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Regulation of calcitriol receptor and its mRNA in normal and renal failure rats

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Regulation of calcitriol receptor and its mRNA in normal and renal failure rats. Homologous up-regulation of calcitriol receptor (VDR) by calcitriol is believed to be a transcriptional event. In this experiment, we studied the effect of calcitriol on VDR in normal and renal failure rats. The time course of the effect of calcitriol on VDR mRNA showed a biphasic change in VDR mRNA in response to calcitriol. The concentration of intestinal VDR mRNA increased at six hours and reached peak levels approximately 15 hours after calcitriol injection. Thereafter, the mRNA began to decrease and by 48 hours the level had declined to below the control values. The VDR levels also increased, though they lagged behind the VDR mRNA, and nearly plateaued at 24 hours after calcitriol treatment. In renal failure, the concentrations of VDR were lower and the levels of VDR mRNA were higher than the respective values of normal rats, suggesting that VDR synthesis was inhibited at post-transcriptional sites. Chronic administration of calcitriol increased the VDR but lowered the VDR mRNA levels in both normal and renal failure rats. Infusion of uremic ultrafiltrate to normal rats resulted in lower VDR and higher VDR mRNA levels similar to those found in rats with renal failure. The results indicate that uremic toxins are responsible for the low VDR and high VDR mRNA in renal failure.

The genomic action of calcitriol is believed to be mediated through a hormone-receptor complex interacting with nuclear chromatin. Interaction of the calcitriol receptor (VDR) with VDR responsive genes produces the bioactive proteins that carry out the biological action of calcitriol [1]. Therefore, regulation of VDR concentration modulates cellular responsiveness to calcitriol, for the genomic action of calcitriol is proportional to the cellular receptor numbers [2]. In renal failure, the concentration of VDR is decreased [3, 4]; therefore, the biological response to calcitriol could be diminished. For example, intestinal calcium absorption in response to a pharmacological dose of calcitriol is lower in nephrectomized rats than in normal rats [5, 6]. Furthermore, parathyroid hormone (PTH) levels are elevated despite normal plasma concentrations of calcitriol [7], and long-term administration of calcitriol fails to normalize PTH levels [8].

Recent studies have shown that calcitriol increases VDR and VDR mRNA concentrations [9, 10], suggesting that calcitriol up-regulates its own receptor. Other studies, however, were unable to show that the up-regulation of VDR is associated with an increased VDR mRNA [11, 12]. Regulation of VDR level in renal failure, however, has not been thoroughly studied. Therefore, in this study, we examined the effect of calcitriol on intestinal VDR and its mRNA in normal rats and in rats with renal failure. Our study showed that calcitriol induced a biphasic change in VDR mRNA. VDR mRNAs increased initially and followed by a decline to levels below the control values. Further, uremic toxins appeared to inhibit the VDR synthesis at post-transcriptional sites.

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Methods

Male Sprague-Dawley rats weighing 200 g were used for the present study. Renal failure was achieved by subtotal nephrectomies under ether anesthesia. One kidney was removed through flank incision and two-thirds of the other kidney was removed three days later. Control rats had sham nephrectomies. Animals were pair-fed a regular Purina rat chow containing 1.0% Ca, 0.8% P, and 4.5 IU per g vitamin D. Intestinal VDR and VDR mRNA and plasma concentrations of creatinine, calcitriol, calcium, and phosphorus were measured at the end of the experiments described below.

Time course of the effect of calcitriol on intestinal VDR and VDR mRNA in normal rats and renal failure rats

Five rats underwent subtotal nephrectomies, and another five rats had sham nephrectomies to serve as controls for the following studies. Animals were pair-fed for one week after the surgery. One control rat and one subtotally nephrectomized rat received vehicle (1.25% ethanol in propylene glycol) through the tail veins. They were killed immediately and intestinal VDR and VDR mRNA were measured to represent the values at time 0. Three control rats and three renal failure rats received 50 ng of calcitriol intravenously (i.v.; this was an arbitrarily chosen dose and equivalent to tenfold calcitriol production rate/day of a 200 g rat [13]), and intestinal VDR and VDR mRNA were measured at time 6, 15, and 24 hours after calcitriol injection (one rat for each time point for both control and renal failure). Another two rats (one control and one renal failure) were intravenously injected with a 50 ng of calcitriol at time 0 and 24 hours. Intestinal VDR and VDR mRNA were measured 48 hours after the first calcitriol injection.

Effect of calcitriol on intestinal VDR and VDR mRNA on normal rats

Five normal rats were injected i.v. with 3 ng of calcitriol once a day for two days and another five rats were injected i.v. with 50 ng of calcitriol once a day for two days. Control rats ($N = 5$) were injected with vehicle once a day for two days to serve as controls. All rats were killed for measurement of intestinal VDR and VDR mRNA 48 hours after the initial injection of vehicle or calcitriol.

Effect of calcitriol on intestinal VDR and VDR mRNA in rats with renal failure

Group 1. Five control rats and five renal failure rats were injected i.v. with vehicle for two days. Five renal failure rats were injected with 3 ng/day of calcitriol for two days. This dose of calcitriol was equivalent to the average difference in calcitriol production rate between the subtotally nephrectomized rats and control animals [13]. Another five renal failure rats received 50 ng/day of calcitriol for two days.

Group 2. Five control rats and six renal failure rats were infused subcutaneously for seven days with vehicle by osmotic minipumps implanted subcutaneously between the scapulae. Another six renal failure rats were infused subcutaneously for seven days with 3 ng/day of calcitriol by osmotic minipumps.

Effect of uremic plasma ultrafiltrate on intestinal VDR and VDR mRNA

We have previously demonstrated that infusion of uremic plasma ultrafiltrate suppressed intestinal VDR concentration [4]. In this experiment, we studied the effect of uremic toxins on VDR mRNA. Four normal rats were infused for 20 hours intravenously through femoral vein catheter with 30 ml of normal plasma ultrafiltrate. Another four normal rats were infused for 20 hours with 30 ml of uremic plasma ultrafiltrate as described previously [4]. Uremic ultrafiltrates were collected at the initiation of dialysis. Normal plasma ultrafiltrates were obtained by filtering pooled heparinized plasma through an identical dialyzer used for collecting uremic ultrafiltrates [14]. The ultrafiltrate electrolytes were adjusted to the following concentrations: Na 131 mEq/liter, K 2.7 mEq/liter, Ca 4.6 mg/dl, Mg 1.66 mg/dl, P 4.35 mg/dl, urea nitrogen 18 mg/dl, and creatinine 0.93 mg/dl for the normal plasma ultrafiltrate, and Na 131 mEq/liter, K 2.7 mEq/liter, Ca 4.4 mg/dl, Mg 1.66 mg/dl, P 4.55 mg/dl, urea nitrogen 113 mg/dl, and creatinine 10.4 mg/dl for the uremic ultrafiltrate.

Measurement 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 intestine was split longitudinally in half. One-half was used for VDR measurement, and the other half was used for VDR mRNA measurement as described below. 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 by 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 (KTEDM) [15]. Cytosol was prepared by centrifugation at 100,000 × g for 45 minutes at 0 to 4°C. Cytosol protein concentration was determined by the Bradford method.

Measurement of intestinal receptor concentration. We have previously determined intestinal VDR binding characteristics [4]. Scatchard analysis indicated a single binding site with an apparent kD of 0.44 nM, a value nearly identical to 0.47 nM reported by others [16]. Therefore, in this study we only measured N_{max} of VDR (total concentration of binding sites). N_{max} of VDR was assessed by incubating cytosolic protein for three hours at 0 to 4°C with a saturating concentration of tritiated calcitriol (5 nM) in the presence or absence of a 100-fold excess of cold calcitriol [4]. Bound hormone was separated from free hormone by adsorption of free hormone to dextran-coated charcoal (0.2% dextran, 2.0% charcoal in 10 mM Tris-HCl, 1.0 mM EDTA, pH 7.4) for 15 minutes on ice, followed by centrifugation at 1,600 × g. Supernatants (0.25 ml) were counted with a scintillation counter. Nonspecific binding was determined in each sample of cytosolic protein from the radioactivity that remained in the bound fraction after incubation in the presence of a 100-fold excess of cold calcitriol. Specific binding of calcitriol was calculated by subtracting nonspecific binding from total binding.

Measurement of VDR mRNA. Intestinal cellular RNA was extracted by the guanidinium thiocyanate-phenol-chloroform extraction method of Chomczynski and Sacchi [17]. RNA was quantitated by determining absorbance of the sample at 260/280 nm (Beckman model DU 62 Spectrophotometer). Polyadenylated RNA was extracted by oligo(dT)-affinity chromatography as described by Maniatis, Fritsch and Sambrook [18]. Two hundred milligrams of oligo(dT)-cellulose (Boehringer Mannheim Biochemicals) were equilibrated in 10 ml 1× binding buffer [100 mM sodium citrate pH 7.0, 0.5 M LiCl, 1 mM EDTA, 0.1% SDS (sodium dodecyl sulfate)] in a disposable column. Two mg of total cellular RNA were brought to a volume of 2.4 ml with sterile water and heated to 65°C for five minutes. The samples were then cooled on ice and 2.4 ml 2 × binding buffer was added to each sample and applied to the oligo(dT)-cellulose column. The effluent was collected and reapplied to the column. Each column was then washed with 20 volumes of 1 × binding buffer. The Polyadenylated RNA was eluted from the column with 4 volumes of elution buffer (10 mM Tris-HCl pH 7.6, 1 mM EDTA, 0.05% SDS). 0.1 volumes of 3 M sodium acetate, pH 5.2, and 2.5 volumes of ice-cold ethyl alcohol were then added to each sample, mixed and placed at −20°C for one hour. The samples were then centrifuged at 10,000 × g for 20 minutes at 4°C, supernatant discarded, and pellet washed with 70% ethyl alcohol. The ethyl alcohol was aspirated and the pellet dried by speedvac. The pellet was dissolved in 50 μl sterile water and its absorbance was determined at 260 and 280 nm.

Northern blot analysis. Poly(A)+ RNA, 5 μg, was fractionated under denaturing conditions on a 1.2% formaldehyde-agarose gel and transferred to Biotrans Nylon Membranes (ICN Corporation, Irvine, California, USA) by diffusion blotting as described by Maniatis et al [18]. VDR mRNA was determined from the migration of 18s and 28s ribosomal RNA. The membranes were baked for two hours at 80°C to cross link RNA to membranes. The membranes were then prehybridized for two hours at 42°C in hybridization solution containing 50% formamide, 5X Denhardts solution, 0.5% SDS, 5X SSC (SSC =

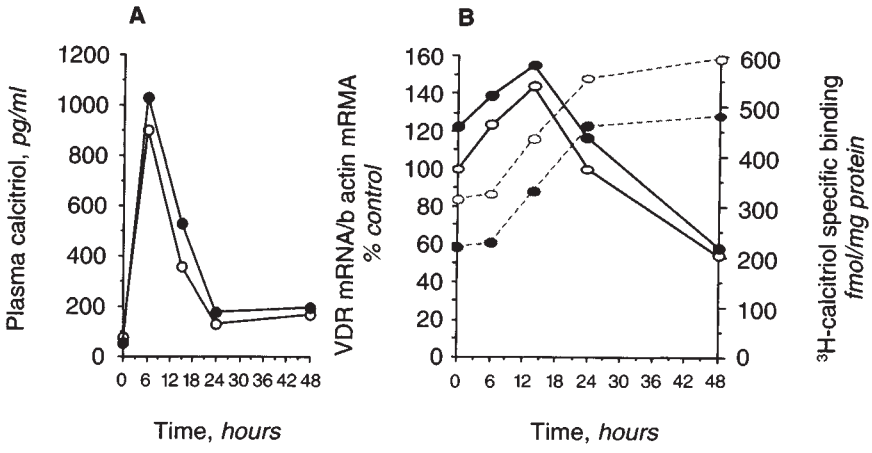

Fig. 1. *Time course of the effect of calcitriol on VDR (- - -) and VDR mRNA (——) in normal (○) and renal failure (●) rats injected with 50 ng/day of calcitriol for two days (B).* VDR and VDR mRNA were measured at time 0, 6, 15, 24, and 48 hours after calcitriol injection (one animal for each time point for control and renal failure). Plasma calcitriol concentrations of each time point of these animals are plotted on A.

0.15 M NaCl, 0.015 M sodium citrate, pH 7.0) and 100 μg/ml sonicated herring sperm DNA. Hybridization was carried out for 18 hours at 42°C in 4 to 10 ml fresh hybridization solution to which 1×10^6 cpm/ml ^{32}P-labeled probe was added. Filters were briefly rinsed in 200 ml 2X SSC at room temperature and then washed four times each for 15 minutes at 42°C in 500 ml of 0.1X SSC, 0.1% SDS. For autoradiographs, the membranes were exposed to XOMAT X-ray films (Eastman Kodak Co., Rochester, New York, USA) with intensifying screens for 2 to 120 hours at −80°C. Northern blot hybridization showed that VDR mRNA ran as a single band at 4.4 kb for each sample before performing dot blot hybridization.

Dot blot hybridization was performed according to the method of Church and Gilbert [19]. Poly(A)+ RNA was serially diluted in 25 mM sodium phosphate buffer pH 7.0 to produce samples containing 0.625, 1.25, 2.5 and 5.0 μg RNA, and blotted onto nylon membranes (Biotrans, ICN Corp., Irvine, California, USA) that had been pre-equilibrated in 25 mM sodium phosphate buffer pH 7.0 using a Minifold I microsample filtration manifold (Schleicher and Schuell, Keene, New Hampshire, USA). The filters were dried at room temperature for thirty minutes and then baked at 80°C for two hours. Prehybridization was carried out as described above. The membranes were then hybridized with the cDNA probe for human VDR (a gift of Dr. Mark R. Haussler, University of Arizona, Tucson, Arizona, USA). A 2.1 kilobase human VDR insert from the EcoR I site of pGem 4 and a 2.1 kilobase chick β-actin cDNA insert from the HIND II site of pBR322 were obtained by restriction enzyme digestion of the respective plasmid preparation. Each cDNA was labeled to a specific activity of 10^8 to 10^9 cpm/mg DNA according to the oligo-priming method of Feinberg and Vogelstein [20] using a random primed DNA labeling kit (Boehringer Mannheim, Indianapolis, Indiana, USA).

The dot intensities of the autoradiographs of varying exposures were quantitated by densitometry with the Bio-Rad Model 620 Video Densitometer equipped with the 2-D Analyst II data analysis software (BIO-RAD Laboratories, Richmond, California, USA). The amount of VDR mRNA in each blot was quantitated relative to the amount of β-actin mRNA. All the results were expressed as percent of the controls obtained from dot blot hybridization.

Other analytical methods. Calcium concentration was measured by atomic absorption spectrophotometry (model 306; Perkin Elmer, Norwalk, Connecticut, USA). The concentration of creatinine and phosphorus were measured as described previously [13]. Plasma calcitriol was measured in duplicate using a radioreceptor assay [21]. The intra-assay coefficients of variation were 5.4% for low control (20 pg/ml, $N = 6$) and 4.7% for high control (100 pg/ml, $N = 6$). The inter-assay coefficients of variation were 7% for low ($N = 12$) and 4.1% for high control ($N = 12$).

All data were expressed as mean ± SEM. Statistical analysis was performed using Student's *t*-test and Duncan's multiple range test. A P value of less than 0.05 was considered significant.

Results

Time course of the effect of calcitriol on intestinal VDR and VDR mRNA in normal rats and renal failure rats

Plasma creatinine of normal rats ranged from 0.57 to 0.63 mg/dl ($N = 5$) and the levels were increased in rats with renal failure ranging from 1.23 to 1.40 mg/dl ($N = 5$). Figure 1 shows the plasma concentrations of calcitriol in rats injected with calcitriol. The level was lower in the rat with renal failure (54.1 pg/ml) than that of the control rat (74.3 pg/ml) at time 0. Following 50 ng calcitriol injection, plasma levels of calcitriol were higher at each time point in rats with renal failure, possibly due to their lower clearance rate of calcitriol [22]. The expression of VDR mRNA of control and renal failure rats increased steadily and peaked at 15 hours, thereafter the levels began to decrease and by 48 hours after calcitriol injection the levels had declined to 54% (normal rat) and 59% (renal failure rat) of their respective controls (at time 0). The increment of VDR lagged slightly behind the rising VDR mRNA. However, the levels increased rapidly during the first 24 hours and nearly plateaued 24 hours after calcitriol injection. The concentrations of VDR remained lower in rats with renal failure compared to normal rats, even though their plasma levels of calcitriol were higher after injection of calcitriol.

Effect of calcitriol on intestinal VDR and VDR mRNA on normal rats

Figure 2 illustrates the results of plasma calcitriol and the levels of VDR mRNA and VDR in normal rats injected with 3 ng/day and 50 ng/day of calcitriol for two days. Plasma

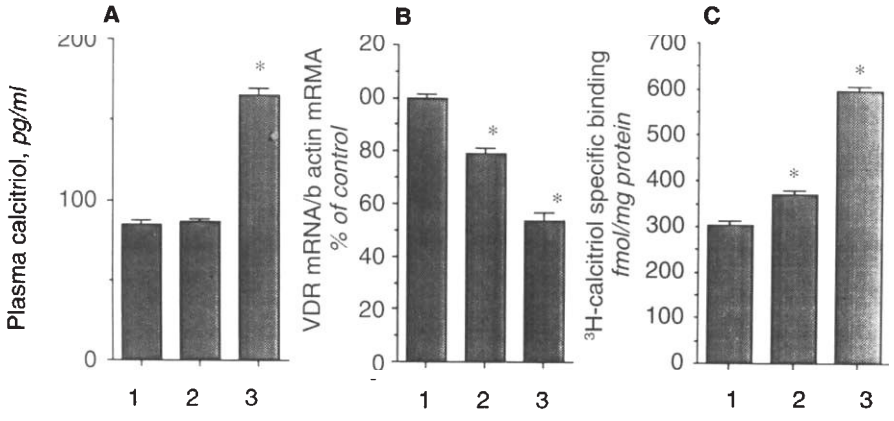

Fig. 2. *Plasma concentration of calcitriol and intestinal VDR mRNA and VDR of normal rats injected with vehicle or calcitriol daily for two days.* 1, normal rats injected with vehicle ($N = 5$); 2, normal rats injected with 3 ng/day calcitriol ($N = 5$); 3, normal rats injected with 50 ng/day calcitriol ($N = 5$). P values refer to comparison between the control (1) and experimental groups (2 or 3). * $P < 0.001$.

Table 1. Plasma concentrations of creatinine, calcium and phosphorus in normal rats injected with calcitriol for two days

	P_{Cr} *mg/dl*	P_{Ca} *mg/dl*	P_P *mg/dl*
1. Normal rats + vehicle × 2 days ($N = 5$)	0.57 ± 0.01	9.28 ± 0.05	7.09 ± 0.16
2. Normal rats + 3 ng/day × 2 days ($N = 5$)	0.57 ± 0.02	9.78 ± 0.07	6.84 ± 0.09
3. Normal rats + 50 ng/day × 2 days ($N = 5$)	0.59 ± 0.02	10.96 ± 0.24	7.21 ± 0.24
P values			
1 vs. 2	NS	<0.001	NS
1 vs. 3	NS	<0.001	NS
2 vs. 3	NS	<0.001	NS

Abbreviations are: P_{Cr}, plasma creatinine; P_{Ca}, plasma calcium; P_P, plasma phosphorus.

concentrations of calcitriol were not increased after 3 ng/day of calcitriol injection (86.4 ± 2.0 vs. 85.0 ± 2.1 pg/ml of controls injected with vehicles). This is probably due to the fact that calcitriol accelerates its own degradation [13], so that the small dose of calcitriol did not raise plasma levels of calcitriol. The levels, however, increased significantly after 50 ng/day of injection (165.5 ± 3.7 pg/ml, $P < 0.001$). Similar to the results shown in Figure 1, 48 hours after calcitriol injection VDR mRNA decreased and VDR increased significantly. The VDR was greater in animals injected with a higher dose of calcitriol (group 2 vs. 3, $P < 0.001$, Fig. 2).

Table 1 summarizes the plasma concentrations of creatinine, calcium and phosphorus of normal rats injected with calcitriol or vehicle. Plasma calcium levels were increased, whereas phosphorus levels did not change after injection of calcitriol.

Effect of calcitriol on intestinal VDR and VDR mRNA in rats with renal failure

The effect of calcitriol on VDR mRNA and VDR in renal failure two days after calcitriol injection are summarized in Figure 3. Plasma levels of calcitriol in animals injected with vehicle were significantly lower in renal failure rats as compared to normal rats (87.6 ± 2.2 vs. 50.6 ± 1.3 pg/ml, $P <$ 0.001). The levels increased significantly in rats injected with 3 ng/day and 50 ng/day of calcitriol (71.4 ± 1.7 pg/ml and 194.3 ± 6.7 pg/ml, both $P < 0.005$), though the levels of rats injected with 3 ng/day were still lower than those of normal rats injected with vehicle ($P < 0.005$).

The concentrations of VDR mRNA in rats injected with vehicle were significantly higher in renal failure (124.8 ± 3.2 vs. 100 ± 4.3%, $P < 0.005$) compared to the controls. Following calcitriol injection, VDR increased and the increase was greater in animals injected with a higher dose of calcitriol. However, VDR mRNA decreased to nearly the same level in renal failure rats injected with 3 ng/day and 50 ng/day of calcitriol.

Plasma concentrations of creatinine, calcium and phosphorus of renal failure rats injected with two days of calcitriol are tabulated in Table 2. Plasma concentration of calcium increased significantly in renal failure rats after calcitriol injection, whereas plasma phosphorus levels were not different between normal rats and renal failure rats with or without calcitriol injection.

Chronic infusion of calcitriol (seven days) and its effect on VDR mRNA and VDR are depicted on Figure 4. Plasma calcitriol of rats with renal failure increased significantly after seven days of calcitriol infusion. However, the levels (72.8 ± 1.5 pg/ml) remained lower than those of the controls infused with vehicle (87.3 ± 0.8 pg/ml, $P < 0.001$). Despite the lower plasma concentrations of calcitriol, the VDR of rats with renal failure (222.2 ± 6.0 fmol/mg protein) increased to levels (365.0 ± 19.0 fmol/mg protein) greater than those of the controls (326.2 ± 10.4 fmol/mg protein, $P < 0.005$) after seven days of calcitriol supplementation. The levels of VDR mRNA of rats with renal failure (137.4 ± 4.5%, $P < 0.001$) were higher than the controls without calcitriol supplementation. The levels (80.5 ± 1.3%) also decreased significantly after calcitriol infusion.

Table 3 summarizes plasma creatinine, calcium and phosphorus of rats infused with 3 ng/day calcitriol for seven days. Plasma calcium and phosphorus levels were not different between renal failure rats infused with vehicle and calcitriol.

Effect of uremic plasma ultrafiltrate on intestinal VDR mRNA and VDR in normal rats

Our previous study [4] led us to believe that uremic toxins are partly responsible for the decreased VDR concentration in renal failure, therefore, we studied the effect of uremic ultrafiltrate on the concentration of VDR mRNA and VDR. Figure 5 summarized the results of the study. Plasma calcitriol [23, 24] and

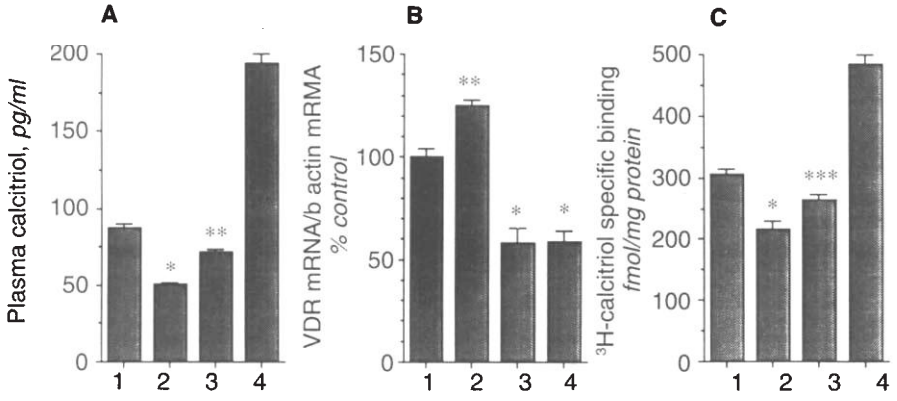

Fig. 3. *Plasma concentration of calcitriol and intestinal VDR mRNA and VDR of normal and renal failure rats injected with vehicle or calcitriol daily for two days.* 1, normal rats injected with vehicle ($N = 5$); 2, renal failure rats injected with vehicle ($N = 5$); 3, renal failure rats injected with 3 ng/day calcitriol ($N = 5$); 4, renal failure rats injected with 50 ng/day calcitriol ($N = 5$). P values refer to comparison between the control (1) and the experimental groups (2, 3 or 4). * $P < 0.001$; ** $P < 0.005$; and *** $P < 0.05$.

Table 2. Plasma concentrations of creatinine, calcium and phosphorus in renal failure rats injected with calcitriol for two days

	P_{Cr} *mg/dl*	P_{Ca} *mg/dl*	P_P *mg/dl*
1. Normal rats + vehicle × 2 days ($N = 5$)	0.55 ± 0.02	9.52 ± 0.04	6.76 ± 0.13
2. Renal failure rats + vehicle × 2 days ($N = 5$)	1.27 ± 0.02	9.44 ± 0.06	6.82 ± 0.14
3. Renal failure rats + 3 ng/day × 2 days ($N = 5$)	1.27 ± 0.04	9.72 ± 0.07	6.47 ± 0.08
4. Renal failure rats + 50 ng/day × 2 days ($N = 5$)	1.30 ± 0.03	10.66 ± 0.05	6.86 ± 0.14
P values			
1 vs. 2	<0.001	NS	NS
1 vs. 3	<0.001	NS	NS
1 vs. 4	<0.001	<0.001	NS
2 vs. 3	NS	<0.005	NS
2 vs. 4	NS	<0.001	NS
3 vs. 4	NS	<0.001	NS

Abbreviations are in Table 1.

intestinal VDR decreased [4] as we have previously demonstrated, whereas VDR mRNA increased significantly after infusion of uremic ultrafiltrate.

Discussion

In chronic renal failure, the concentration of VDR has been reported to be decreased in parathyroid gland [3, 25, 26] and intestine [4]. Several possible mechanisms are proposed for decreased concentration of VDR in renal failure: (1) Because calcitriol is known to up-regulate its own receptor [27], the low plasma calcitriol concentration in renal failure could down-regulate the VDR. (2) Accumulation of uremic toxins in renal insufficiency could reduce VDR concentration, as we have found that infusion of uremic ultrafiltrate to normal rats suppresses the concentration of intestinal VDR [4]. (3) A high level of plasma PTH in renal failure may decrease the concentration of VDR. The hormone down-regulates VDR and VDR mRNA *in vitro* in ROS 17/2.7 cells. It also blocks calcitriol-induced up-regulation of VDR of intestine and kidney in normal rats [28]. Furthermore, elevation of PTH secondary to calcium deficiency is associated with a significant down-regulation of kidney VDR despite a high concentration of plasma calcitriol [29]. Taken together, uremic toxins, a high concentration of plasma PTH, and a low concentration of plasma calcitriol could reduce VDR level in renal failure.

Homologous up-regulation of VDR by calcitriol is believed to be a transcriptional event, although this issue remains controversial. Strom et al have demonstrated that calcitriol increases the concentration of intestinal receptor as well as receptor mRNA in vitamin D depleted rats [10]. Others, however, were unable to demonstrate calcitriol induced up-regulation of VDR in cultured bovine parathyroid cells [30]. Furthermore, up-regulation of VDR is not always associated with an increased intestinal VDR mRNA *in vivo* [11]. In tissue culture system, calcitriol increased both VDR and VDR mRNA in ROS cells 18 hours after calcitriol treatment [28]. Our time course effect of calcitriol on intestinal VDR mRNA (Fig. 1) showed a biphasic change in VDR mRNA in response to calcitriol. The concentration of intestinal VDR mRNA increased at six hours and reached peak levels approximately 15 hours after calcitriol injection. Thereafter, the mRNA began to decrease and by 48 hours the level had declined to levels below the control values. The reason for the decreased VDR mRNA after calcitriol treatment is not clear. Lower levels of VDR mRNA after calcitriol treatment have also been reported previously by other investigators [31, 32].

A recent study has shown that there was no difference in the levels of VDR mRNA in parathyroid glands between control and 5/6 subtotally nephrectomized rats [32]. In contrast, we have shown that the levels of intestinal VDR mRNA were higher in rats with renal failure than those of control rats. The reason for this discrepancy could be due to different organs studied. The levels of intestinal VDR mRNA were also elevated in normal rats infused with uremic ultrafiltrate, suggesting that uremic toxins were responsible for the elevated intestinal VDR mRNA level in renal failure. Despite the higher level of intestinal VDR mRNA the concentrations of intestinal VDR in renal failure were lower than those of control rats. This finding appears to indicate that VDR synthesis in renal failure is inhibited at post-transcriptional site.

The concentrations of intestinal VDR also increased, though lagged few hours behind the VDR mRNAs, following calcitriol treatment. The levels nearly plateaued at 24 hours even though VDR mRNAs were declining during this period (Fig. 1). The time course effect of calcitriol suggests that the initial regulation of intestinal VDR appeared to be a transcriptional process, but it is not clear why VDR continued to increase inspite of

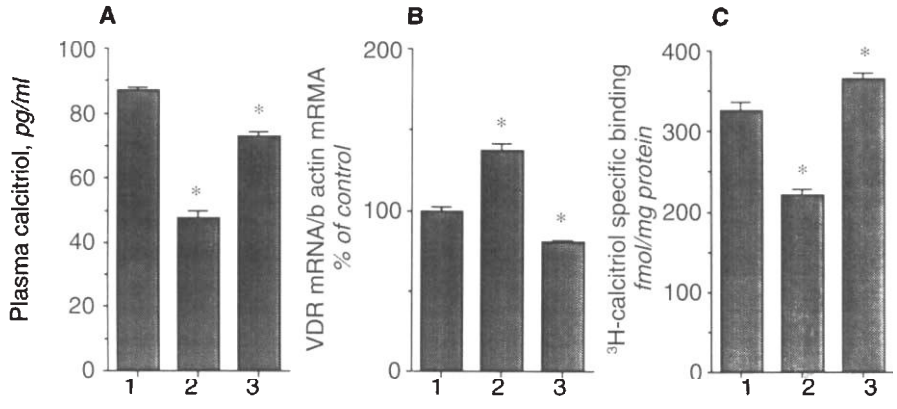

Fig. 4. *Plasma concentration of calcitriol and intestinal VDR mRNA and VDR of normal rats infused with vehicle or calcitriol daily for seven days.* 1, normal rats infused with vehicle ($N = 5$); 2, renal failure rats infused with vehicle ($N = 6$); 3, renal failure rats infused with 3 ng/day calcitriol ($N = 6$). *P* values refer to comparison between the control (1) and the experimental groups (2 or 3). * $P < 0.001$.

Table 3. Plasma concentrations of creatinine, calcium and phosphorus in renal failure rats infused with calcitriol for seven days

	P_{Cr} *mg/dl*	P_{Ca} *mg/dl*	P_P *mg/dl*
1. Normal rats + vehicle × 7 days ($N = 5$)	0.59 ± 0.02	9.78 ± 0.09	6.54 ± 0.22
2. Renal failure rats + vehicle × 7 days ($N = 6$)	1.28 ± 0.03	9.53 ± 0.08	6.38 ± 0.18
3. Renal failure rats + 3 ng/day × 7 days ($N = 6$)	1.27 ± 0.02	9.50 ± 0.15	6.94 ± 0.15
P values			
1 vs. 2	<0.001	NS	NS
1 vs. 3	<0.001	NS	NS
2 vs. 3	NS	NS	NS

Abbreviations are in Table 1.

declining VDR mRNA. Calcitriol could reduce VDR degradation thereby increasing its concentration, as it has been shown that calcitriol prolongs the half-life of VDR in LLC-PK1 cells [33].

In the present study we have also shown that calcitriol up-regulated intestinal VDR in renal failure. Supplementation of 3 ng/day of calcitriol for two days increased VDR levels in renal failure, though the levels remained lower than the controls. Calcitriol injection also slightly increased the plasma concentration of calcium in these animals (Table 2). Raising plasma calcium concentration could up-regulate VDR, as it has been demonstrated that normalizing plasma calcium levels in vitamin D deficient rat either by dietary calcium or vitamin D supplementation increased the concentration of renal VDR [34]. Changes in plasma phosphorus, however, did not alter the renal VDR concentration [34]. Our failure to up-regulate intestinal VDRs in renal failure rats to the control levels (Fig. 3) could be due to an inadequate supplementation of calcitriol, as their plasma levels remained lower than the controls. The high PTH level [29] and the presence of uremic toxins [4] in renal failure may also block calcitriol induced VDR synthesis. However, the concentrations of intestinal VDR exceeded those of control animals after seven days of supplementation, even though the plasma levels of calcitriol remained lower. Apparently the synthesis of intestinal VDR overcame the inhibitory effect of PTH and uremic toxins after a long-term supplementation of calcitriol.

We have also confirmed our previous study [4] that uremic toxins reduce intestinal VDR concentration. Infusion of uremic ultrafiltrate for 20 hours to normal rats reduces their VDR concentration [4]. Although uremic ultrafiltrate suppresses calcitriol synthesis [14], decreased calcitriol synthesis can not entirely account for the decreased VDR concentration, because the up-regulation of VDR by a pharmacological dose of calcitriol (20-fold of the daily production rate) is also blocked by uremic toxins [4]. Furthermore, as shown in Figure 1, the concentrations of VDR remained lower in rats with renal failure than those of normal rats even though the plasma levels of calcitriol were higher in renal failure rats injected with 50 ng/day of calcitriol. This supports the contention that uremic toxins inhibited the up-regulation of calcitriol induced VDR.

In summary, in renal failure the concentrations of intestinal VDR were lower and the levels of VDR mRNA higher than the respective values of normal rats. These findings suggest that VDR synthesis is inhibited at translational sites in renal failure. Calcitriol increased the intestinal VDR levels in normal and renal failure rats. However, the response of intestinal VDR mRNA to calcitriol is biphasic. The VDR mRNA levels increased initially but decreased later in normal as well as renal failure rats. Infusion of uremic ultrafiltrate to normal rats resulted in lower intestinal VDR and higher VDR mRNA levels, suggesting that uremic toxins are responsible for the lower intestinal VDR and higher VDR mRNA in renal failure. The reasons for higher VDR mRNA in renal failure despite lower plasma calcitriol is not clear. Nuclear run-on experiments may clarify this paradoxical finding.

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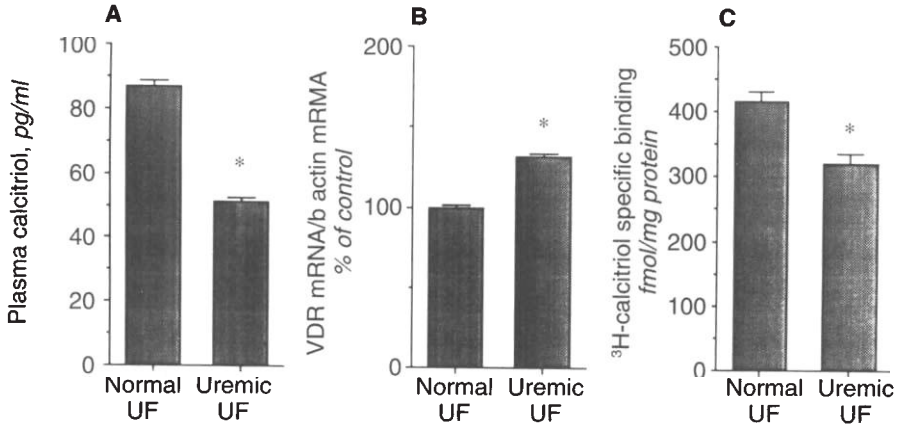

Fig. 5. *Plasma concentration of calcitriol and intestinal VDR mRNA and VDR of normal rats infused for 20 hours with 30 ml normal (N = 4) or uremic (N = 4) ultrafiltrate. * P <* 0.001.

References

1. PIKE JW: Emerging concepts on the biologic role and mechanism of action of 1,25-dihydroxyvitamin D3. *Steroids* 49:3–27, 1987
2. HIRST M, FELDMAN D: Regulation of 1,25$(OH)_2$ vitamin D_3 receptor content in cultured LLC-PK1 kidney cells limits hormonal responsiveness. *Biochem Biophys Res Commun* 116:121–127, 1983
3. KORKOR AB: Reduced binding of [^{3}H]1,25-dihydroxyvitamin D_3 in the parathyroid glands of patients with renal failure. *N Engl J Med* 316:1573–1577, 1987
4. HSU CH, PATEL RS, VANHOLDER R: Mechanism of decreased intestinal calcitriol receptor concentration in renal failure. *Am J Physiol* 264:F662–F669, 1993
5. WALLING MW, KIMBERG DV, WASSERMAN RH, FEINBERG RR: Duodenal active transport of calcium and phosphate in vitamin D-deficient rats: Effects of nephrectomy, Cestrum diurnum, and 1 alpha,25-dihydroxyvitamin D_3. *Endocrinology* 98:1130–1134, 1976
6. WONG RG, NORMAN AW, REDDY CR, COBURN JW: Biologic effects of 1,25-dihydroxycholecalciferol (a highly active vitamin D metabolite) in acutely uremic rats. *J Clin Invest* 51:1287–1291, 1972
7. FUKAGAWA M, KANAME S, IGARASHI T, OGATA E, KUROKAWA K: Regulation of parathyroid hormone synthesis in chronic renal failure in rats. *Kidney Int* 39:874–881, 1991
8. ANDRESS DL, NORRIS KC, COBURN JW, SLATOPOLSKY EA, SHERRARD DJ: Intravenous calcitriol in the treatment of refractory osteitis fibrosa of chronic renal failure [see comments]. *N Engl J Med* 321:274–279, 1989
9. MCDONNELL DP, MANGELSDORF DJ, PIKE JW, HAUSSLER MR, O'MALLEY BW: Molecular cloning of complementary DNA encoding the avian receptor for vitamin D. *Science* 235:1214–1217, 1987
10. STROM M, SANDGREN ME, BROWN TA, DELUCA HF: 1,25-Dihydroxyvitamin D_3 up-regulates the 1,25-dihydroxyvitamin D_3 receptor *in vivo*. *Proc Natl Acad Sci USA* 86:9770–9773, 1989
11. FAVUS MJ, MANGELSDORF DJ, TEMBE V, COE BJ, HAUSSLER MR: Evidence for *in vivo* upregulation of the intestinal vitamin D receptor during dietary calcium restriction in the rat. *J Clin Invest* 82:218–224, 1988
12. HUANG YC, LEE S, STOLZ R, GABRIELIDES C, PANSINI-PORTA A, BRUNS ME, BRUNS DE, MIFFIN TE, PIKE JW, CHRISTAKOS S: Effect of hormones and development on the expression of the rat 1,25-dihydroxyvitamin D_3 receptor gene. Comparison with calbindin gene expression. *J Biol Chem* 264:17454–17461, 1989
13. PATEL S, SIMPSON RU, HSU CH: Effect of vitamin D metabolites on calcitriol metabolism in experimental renal failure. *Kidney Int* 36:234–239, 1989
14. HSU CH, PATEL S: Uremic plasma contains factors inhibiting 1α-hydroxylase activity. *J Am Soc Nephrol* 3:947–952, 1992
15. HIRST M, FELDMAN D: Cleavage of the rat intestinal 1,25-dihydroxyvitamin D_3 receptor by an endogenous protease to a form with defective DNA binding. *Arch Biochem Biophys* 250:153–161, 1986
16. FELDMAN D, MCCAIN TA, HIRST MA, CHEN TL, COLSTON KW: Characterization of a cytoplasmic receptor-like binder for 1 alpha, 25-dihydroxycholecalciferol in rat intestinal mucosa. *J Biol Chem* 254:10378–10384, 1979
17. CHOMCZYNSKI P, SACCHI N: Single-step method of RNA isolation by acid guanidinium thiocyanate-phenol-chloroform extraction. *Anal Biochem* 162:156–159, 1987
18. MANIATIS T, FRITSCH C, SAMBROOK J: *Molecular Cloning—A Laboratory Manual*. New York, Cold Spring Harbor, 1989, (Chapt 7) pp. 7.1–7.84
19. CHURCH GM, GILBERT W: Genomic sequencing. *Proc Natl Acad Sci USA* 81:1991–1995, 1984
20. FEINBERG AP, VOGELSTEIN B: A technique for radiolabeling DNA restriction endonuclease fragmentstivity. *Anal Biochem* 137:266–267, 1984
21. HOLLIS BW: Assay of circulating 1,25-dihydroxyvitamin D involving a novel single-cartridge extraction and purification procedure. *Clin Chem* 32:2060–2063, 1986
22. HSU CH, PATEL SR, YOUNG EW, SIMPSON RU: Production and degradation of calcitriol in renal failure rats. *Am J Physiol* 253: F1015–F1019, 1987
23. HSU CH, PATEL S, BUCHSBAUM BL: Calcitriol metabolism in patients with chronic renal failure. *Am J Kidney Dis* 17:185–190, 1991
24. HSU CH, VANHOLDER R, PATEL S, DE SMET RR, SANDRA P, RIGOIR SMG: Subfractions in uremic plasma ultrafiltrate inhibit calcitriol metabolism. *Kidney Int* 40:868–873, 1991
25. MERKE J, HUGEL U, ZLOTKOWSKI A, SZABO A, BOMMER J, MALL G, RITZ E: Diminished parathyroid 1,25$(OH)_2D_3$ receptors in experimental uremia. *Kidney Int* 32:350–353, 1987
26. BROWN AJ, DUSSO A, LOPEZ-HILKER S, LEWIS-FINCH J, GROOMS P, SLATOPOLSKY E: 1,25-$(OH)_2$D receptors are decreased in parathyroid glands from chronically uremic dogs. *Kidney Int* 35:19–23, 1989
27. COSTA EM, FELDMAN D: Homologus up-regulation of the 1,25$(OH)_2$ vitamin D_3 receptor in rats. *Biochem Biophys Res Commun* 137:742–747, 1986
28. REINHARDT TA, HORST RL: Parathyroid hormone down-regulates 1,25-dihydroxyvitamin D receptors (VDR) and VDR messenger ribonucleic acid *in vitro* and blocks homologous up-regulation of VDR *in vivo*. *Endocrinology* 127:942–948, 1990
29. GOFF JP, REINHARDT TA, BECKMAN MJ, HORST RL: Contrasting effects of exogenous 1,25-dihydroxyvitamin D [1,25-$(OH)_2$D] versus endogenous 1,25-$(OH)_2$D, induced by dietary calcium restriction, on vitamin D receptors. *Endocrinology* 126:1031–1035, 1990
30. BROWN AJ, BERKOBEN M, RITTER CS, SLATOPOLSKY E: Binding and metabolism of 1,25-dihydroxyvitamin D_3 in cultured parathyroid cells. *Endocrinology* 130:276–281, 1992
31. NAVEH MT, MARX R, KESHET E, PIKE JW, SILVER J: Regulation of 1,25-dihydroxyvitamin D_3 receptor gene expression by 1,25-dihydroxyvitamin D_3 in the parathyroid *in vivo*. *J Clin Invest* 86:1968–1975, 1990

32. SHVIL Y, NAVEH-MANY T, BARACH P, SILVER J: Regulation of parathyroid cell gene expression in experimental uremia. *J Am Soc Nephrol* 1:99–104, 1990
33. COSTA EM, BLAU HM, FELDMAN D: Measurement of 1,25-dihydroxyvitamin D_3 receptor turnover by dense amino acid labeling: Changes during receptor up-regulation by vitamin D metabolites. *Endocrinology* 120:1173–1178, 1987
34. SANDGREN ME, DELUCA HF: Serum calcium and vitamin D regulate 1,25-dihydroxyvitamin D_3 receptor concentration in rat kidney *in vivo*. *Proc Natl Acad Sci USA* 87:4312–4314, 1990