptor binding on DNA [Schräder et al., 1996], these dimerization interfaces are not used, which may be an explanation for its resistance against uremic solutions. By contrast, the LBD does not appear to be an important target for the effects of uremic solutions, because the analysis of the ligand-stabilized conformations of the VDR in the presence and absence of uremic solutions by limited protease digestion [Nayeri and Carlberg, 1997] did not demonstrate a significant effect (data not shown). Taken together, the question of the precise mechanisms of the inhibitory effect of uremic solutions remains unsolved. Presently, there is no direct evidence that VDR and the other nuclear receptors are covalently modified by uremic solutions. In contrast, it could be hypothesized that salt components of an uremic solution (e.g., divalent ions), that are different to normal serum, influence the surface charge of the nuclear receptor and in this way its ability to interact with DNA as well as with other proteins. For example, it is known that salt concentration is the major determinant for the strength and ligand dependence of the interaction of VDR and other nuclear receptors with their cognate DNA binding sites [Kimmel-Jehan et al., 1997]. Therefore, the concept would not be exclusively linked to components of uremic solutions derived from pathological situations, but also to components of all extra- and intracellular fluids that may interact with nuclear receptors. With this in mind, the often used term "uremic toxin" [Ringoir, 1997] was avoided in this study, as it is likely that the modulation of the activity of a nuclear receptor via changing its surface charge is part of the normal regulation process. Investigations addressing this point are in progress.

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