Abnormalities of the vitamin D receptor in uraemia

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Abstract. The 1,25-dihydroxyvitamin D₃ (1,25(OH)₂D₃) receptor mediates many of the actions of 1,25(OH)₂D₃ in cells of target tissues. Abnormalities in vitamin D receptor concentration and regulation exist in tissues of subjects with chronic renal failure and end-stage renal disease. There is evidence that inhibitors of vitamin D receptor-1,25(OH)₂D₃ binding and 1,25(OH)₂D₃ action are present in dialysates and sera of individuals with end-stage renal disease. The biological significance of decreased vitamin D receptor number and altered vitamin D receptor regulation in uraemia is unclear at present as responses to 1,25(OH)₂D₃ in uraemia have not been systematically compared with responses in normal subjects.

Key words: 1,25(OH)₂D₃; calcitrol; calcium; vitamin D receptor

Introduction

In this article I will review: (1) the interrelationships between various calciotropic hormones; (2) the mechanism of action of 1,25-dihydroxyvitamin D₃ (1,25(OH)₂D₃); (3) the structure and distribution of the vitamin D receptor in various tissues; (4) the regulation of vitamin D receptor levels in various tissues in normal situations; and (5) the regulation of the vitamin D receptor in uraemia.

The physiology of calciotropic hormones

Briefly, in hypocalcaemic situations, both PTH and 1,25(OH)₂D₃ play important roles in normalizing serum calcium concentrations [1-3]. A decrement in serum calcium is sensed via calcium receptors and causes the release of preformed PTH [4-6]. PTH increases the mobilization of soft tissue calcium and phosphorus and also increases the reabsorption of calcium in the nephron. In addition, PTH increases the synthesis of 1,25(OH)₂D₃ by stimulating the 25(OH)D₃ 1α-hydroxylase enzyme in the proximal tubule of the kidney. 1,25(OH)₂D₃ increases the active absorption of calcium from the intestine and also promotes bone and soft tissue calcium mobilization. Thus, both PTH and 1,25(OH)₂D₃ normalize serum calcium concentrations. Normal serum calcium concentrations inhibit the secretion of PTH, and 1,25(OH)₂D₃ inhibits the synthesis of PTH by directly altering PTH gene transcription.

The mechanism of action of 1,25(OH)₂D₃

1,25(OH)₂D₃ acts in various tissues by both nuclear (genomic) and membrane-associated (non-genomic) mechanisms [7,8]. Genomic events occur after the hormone associates with its intracellular receptor, the 1,25(OH)₂D₃ receptor, and non-genomic events occur as a result of direct effects on membrane channels of proteins [7,8]. A bulk of the activities of 1,25(OH)₂D₃ occur as a result of genomic actions, and include the control of the synthesis of a number of genes such as those for calbindin-D₂₈K, calbindin-D₉K, osteopontin, 25(OH)D₃, 24-hydroxylase cytochrome P-450, the gene for the vitamin D receptor itself, collagen and PTH [9-36]. The central role for the genomic actions of the 1,25(OH)₂D₃ receptor is underscored by the study of individuals with vitamin D-dependency rickets type II [37,38]. These individuals have mutations in the vitamin D receptor and have overt rickets. They respond only to exceptionally high concentrations of 1,25(OH)₂D₃.

Cellular distribution of the vitamin D receptor

The vitamin D receptor is widely distributed in many epithelial tissues, such as the absorptive cells of the intestine, the proximal and distal tubular cells of the kidney, and in epithelial cells of the reproductive organs [37,38]. The receptor is also present in various endocrine cells such as those of the pituitary gland, the pancreas, and parathyroid glands [37,41]. The receptor is also present in bone cells, particularly osteoblast-like cells.
The structure of the vitamin D receptor

The vitamin D receptor is a single chain polypeptide of approximately 50,000 Da molecular weight [37,38,42–48]. The human vitamin D receptor closely resembles the thyroid hormone receptor and the receptors for retinoic acid. There are more distant homologies with the progesterone receptor, the glucocorticoid receptors and the oestrogen receptor. The human vitamin D receptor is a 427 amino acid peptide with a DNA binding domain that extends from residues 25 to 112, and a sterol binding domain that extends from approximately residue 200 to residue 400. The DNA binding domain of the vitamin D receptor has two ‘zinc fingers’ which are responsible for the binding of the vitamin D receptor to DNA in vitamin D responsive genes. Several mutations of the vitamin D receptor DNA binding domain have been detected in individuals with vitamin D-dependence rickets type II [38,46]. The vitamin D receptor binds to vitamin D regulatory elements, the minimal structure of which is shown in Table 1 [49]. Vitamin D regulatory elements in various vitamin D-responsive genes are shown directly below the structure of the minimal vitamin D regulatory element in Table 1 [49–54]. In addition, there is evidence that other vitamin D regulatory elements that do not follow the direct repeat motif are responsible for the binding of the vitamin D receptor to DNA [55–59]. The vitamin D receptor binds to DNA both as a heterodimer in association with RXRβ or the thyrxine receptor or as a homodimer comprised of two vitamin D receptors. DNA binding motifs that allow the binding of the vitamin D receptor to DNA elements of genes regulated by 1,25(OH)2D3 do not necessarily follow the direct repeat sequence shown in Table 1. Several other sequences allow the binding of vitamin D receptor to various genes.

Table 1. Minimal vitamin D regulatory elements (VDREs) present in vitamin D regulated genes

<table>
<thead>
<tr>
<th>Minimal vitamin D regulatory element</th>
<th>aggtca</th>
<th>agg</th>
<th>aggtca</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rat osteocalcin gene VDRE</td>
<td>ggggga</td>
<td>atg</td>
<td>gggacca</td>
</tr>
<tr>
<td>Human osteocalcin gene VDRE</td>
<td>aggtga</td>
<td>cag</td>
<td>aggtca</td>
</tr>
<tr>
<td>9K calcium binding protein</td>
<td>aggtgtg</td>
<td>sgg</td>
<td>aaggcc</td>
</tr>
</tbody>
</table>

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Regulation of the vitamin D receptor in normal or physiological states

I will discuss the regulation of the vitamin D receptor in four tissues that are germane to renal failure and uraemia. The regulation of the receptor under normal conditions in these situations will be discussed. 1,25(OH)2D3 and dietary calcium both appear to be important regulators of the vitamin D receptor in the kidney and in the parathyroid glands; in the intestine, data on the effects of 1,25(OH)2D3 on vitamin D receptor regulation are less clear [60–66]. In bone cells in culture, 1,25(OH)2D3 and PTH increase the synthesis of the vitamin D receptor [67–69]. It is important to realize that there are differences between what occurs in vivo as a result of a physiological alteration that increases 1,25(OH)2D3 concentrations and what occurs following the infusion of 1,25(OH)2D3 to a vitamin D-deplete or vitamin D-replete animals. Table 2 shows the effects of the administration of 1,25(OH)2D3 to rats receiving a normal vitamin D-containing diet. The administration of 1,25(OH)2D3 results in an increase in duodenal vitamin D receptor, and an increase in kidney vitamin D receptor. On the other hand, as shown in Table 3, adaptation to a low calcium diet, a manoeuvre that is known to increase the synthesis of 1,25(OH)2D3 does not result in an increase in the amount of the vitamin D receptor in the duodenum or the kidney (in fact there may be a decrease in vitamin D receptor concentrations in the kidney following adaptation to a low calcium diet). This has been confirmed by Christakos and her colleagues who

Table 2. Mean (±SEM) plasma calcium, phosphorous, and 1,25(OH)2D3 and uncoupled vitamin D receptor (VDR) content of duodenum and kidney of rats receiving 36 ng 1,25(OH)2D3/day for 7 days and control rats (n = 6)

<table>
<thead>
<tr>
<th>Diet</th>
<th>Day</th>
<th>Plasma Ca (mg/dl)</th>
<th>Plasma phosphorous (mg/dl)</th>
<th>Plasma 1,25(OH)2D3 (pg/ml)</th>
<th>Duodenum VDR (fmol/mg protein)</th>
<th>Kidney VDR (fmol/mg protein)</th>
</tr>
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<tr>
<td>Control</td>
<td>2</td>
<td>10.78 ±0.11</td>
<td>8.23 ±0.27</td>
<td>53 ±6</td>
<td>351 ±16</td>
<td>60 ±5</td>
</tr>
<tr>
<td>1,25(OH)2D3-treated</td>
<td>2</td>
<td>13.40 ±0.17**</td>
<td>7.75 ±0.24</td>
<td>261 ±0.17**</td>
<td>510 ±21**</td>
<td>194 ±23**</td>
</tr>
</tbody>
</table>

**P<0.001.
Reproduced with permission from [60].

Table 3. Mean (±SEM) unoccupied vitamin D receptor content (expressed as femtomoles of 1,25(OH)2D3[3H] bound per mg cytosol protein) of duodenal and renal tissue from rats fed a 1% or 0.02% calcium diet after 2, 7, 14, and 21 days of dietary treatment

<table>
<thead>
<tr>
<th>Diet</th>
<th>Day</th>
<th>Duodenum 1% calcium</th>
<th>Duodenum 0.02% calcium</th>
<th>Kidney 1% calcium</th>
<th>Kidney 0.02% calcium</th>
</tr>
</thead>
<tbody>
<tr>
<td>1% calcium</td>
<td>2</td>
<td>341 ±26</td>
<td>365 ±27</td>
<td>ND**</td>
<td>ND**</td>
</tr>
<tr>
<td>0.02% calcium</td>
<td>21</td>
<td>259 ±26</td>
<td>267 ±28</td>
<td>163 ±11</td>
<td>124 ±8</td>
</tr>
</tbody>
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*P<0.05; **P<0.001.
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Abnormalities in the vitamin D receptor in uraemia

Table 4. Mean (±SEM) plasma calcium, phosphorous, and 1,25(OH)2D3 and uncoupled vitamin D receptor (VDR) content of duodenum and kidney of rats receiving 36 ng 1,25(OH)2D3/day for 7 days and control rats (n = 6)

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**P<0.001.
Reproduced with permission from [60].
showed that the administration of 1,25(OH)₂D₃ resulted in an increase in rat intestinal calbindin-D₉k mRNA, but no increase in vitamin D receptor mRNA [61]. Slatopolsky and his colleagues have shown a lack of effect of 1,25(OH)₂D₃ on intestinal vitamin D receptor mRNA in animals on a low calcium diet, a normal calcium diet, or a high calcium diet [65]. It is important to recognize that vitamin D mRNA concentrations may not be equivalent to the amount of functional receptor present in a tissue as there might be ligand-induced stabilization of receptor protein resulting in an increased amount of receptor in that tissue following the administration of the ligand. Changes in the level of vitamin D receptor in the kidney in response to various dietary conditions and oral treatment with vitamin D or the administration of 1,25(OH)₂D₃ by mini-osmotic pump have been examined by DeLuca and his colleagues [62,63]. Weaning rats were given a vitamin D-deficient diet containing either low or normal amounts of calcium. Rats then either received no vitamin D₃, a supplement of vitamin D₃ orally three times per week, or 60 pmol 1,25(OH)₂D₃ per day by mini-osmotic pump. In the presence of normal dietary calcium, the administration of vitamin resulted in an increase in renal vitamin D receptor content. The administration of 1,25(OH)₂D₃ to animals administered a vitamin D-deficient 1.2% calcium diet also resulted in an increase in vitamin D receptor content. In contrast, in the presence of a low calcium diet neither vitamin D nor 1,25(OH)₂D₃ increased receptor content in the kidney. Christakos and others [61] examined mRNA concentrations for the 1,25(OH)₂D₃ receptor in kidneys of rats given 1,25(OH)₂D₃. They observed no change in mRNA for the vitamin D receptor in rats raised on a low calcium diet. Dietary phosphate also appears to have a stabilizing effect on 1,25(OH)₂D₃ receptor concentrations.

In bone cells, 1,25(OH)₂D₃ appears to increase vitamin D receptor mRNA levels. PTH at a dose of 1 nM and 10 nM also increases vitamin D receptor mRNA concentrations in bone cells [68]. The effect of 1,25(OH)₂D₃ on vitamin D receptors has also been examined in yeast cells. Here, the addition of 1,25(OH)₂D₃ results in a 5-fold increase in the amount of the 1,25(OH)₂D₃ receptor 6 and 8 h after the administration of ligand [70]. In fibroblast-like cells maintained in culture the addition of 1,25(OH)₂D₃ at a concentration of 10 nM results in an increase in the amount of vitamin D receptor protein in these cells approximately 2-4 h after the administration of ligand [71]. On the other hand, mRNA concentrations are not substantially increased in these cells, suggesting that the ligand has a predominant effect on the stabilization of pre-existing vitamin D receptor protein as opposed to an increase in the synthesis of new protein via an increase in synthesis of mRNA.

In the parathyroid glands, 1,25(OH)₂D₃ causes a suppression of PTH pre-pro-mRNA content [64–66]. This is associated with a decrease in the secretion rate of PTH and the effect appears to be reversible. The effect can be demonstrated both in vitro as well as in vivo. The effects of varying dietary calcium levels alone on vitamin D receptor mRNA in the parathyroid gland of vitamin D₃-deficient chicks has been examined [64]. The administration of diets containing greater amounts of calcium results in an increase in the mRNA for the vitamin D receptor. Examination of the interrelationships between 1,25(OH)₂D₃ administration and dietary calcium shows that the stimulatory effect of 1,25(OH)₂D₃ on vitamin D receptor mRNA content only occurs when animals are fed diets high in calcium.

In summary, vitamin D receptor concentrations are increased in various tissues by 1,25(OH)₂D₃ and by calcium. It appears that the effect of 1,25(OH)₂D₃ is most marked when adequate amounts of calcium are present.

Vitamin D receptor in uraemia

Several investigators have examined basal concentrations of vitamin D receptor in parathyroid glands of uraemic animals and humans [72,73]. Basal levels of vitamin D receptors are reduced in these glands in the presence of uraemia. Basal levels of the vitamin D receptor in the intestine of uraemic animals are not altered. Korkor examined the amount of vitamin D receptor present in the parathyroid glands of patients with chronic renal failure, post-kidney transplantation, and in primary hyperparathyroidism [72]. The amount of receptor present in the glands of patients with chronic renal failure was substantially less than that in other states. These results have been confirmed by studies in uraemic animals [73]. Koyama et al. have examined the amount of vitamin D receptor protein present in duodenal cells of uraemic rats [74]. The amount of vitamin D receptor protein appears to be reduced. The administration of 1,25(OH)₂D₃ to such animals is associated with no change in vitamin D receptor protein but an increase in vitamin D receptor mRNA.

In addition to observations concerning the amount of receptor present in various tissues, Patel and others have shown that uraemic ultrafiltrates contain a factor that inhibits the interaction of vitamin D receptors with DNA cellulose [75,76]. They have also shown that vitamin D receptor extracted from rats with renal failure shows impaired binding to a vitamin D regulatory element [77]. They have shown that this effect is recapitulated by the incubation of normal vitamin D receptor with an uraemic plasma ultrafiltrate.

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