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1,25,28-TRIHYDROXYVITAMIN D_2 UP-REGULATES RENAL VITAMIN D RECEPTOR WITHOUT CAUSING HYPERCALCEMIA

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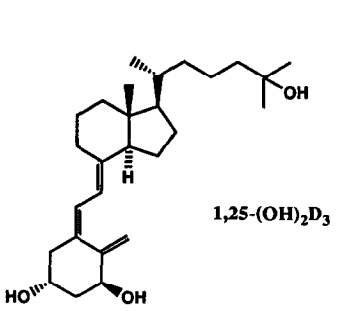
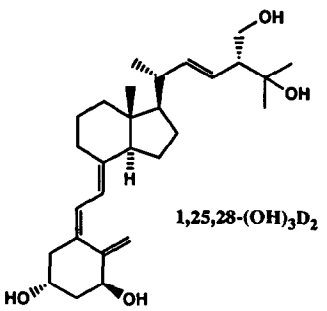
Abstract. Treatment of rats with 1,25,28-trihydroxyvitamin D_2 caused significant up-regulation in kidney vitamin D receptor in the absence of hypercalcemia. This result suggests that this relatively non-calcemic analogue (and possibly other analogues) of vitamin D may be used in stimulating vitamin D-regulated genetic events not associated with stimulation of active calcium transport *in vivo*.

The vitamin D hormone, 1,25-dihydroxyvitamin D_3 ($1,25(OH)_2D_3$), stimulates calcium and phosphorus homeostatic mechanisms in its classical target tissues; intestine, kidney, and bone. Tissue responsiveness to $1,25(OH)_2D_3$ is dependent on the circulating level of the hormone in the extracellular milieu and the presence of vitamin D receptors (VDR) in the target tissue.[1-3] Several hormones[4-6] and various physiological states[7,8] have been shown to alter tissue VDR number, thus modulating tissue responsiveness to $1,25(OH)_2D_3$. *In vitro*[9] and *in vivo*[10] studies have demonstrated that treatment with $1,25(OH)_2D_3$ is able to cause up-regulation of VDR in a variety of tissues. This treatment also causes hypercalcemia in the animals. Dietary calcium restriction to stimulate endogenous production of $1,25(OH)_2D_3$ without hypercalcemia fails to cause up-regulation of VDR.[8] Sandgren and DeLuca[11] suggest that the plasma calcium concentration increase following treatment with vitamin D_3 is responsible for up-regulation of VDR in tissues. However, Reinhardt and Horst[10] presented strong evidence that parathyroid hormone prevents up-regulation of VDR by $1,25(OH)_2D_3$. In this paper, we present evidence that *in vivo* administration of a non-hypercalcemic vitamin D analogue, $1,25,28(OH)_3D_2$ synthesized in our laboratory,[12] up-regulates renal VDR and that an increase in plasma calcium is not the driving force for up-regulation of renal VDR.



$1,25\text{-}(OH)_2D_3$



$1,25,28\text{-}(OH)_3D_2$

Materials and Methods

The ability of $1,25,28(OH)_3D_2$ to stimulate bone calcium resorption (BCR) and intestinal calcium absorption (ICA) relative to $1,25(OH)_2D_3$ was tested in vitamin D-deficient male Holtzman rats as previously described.[13] We also studied the relative effects of $1,25,28(OH)_3D_2$ on plasma calcium and VDR up-regulation in the kidney in ovariectomized nine-month-old female Fischer-344 rats treated with either 7 micrograms of $1,25,28(OH)_3D_2$ or 7.5 nanograms of $1,25(OH)_2D_3$ dissolved in corn oil (n=6/group) administered per os daily for 90 days. The Fischer-344 rats were maintained on rat chow that was 1% Ca and 0.45% P (Teklad 90268, Teklad Premiere, Madison, WI). Rats in all experiments were killed by inhalation of CO_2-O_2 (50:50) followed by guillotining. Plasma and the tissues were obtained from each rat. Plasma was analyzed for calcium content by atomic absorption spectrophotometry.[14] Renal VDR was determined by ligand-binding assay as described previously.[8] Student's unpaired *t*-test[15] was used to test for significant differences in plasma calcium or renal VDR as a result of $1,25,28(OH)_3D_2$ treatment.

Results and Discussion

The data (Table 1) show that the $1,25,28(OH)_3D_2$ at the highest dose (200 ng) was ineffective at stimulating either BCR or ICA. The $1,25(OH)_2D_3$, however, caused significant elevations in both parameters when given at a dose of 12.5 ng. In these bioassays, $1,25,28(OH)_3D_2$ therefore was at least 15-fold less active than $1,25(OH)_2D_3$.

TABLE 1. Biological evaluation of $1,25,28(OH)_3D_2$ using the rat bioassay

Steroid	Dose (ng/rat)	^{45}Ca (serosal)/ ^{45}Ca (mucosal) (ICA)	Serum Ca (mg/dl) (BCR)
Control	-	2.02 ± 0.45	4.1 ± 0.4
$1,25(OH)_2D_3$	12.5	4.46 ± 0.96*	4.6 ± 0.2*
$1,25,28(OH)_3D_2$	25	2.17 ± 0.59	4.1 ± 0.3
	50	2.08 ± 0.67	4.2 ± 0.5
	100	2.22 ± 0.04	3.8 ± 0.2
	200	3.21 ± 1.23	4.0 ± 0.3

* Different from controls ($P < 0.05$)

In the long-term experiment with ovariectomized Fisher-344 rats, treatment with 7 micrograms $1,25,28(OH)_3D_2$ daily for 90 days resulted (Table 2) in a significant ($P < 0.05$) increase in renal VDR concentration (249 ± 34 fmol/mg in treated rats *versus* 87 ± 3 fmol/mg in controls and 98 ± 11 in $1,25(OH)_2D_3$-treated rats). This degree of elevation of renal VDR is comparable to changes in renal VDR observed in rats treated parenterally with $1,25(OH)_2D_3$.[8] Despite its ability to up-regulate renal VDR, the $1,25,28(OH)_3D_2$ treatment had little effect on plasma calcium. Plasma calcium concentration in control rats (10.09 ± 0.16 mg/dl) was similar, and not different ($P < 0.05$) from that of $1,25,28(OH)_3D_2$-treated (10.53 ± 0.14 mg/dl) and $1,25(OH)_2D_3$-treated rats (10.55 ± 0.21 mg/dl).

TABLE 2. Plasma calcium and kidney VDR in rats treated with $1,25,28(OH)_3D_2$ and $1,25(OH)_2D_3$

	Plasma Ca (mg/dl)	Kidney VDR (fmol/mg protein)
Control	10.09 ± 0.16	87 ± 3
$1,25(OH)_2D_3$	10.55 ± 0.21	98 ± 11
$1,25,28(OH)_3D_2$	10.53 ± 0.14	249 ± 34*

* Significantly different from controls and $1,25(OH)_2D_3$-treated rats ($P < 0.05$).

These data support the hypothesis that VDR regulation by vitamin D metabolites is independent of plasma calcium concentration. Similarly, Uhland-Smith and DeLuca,[16] using an immunoradiometric assay for VDR, have demonstrated that renal VDR can be up-regulated following intravenous administration of $1,25(OH)_2D_3$ without having increased plasma calcium in short-term studies (24 hours). In earlier studies, Goff *et al.*,[8] reported that treatment of rats with 36 ng $1,25(OH)_2D$/day for 7 days (via mini-osmotic pump) increased renal VDR from 60 fmol/mg in control rats to 194 fmol/mg. At the same time, plasma calcium increased from 10.78 mg/dl to 13.4 mg/dl. The results of the present study suggest that the elevation in kidney VDR in the Goff studies was likely a result of a direct effect of $1,25(OH)_2D_3$ on the synthesis of new receptor, rather than an effect dependent on hypercalcemia.

The unique ability of $1,25,28(OH)_3D_2$ to up-regulate renal VDR without causing significant hypercalcemia should prove a valuable tool in further studies of VDR regulation and the interaction between VDR concentration and the biological response of the tissues. This compound may also provide

an alternative to $1,25(OH)_2D_3$ for regulation of vitamin D responsive genes without causing hypercalcemic side effects.

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