COMMENTARY

1α-Hydroxylase and the action of vitamin D

M Hewison, D Zehnder, R Bland and **P M Stewart**

Division of Medical Sciences, University of Birmingham, Birmingham B15 2TH, UK

(Requests for offprints should be addressed to M Hewison, Division of Medical Sciences, Queen Elizabeth Hospital, Birmingham B15 2TH, UK; Email: M.Hewison@bham.ac.uk)

ABSTRACT

The active form of vitamin D, 1,25-dihydroxyvitamin D_3 ($1,25(OH)_2D_3$), is a pleiotropic hormone whose actions include the regulation of calcium homeostasis, control of bone cell differentiation and modification of immune responses. Synthesis of $1,25(OH)_2D_3$ from the major circulating metabolite, 25-hydroxyvitamin D_3 ($25(OH)D_3$), is catalysed by a mitochondrial cytochrome P450 enzyme, 25-hydroxyvitamin D-1α-hydroxylase (1α-OHase). Although 1α-OHase is expressed predominantly in the kidney, extra-renal production of $1,25(OH)_2D_3$ has also been demonstrated in tissues such as lymph nodes and skin. The tight regulation of 1α-OHase which occurs in both renal and peripheral tissues has made studies of the expression and regulation of this enzyme remarkably difficult. However, the recent cloning of mouse, rat and human cDNAs for 1α-OHase (CYP1α/Cyp1α) has enabled a more thorough characterization of this enzyme. In particular, analysis of the CYP1α gene has identified mutations causing the inherited disorder vitamin D-dependent rickets type 1, also known as pseudo-vitamin D deficiency rickets. Studies from our own group have focused on the distribution of 1α-OHase in both renal and extra-renal tissues. Data indicate that the enzyme is expressed throughout the nephron, suggesting discrete endocrine and paracrine/autocrine functions. Further immunohistochemical analyses have shown that the enzyme is widely distributed in extra-renal tissues, and this appears to be due to the same gene product as the kidney. Collectively, these observations have raised important new questions concerning the role of 1α-OHase in vitamin D signalling at a local level. The relationship between expression of protein for 1α-OHase and enzyme activity has yet to be fully characterized and may be dependent on membrane proteins such as megalin. Similarly, elucidation of the mechanisms involved in differential regulation of renal and extra-renal $1,25(OH)_2D_3$ production will be essential to our understanding of the tissue-specific functions of 1α-OHase. These and other issues are discussed in the current review.
Journal of Molecular Endocrinology (2000) **25,** 141–148

INTRODUCTION

Vitamin D is a seco-steroid whose actions are dependent on selective metabolic steps catalysed by cytochrome P450 enzymes. The first of these steps occurs in the liver and involves the enzyme 25-hydroxylase, which catalyses synthesis of 25-hydroxyvitamin D_3 ($25(OH)D_3$), the major circulating form of vitamin D. The $25(OH)D_3$ molecule then acts as the substrate for other hydroxylase enzymes, the most important of these being 25-hydroxyvitamin D_3-1α-hydroxylase (1α-hydroxylase, 1α-OHase) and vitamin D_3-24-hydroxylase (24-OHase). Although both of these enzymes are strongly expressed in the kidney they have also been detected in a variety of extra-renal tissues. The function of 24-OHase has yet to be fully determined. On the one hand, the enzyme may function as a negative regulatory enzyme by synthesizing relatively inactive vitamin D metabolites such as 1,24,25-trihydroxyvitamin D_3 (1,24, $25(OH)_2D_3$). Alternatively, recent studies have suggested that, in specific tissues, 24-OHase plays a more active role by generating local concentrations

This commentary was presented at the Symposium on Enzymes and Steroid Hormone action at the 19th Joint Meeting of the British Endocrine Societies with EFES, March 2000, Birmingham, UK.

0952–5041/00/025–141

of the metabolite 24,25-dihydroxyvitamin D_3 ($24,25(OH)_2D_3$) (St-Arnaud & Glorieux 1998). The function of 1α-OHase is more specific in that the enzyme catalyses the production of active, hormonal 1,25-dihydroxyvitamin D_3 ($1,25(OH)_2D_3$) (Henry 1997). Classically, $1,25(OH)_2D_3$ plays a pivotal role in maintaining serum calcium homeostasis by modulating calcium/phosphate absorption, and parathyroid hormone (PTH) secretion (Reichel *et al*. 1989). More recently, $1,25(OH)_2D_3$ has also been shown to act as a potent stimulator of cell differentiation, influencing the development of bone and immune cell lineages (Hewison & O'Riordan 1997). The actions of $1,25(OH)_2D_3$ are mediated primarily through interaction with the intracellular vitamin D receptor (VDR) (Haussler *et al*. 1998), although several recent reports have highlighted rapid non-genomic effects of $1,25(OH)_2D_3$, particularly in cells such as growth-plate chondrocytes (Sylvia *et al*. 1998). Much attention has focused on the role of membrane and nuclear receptors as mediators of both classical and non-classical actions of $1,25(OH)_2D_3$. However, it is important to recognize that the efficacy of these signalling pathways is largely dependent on the availability of active $1,25(OH)_2D_3$ and its subsequent metabolism. In particular, characterization of the expression and regulation of 1α-OHase at both renal and extra-renal sites has provided new insights into the function of $1,25(OH)_2D_3$.

The 1α-OHase gene

Studies of the mechanisms involved in regulating $1,25(OH)_2D_3$ availability at both a local and a systemic level have been greatly facilitated by the recent cloning of mouse, rat and human cDNA and genomic clones for 1α-OHase (CYP1α or Cyp1α). The first of these was reported by Takeyama *et al*. (1997), who used a VDR knockout mouse model to isolate a candidate cDNA. The absence of functional VDR in the knockout mouse results in the loss of feedback control of renal $1,25(OH)_2D_3$ production, and leads to constitutive over-expression of 1α-OHase. Using this model, a candidate 2·5 kb cDNA was isolated which corresponded to a 507-amino-acid cytochrome P450-like protein, with a predicted size of 55 kDa. The mouse 1α-OHase protein was shown to be homologous to other members of the cytochrome P450 family and, as such, has several key regions of amino acids. The mitochondrial target sequence (amino acids 13–18) showed 41·7% homology with rat 25-hydroxylase (Cyp27) and 31·6% homology with mouse 24-OHase (Cyp24). The sterol-binding domain (amino acids 367–382) was 93% homologous with rat Cyp27 and 60% with mouse Cyp24. However, the region with greatest overall homology was the haem-binding domain (70% with rat, 80% with mouse). Subsequent cloning of a human CYP1α cDNA was achieved using mRNA from a non-classical source of the enzyme. Fu *et al*. (1997) used cultured human keratinocytes, which have previously been shown to synthesize $1,25(OH)_2D_3$ (Pillai *et al*. 1988). Cloning of a candidate CYP1α cDNA was achieved using PCR degenerate primers corresponding to the haem-binding domains of human CYP24 and CYP27. A full-length cDNA of 2·4 kb was shown to encode a 508-amino-acid protein with a predicted size of 56 kDa. Overall sequence identity to related human cytochrome P450 proteins ranged from 39% (CYP27) to 33% (11β-hydroxylase). Characterization of the CYP1α gene, which spans approximately 6 kb, consists of 9 exons and has approximately 500 bp of 5′ untranslated mRNA, has also highlighted the homology between CYP1α and its CYP27 counterpart (Monkawa *et al*. 1997). Both of these genes have the same number of exons and also have indentical intronic insertions.

In parallel with the original cloning of the human gene, St-Arnaud *et al*. (1997) isolated the cDNA for rat Cyp1α, which has 82·5% identity to the human cDNA. This report also confirmed the location of the human CYP1α gene on chromosome 12q13·1-q13·3, providing further evidence that abnormal CYP1α gene expression is the cause of hereditary pseudovitamin D-deficiency rickets (PDDR). Also known as vitamin D-dependent rickets type 1 (VDDR 1), PDDR is an autosomal recessive disorder characterized by low serum calcium, secondary hyperparathyroidism and low circulating levels of $1,25(OH)_2D_3$. The disease locus for PDDR had been mapped previously to 12q13-q14, but the characterization of a specific genetic defect associated with the disorder only became possible following the cloning of the CYP1α gene. The first description of a mutation in the CYP1α gene associated with defective CYP1α activity was carried out using mRNA isolated using cultured keratinocytes from a PDDR patient (Fu *et al*. 1997). The absence of CYP1α activity in these cells was associated with deletion/frameshift mutations at codons 211 or 231, indicating that the patient was a compound heterozygote for two null mutations. Subsequent to this study, several other reports have been published which have documented families with mutations in the CYP1α gene (Kitanaka *et al*. 1998, Wang *et al*. 1998, Yoshida *et al*. 1998).

Most notable amongst these studies is a report by Mawer and colleagues, who used activated monocytes from PDDR patients to characterize abnormalities in $1,25(OH)_2D_3$ production (Smith

et al. 1999). This study identified a further two unrelated families with the 7 bp insertion in exon 8. In particular, the authors noted that synthesis of $1,25(OH)_2D_3$ in activated macrophages from PDDR patients was lower than that in controls, and the CYP1α mutations were detectable using cDNA from these cells. These data provide further evidence of a common genetic origin for 1α-OHase activity at both renal and extra-renal sites.

Renal distribution of 1α-OHase

The availability of sequence information for CYP1α has also facilitated the development of new molecular tools for further analysis of the expression and regulation of $1,25(OH)_2D_3$ synthesis by both renal and non-renal cells. Specifically, we have developed polyclonal antisera to both human and mouse 1α-OHase which have allowed us to document, for the first time, the precise distribution of this enzyme in the kidney and other tissues. Data using normal human kidneys confirmed the expression of mRNA and protein for 1α-OHase in proximal tubules (Zehnder *et al*. 1999). However, protein and mRNA were also expressed in distal tubules and in collecting ducts. The specificity of 1α-OHase expression in the kidney was emphasized by stringent controls for both the *in situ* hybridization and the immunohistochemistry analyses. The other key sites of 1α-OHase expression along the nephron were the medullary collecting ducts and papillary epithelium. RT-PCR, Western blots and enzyme-activity studies using primary cell culture confirmed the presence of 1α-OHase in human cortical and medullary renal tissue, with mRNA and protein corresponding to the size of previously reported species for the enzyme.

Although previous studies of the renal function of vitamin D have focused primarily on the production and function of $1,25(OH)_2D_3$ in proximal tubules, there is increasing evidence of a role for this hormone in more distal parts of the nephron. In view of studies with vitamin D deficient animals, it seems likely that production of $1,25(OH)_2D_3$ in the proximal tubules acts in an endocrine fashion to support circulating levels of $1,25(OH)_2D_3$, whereas more distal areas of the nephron fulfil an autocrine or paracrine function. Previous studies have shown that $1,25(OH)_2D_3$, as well as calcitonin and PTH, stimulate calcium absorption in the distal nephron (Bouhtiauy *et al*. 1993, Friedman & Gesek 1993, You *et al*. 1997, Hoenderop *et al*. 1999). It is also important to recognize that in some cases the impact of $1,25(OH)_2D_3$ on renal function may occur through indirect mechanisms. In particular, the observation that the calcium-sensing receptor is primarily regulated by $1,25(OH)_2D_3$, and not PTH or calcium, suggests that this may be the key target for local 1α-OHase activity in the distal nephron (Brown *et al*. 1996).

Extra-renal expression of 1α-OHase

The original description of extra-renal 1α-OHase expression was based on studies of the granulomatous disease sarcoidosis, which frequently presents with associated hypercalcaemia (Papapoulos *et al*. 1979, Barbour *et al*. 1981). Enzyme-activity analyses using lymph-node homogenates and pulmonary alveolar macrophages from patients with sarcoidosis showed high levels of 1α-OHase activity (Adams *et al*. 1983, Adams & Gacad 1985, Reichel *et al*. 1987). Furthermore, addition of exogenous $1,25(OH)_2D_3$ did not appear to inhibit macrophage 1α-OHase as is classically observed with its renal counterpart. This would explain the apparently unregulated synthesis of $1,25(OH)_2D_3$ which is characteristic of the more severe forms of this disease, but also suggested that the expression and regulation of 1α-OHase in extra-renal tissues was different from that observed with the kidney enzyme. Prior to the cloning of the 1α-OHase gene it seemed likely that extra-renal production of $1,25(OH)_2D_3$ was due to a separate enzyme. However, as illustrated above, it now appears that renal and extra-renal 1α-OHase activity is due to a single gene product. On the basis of these observations, we have used immunohistochemistry and Western analyses with renal 1α-OHase antisera to characterize the extra-renal distribution of the enzyme in human tissues (Zehnder *et al*. 2000). As would be predicted from previous *in vitro* studies, 1α-OHase was detectable in tissues such as normal skin (stratum basalis) and sarcoid lymph nodes. In the latter, staining for 1α-OHase was coincident with expression of the cell-surface antigen CD68, which is a macrophage marker. 1α-OHase was also highly expressed in skin from sarcoid patients. In this case, the enzyme was observed predominantly in inflammatory infiltrates, with expression in the stratum basalis remaining normal. In contrast, analysis of psoriatic skin indicated that 1α-OHase was expressed in keratinocytes throughout the dysregulated stratum spynosum. This observation is somewhat paradoxical in view of the fact that current therapy for psoriasis includes the use of $1,25(OH)_2D_3$ analogues as antiproliferative agents (Bikle 1995). Immunohistochemistry also confirmed previous enzyme-activity studies which indicated that 1α-OHase is expressed in decidual cells (Weisman *et al*. 1979, Glorieux *et al*. 1995). However, the enzyme was also detectable in

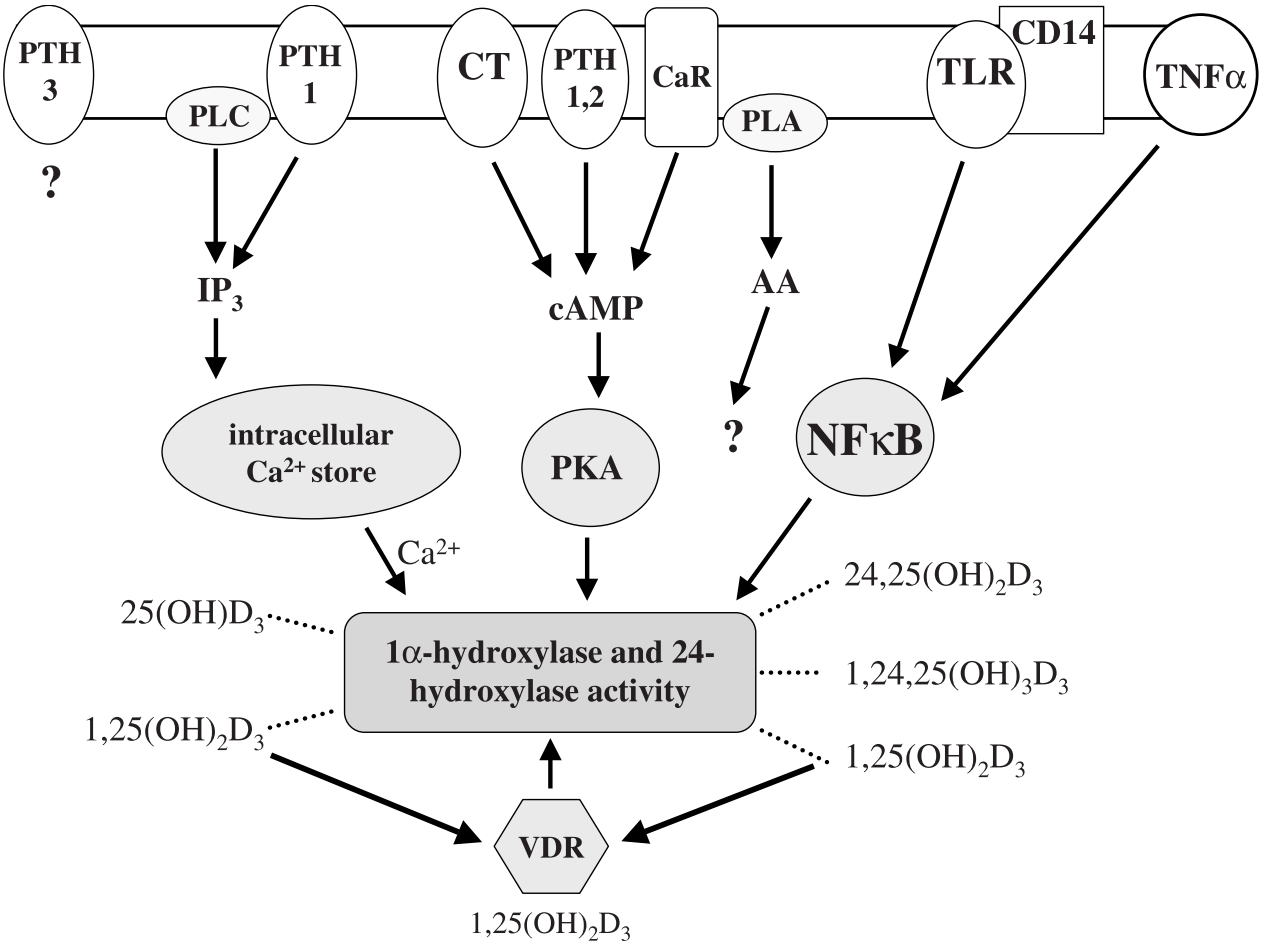

FIGURE 1. Schematic representation of the possible mechanisms involved in the regulation of 1α-OHase activity. Membrane receptors include the following: parathyroid hormone (PTH) receptors 1, 2 and 3; the calcitonin (CT) receptor; the calcium (Ca^{2+})-sensing receptor; the toll-like receptor (TLR); the lipopolysaccharide receptor (CD14); the tumour necrosis factor-α (TNFα) receptor. Associated kinase activity includes phospholipase C (PLC), phospholipase A (PLA) and phosphokinase A (PKA). Second messengers include cyclic AMP (cAMP), calcium (Ca^{2+}) and nuclear factor κ B (NFκB).

trophoblasts and syncytiotrophoblasts, suggesting potentially diverse functions for 1α-OHase in placentation and feto–placental calcium homeostasis (Kovacs & Kronenberg 1997). Novel sites for 1α-OHase expression included the parathyroids, pancreas, adrenal medulla, colon and cerebellum, with negative tissues including the heart, liver and adrenal cortex. In all cases, Western blot analyses suggested that expression of 1α-OHase was due to the reported protein species.

The regulation of vitamin D metabolism

The apparent widespread distribution of protein and mRNA for 1α-OHase in both renal and extra-renal tissues has raised important questions concerning the local enzyme activity at these sites. The relationship between expression of 1α-OHase and actual synthesis of $1,25(OH)_2D_3$ in a particular tissue probably involves two specific mechanisms, the first of these being substrate access, and the second being auto-regulation of 1α-OHase activity by $1,25(OH)_2D_3$ itself. The former questions the assumption that, in common with other steroid hormones, $1,25(OH)_2D_3$ enters cells by a passive mechanism by virtue of its lipophilic nature. The latter raises the possibility that local 1α-OHase activity in extra-renal tissues is under even tighter control than that observed with the endocrine enzyme and, thus, local production of $1,25(OH)_2D_3$ *in vivo* may be difficult to detect.

Circulating vitamin D metabolites can bind to a variety of serum proteins, but by far the most important of these is the vitamin D-binding protein (DBP), which is synthesized in the liver. Previous studies *in vitro* have suggested that DBP-bound vitamin D metabolites have limited access to target cells and, as such, the free forms of vitamin D metabolites, with greater apparent accessibility to target cells, are more biologically active (Bikle & Gee 1989). However, analysis of the DBP null mouse indicated that these animals were less susceptible than the wild type to vitamin D-induced hypercalcaemia (Safadi *et al*. 1999). The DBP null mice also developed vitamin D deficiency much earlier than their normal litter-mates. Taken together, these findings suggest that, in addition to its function as a transport protein, DBP may play an active role in directing vitamin D responses. In particular, because of its relatively high capacity for binding $25(OH)D_3$, DBP is likely to be a key determinant of the availability of substrate to 1α-OHase. Recent studies have shown that DBP and DBP-bound vitamin D metabolites are filtered through the glomerulus and reabsorbed by the luminal endocytic receptor megalin (gp330) in the proximal tubules (Nykjaer *et al*. 1999). Megalin belongs to the low-density-lipoprotein receptor gene family (Saito *et al*. 1994) and is expressed in a variety of tissues (Lundgren *et al*. 1997). The precise physiological role of this receptor has yet to be fully clarified, but, importantly, megalin-null mice have high urinary excretion of $25(OH)D_3$ and DBP with associated bone disease (Nykjaer *et al*. 1999). Megalin has also been shown to be located in the brush border membrane of the proximal tubules, whereas cells of the distal nephron appear to be megalin-negative (Lundgren *et al*. 1997). Thus, megalin-mediated endocytosis of DBP-bound $25(OH)D_3$ may act as an additional mechanism controlling tissue-specific synthesis of $1,25(OH)_2D_3$ by modulating the availability of substrate to the 1α-OHase protein. This may provide a partial explanation for the discrepancy between widespread 1α-OHase protein expression along the nephron and more discrete patterns of actual enzyme activity *in vivo*.

Previous studies have shown that, during vitamin D sufficiency, $1,25(OH)_2D_3$ production by the kidneys is very tightly regulated, but there is a striking up-regulation of 1α-OHase activity in proximal tubule cells in vitamin D-deficient states (Brunette 1977, Kawashima *et al*. 1981). This response appears to be a function of several direct and indirect mechanisms, including changes in accessory proteins such as ferrodoxin, or alterations in VDR or 24-OHase expression. Studies *in vivo* suggest that the key activator of 1α-OHase is a PTH and that this effect is mediated, at least in part, by target-cell induction of cAMP (Henry & Luntao 1989) (see Figure 1). More recent reports have highlighted potential cAMP response elements in downstream areas (–1·4 kb) which are PTH-responsive in promoter–reporter assays (Brenza *et al*. 1998, Kong *et al*. 1999). In both of these studies, the authors were unable to show any self-regulation of basal CYP1α promoter activity, and no vitamin D response elements (VDREs) were identified in the 1·4 kb fragment. However, in each case $1,25(OH)_2D_3$ was able to suppress PTH-induced transactivation. This suggests either that the CYP1α gene promoter has an atypical VDRE, or that $1,25(OH)_2D_3$ achieves its effects by an indirect mechanism. These reports contrast with analysis of the murine promoter, which demonstrated both positive (PTH) and negative ($1,25(OH)_2D_3$) responsiveness in a region downstream of –0·9 kb (Murayama *et al*. 1998). In this study, calcitonin was shown to be a potent stimulator of 1α-OHase expression, supporting previous reports in which calcitonin was shown to stimulate 1α-OHase mRNA and activity under normocalcaemic conditions (Shinki *et al*. 1999). This suggests that calcitonin, acting via distal areas of the nephron, may play an important role in the 'fine-tuning' of serum $1,25(OH)_2D_3$ levels during vitamin D sufficiency.

Amongst the most prominent inhibitors of 1α-OHase are calcium, phosphate and $1,25(OH)_2D_3$ itself, the latter also stimulating an increase in 24-OHase activity (Murayama *et al*. 1999). It seems likely that many of these effects are mediated indirectly through modulation of PTH production and secretion. However, as a consequence of the tight regulation of $1,25(OH)_2D_3$ production, analysis of the precise mechanisms involved in controlling 1α-OHase has proved difficult. In recent studies using a transformed human proximal tubule cell line, HKC-8, we confirmed the cAMP-mediated up-regulation of 1α-OHase expression as well as inhibition of expression by $1,25(OH)_2D_3$ (Bland *et al*. 1999). However, we also noted that the most potent and rapid modulation of 1α-OHase expression and activity occurred following changes in extra-cellular calcium. Relatively high levels of calcium (2 mM versus 1 mM) reduced the synthesis of $1,25(OH)_2D_3$, whereas relatively low levels (0·5 mM versus 1 mM) increased the enzyme activity. These responses occurred within 4 h but were transient, with activities returning to normal at 24 h. These observations, coupled with the widespread expression of calcium-sensing receptors along the nephron, suggest that changes in

local calcium sensing may act as a major determinant of tissue-specific $1,25(OH)_2D_3$ production (Figure 1).

Another approach to the *in vitro* analysis of 1α-OHase has been to use preparations of keratinocytes or activate macrophages as a source of 1α-OHase activity. The main difficulty associated with these model systems is that current evidence suggests that there are substantial differences between the regulation of 1α-OHase in the kidney and that in extra-renal sites. For example, the synthesis of $1,25(OH)_2D_3$ by activated macrophages is not inhibited by $1,25(OH)_2D_3$, and this appears to be the basis for the unregulated 1α-OHase activity associated with granulomatous diseases such as sarcoidosis, and which frequently leads to hypercalcaemia in these patients (Papapoulos *et al*. 1979, Barbour *et al*. 1981, Adams *et al*. 1983, Adams & Gacad 1985, Reichel *et al*. 1987). This is difficult to explain, particularly in view of recent studies which suggest that renal and extra-renal 1α-OHase are due to the same gene product. Macrophage-like cells are known to express VDR, and several groups (including ourselves) have demonstrated functional responses to $1,25(OH)_2D_3$ in these cells (Hewison & O'Riordan 1997). Therefore, the most likely explanation is that induction of extra-renal 1α-OHase involves regulatory pathways that differ from renal, cAMP-mediated mechanisms and are less sensitive to autoregulation by $1,25(OH)_2D_3$. Induction of extra-renal 1α-OHase frequently involves antigenic activators such as lipopolysaccharide or inflammatory mediators such as interferon-γ. Because these agents signal via nuclear factor κB we can postulate that this pathway activates 1α-OHase in a manner unlike that of calciotrophic factors and, as a consequence, shows differential sensitivity to feedback control by $1,25(OH)_2D_3$. Further analysis of the signal-transduction pathways involved in regulating 1α-OHase will be crucial to our understanding of the way in which $1,25(OH)_2D_3$ functions in extra-renal tissues (see Figure 1).

Summary

In recent years, our understanding of the molecular endocrinology of vitamin D metabolism has increased dramatically. Mutations in the CYP1α gene have been shown to be associated with rare inherited disorders of $1,25(OH)_2D_3$ production; current data suggest that a single gene product is responsible for renal and extra-renal 1α-OHase expression. Analysis of the renal and extra-renal expression of 1α-OHase has provided the first definitive picture of the tissue distribution of 1α-OHase protein. Data indicate that the enzyme is expressed throughout the kidney, suggesting novel roles for local synthesis of $1,25(OH)_2D_3$ (particularly in the distal nephron). Extra-renal studies have confirmed the presence of 1α-OHase in tissues such as the skin and lymph nodes but also highlight novel sites such as the pancreas and the colon. Future analysis of the mechanisms involved in the production of $1,25(OH)_2D_3$ will provide a clearer picture of the importance of 1α-OHase as a modulator of both renal and extra-renal tissue function.

ACKNOWLEDGEMENTS

Studies described in this review are supported by the Medical Research Council (P M S) and the National Kidney Research Fund (R B).

REFERENCES

Adams JS & Gacad MA 1985 Characterization of 1-alpha-hydroxylation of vitamin D_3 sterols by cultured alveolar macrophages from patients with sarcoidosis. *Journal of Experimental Medicine* **161** 755–765.

Adams JS, Sharma OP, Gacad MA & Singer FR 1983 Metabolism of 25-hydroxyvitamin D_3 by cultured pulmonary alveolar macrophages. *Journal of Clinical Investigation* **72** 1856–1860.

Barbour GL, Coburn JW, Slatopolsky E, Norman AW & Horst RL 1981 Hypercalcemia in an anephric patient with sarcoidosis: evidence for extra-renal generation of 1,25-dihydroxyvitamin D. *New England Journal of Medicine* **305** 440–443.

Bikle DD 1995 $1,25(OH)_2D_3$-regulated human keratinocyte proliferation and differentiation – basic studies and their clinical application. *Journal of Nutrition* **125** s1709–s1714.

Bikle DD & Gee E 1989 Free and not total 1,25-dihydroxyvitamin D regulates 25-dihydroxyvitamin D metabolism by keratinocytes. *Endocrinology* **124** 649–654.

Bland R, Walker E, Hughes SV, Stewart PM & Hewison M 1999 Constitutive expression of 25-hydroxyvitamin D-1α-hydroxylase in a transformed human proximal tubule cell line: evidence for direct regulation of vitamin D metabolism by calcium. *Endocrinology* **140** 2027–2034.

Bouhtiauy I, Lajeunesse D & Brunette MG 1993 Effect of vitamin D depletion on calcium transport by the luminal and basolateral membranes of the proximal and distal nephrons. *Endocrinology* **132** 115–120.

Brenza HL, Kimmel-Jehan C, Jehan F, Shinki T, Wakino S, Anazawa H *et al*. 1998 Parathyroid hormone activation of the 25-hydroxyvitamin D_3-1 alpha-hydroxylase gene promoter. *PNAS* **95** 1387–1391.

Brown AJ, Zhong M, Finch J, Ritter C, McCracken R, Morrissey J & Slatapolsky E 1996 Rat calcium-sensing is regulated by vitamin D but not calcium. *American Journal of Physiology* **270** F454–F460.

Brunette MG, Chan M, Ferriere C, Roberts KD 1978 Site of $1,25(OH)_2$ vitamin D_3 synthesis in the kidney. *Nature* **276** 287–289.

Friedman P & Gesek FA 1993 Vitamin D_3 accelerates PTH-dependent calcium transport in distal convoluted tubule cells. *American Journal of Physiology* **265** F300–F308.

Fu KG, Lin D, Zhang MYH, Bikle DD, Shackleton CHL, Miller WL & Portale AA 1997 Cloning of human 25-hydroxyvitamin D-1α-hydroxylase and mutations causing vitamin D-dependent Rickets Type I. *Molecular Endocrinology* **11** 1961–1970.

Glorieux FH, Arabian A, Devlin EE 1995 Pseudo-vitamin D deficiency: absence of 25-hydroxyvitamin D 1 alpha-hydroxylase in human placental decidual cells. *Journal of Clinical Endocrinology and Metabolism* **80** 2255–2258.

Haussler MR, Whitfield GK, Haussler CA, Hsieh JC, Thompson PD, Selznick SH, Dominguez CE & Jurutka PW 1998 The nuclear vitamin D receptor: biological and molecular regulatory properties revealed. *Journal of Bone and Mineral Research* **13** 325–349.

Henry HL 1997 The 25-hydroxyvitamin D 1α-hydroxylase. In *Vitamin D*, pp 57–68. Eds D Feldman, FH Glorieux & JW Pike. San Diego: Academic Press.

Henry HL & Luntao EM 1989 Interactions between intracellular signals involved in the regulation of 25-hydroxyvitamin D_3 metabolism. *Endocrinology* **124** 2228–2234.

Hewison M & O'Riordan JLH 1997 Immunomodulatory and cell differentiation effects of vitamin D. In *Vitamin D*, pp 447–462. Eds D Feldman, FH Glorieux & JW Pike. San Diego: Academic Press.

Hoenderop JGJ, DePont JHHM, Bindels RJM & Willems PHGM 1999 Hormone-stimulated Ca^{2+} reabsorption in rabbit kidney cortical collecting system is cAMP-independent and involves phorbol ester-insensitive PKC isotype. *Kidney International* **55** 225–233.

Kawashima H, Torikai S, Kurokawa K 1981 Localization of 25-hydroxyvitamin D_3 1α-hydroxylase and 24-hydroxylase along the rat nephron. *PNAS* **78** 1199–1203.

Kitanaka S, Takeyama K, Murayama A, Sato T, Okumura K, Nogami M, Hasegawa Y, Niimi H, Yanagisawa J, Tanaka T & Kato S 1998 Inactivating mutations in the 25-hydroxyvitamin D_3 1 alpha-hydroxylase gene in patients with pseudovitamin D-deficiency rickets. *New England Journal of Medicine* **338** 653–661.

Kong XF, Zhu XH, Pei YL, Jackson DM & Holick MF 1999 Molecular cloning, characterization, and promoter analysis of the human 25-hydroxyvitamin D_3-1 alpha-hydroxylase gene. *PNAS* **96** 6988–6993.

Kovacs CS & Kronenberg HM 1997 Maternal–fetal calcium and bone metabolism during pregnancy, puerperium and lactation. *Endocrine Reviews* **18** 832–872.

Lundgren S, Carling T, Hjalm G, Juhlin C, Rastad J, Pihlgren U, Rask L, Akerstrom G & Hellman P 1997 Tissue distribution of human gp330/megalin, a putative Ca^{2+} sensing protein. *Journal of Histochemistry and Cytochemistry* **45** 383–392.

Monkawa T, Yoshida T, Wakino S, Shinki T, Anazawa H, DeLuca HF, Suda T, Hayashi M & Saruta T 1997 Molecular cloning of cDNA and genomic DNA for human 25-hydroxyvitamin D3 1α-hydroxylase. *Biochemical and Biophysical Research Communications* **239** 527–533.

Murayama A, Takeyama K, Kitanaka S, Kodera Y, Hosoya T & Kato S 1998 The promoter of the human 25-hydroxyvitamin D_3 1 alpha-hydroxylase gene confers positive and negative responsiveness to PTH, calcitonin, and $1\alpha,25(OH)_2D_3$. *Biochemical and Biophysical Research Communications* **249** 11–16.

Murayama A, Takeyama K-I, Kitanaka S, Kodera Y, Kawaguchi Y, Hosoya T & Kato S 1999 Positive and negative regulation of the renal 25-hydroxyvitamin D_3 1α-hydroxylase gene by parathyroid hormone, calcitonin, and $1\alpha,25(OH)_2D_3$ in intact animals. *Endocrinology* **140** 2224–2231.

Nykjaer A, Dragun D, Walther D, Vorum H, Jacobsen C, Herz J, Melsen F, Christensen E & Willnow T 1999 An endocytic pathway essential for renal uptake and activation of the steroid 25(OH)vitamin D_3. *Cell* **96** 507–515.

Papapoulos SE, Clemens TL, Fraher LJ, Lewin IG, Sandler LM & O'Riordan JLH 1979 1,25-dihydroxycholecalciferol in the pathogenesis of the hypercalcaemia of sarcoidosis. *Lancet* **1** 627–630.

Pillai S, Bikle DD & Elias PM 1988 1,25-Dihydroxyvitamin D production and receptor-binding in human keratinocytes varies with differentiation. *Journal of Biological Chemistry* **263** 5390–5395.

Reichel H, Koeffler HP, Bishop JE & Norman AW 1987 25-hydroxyvitamin D_3 metabolism by lipopolysaccharide-stimulated normal human macrophages. *Journal of Clinical Endocrinology and Metabolism* **64** 1–9.

Reichel H, Koeffler HP & Norman AW 1989 The role of the vitamin D endocrine system in health and disease. *New England Journal of Medicine* **320** 980–991.

Safadi F, Thornton P, Magiera H, Hollis B, Gentile M, Haddad J, Liebhaber S & Cooke N 1999 Osteopathy and resistance to vitamin D toxicity in mice null for vitamin D binding protein. *Journal of Clinical Investigation* **103** 239–251.

St-Arnaud R & Glorieux FH 1998 24,25-Dihydroxyvitamin D – active metabolite or inactive catabolite. *Endocrinology* **139** 3371–3374.

St-Arnaud R, Messerlian S, Moir JM, Omdahl JL & Glorieux FH 1997 The 25-hydroxyvitamin D 1-alpha-hydroxylase gene maps to the pseudovitamin D deficiency rickets (PDDR) disease locus. *Journal of Bone and Mineral Research* **12** 1552–1559.

Saito A, Pietromanaco S, Lao AK-C & Farquhar MG 1994 Complete cloning and sequencing of rat gp330/'megalin', a distinctive member of the low density lipoprotein receptor gene family. *PNAS* **91** 9725–9729.

Shinki T, Ueno Y, DeLuca HF & Suda T 1999 Calcitonin is a major regulator for the expression of renal 25-hydroxyvitamin D_3 1α-hydroxylase gene in normocalcemic rats. *PNAS* **96** 8253–8258.

Smith SJ, Rucka AK, Berry JL, Davies M, Mylchreest S, Paterson CR, Heath DA, Tassabehji M, Read AP, Mee AP & Mawer EB 1999 Novel mutations in the 1 alpha-hydroxylase (P450 cl) gene in three families with pseudovitamin D-deficiency rickets resulting in loss of functional enzyme activity in blood-derived macrophages. *Journal of Bone and Mineral Research* **14** 730–739.

Sylvia VL, Schwartz Z, Curry DB, Chang Z, Dean DD & Boyan BD 1998 $1,25(OH)_2D_3$ regulates protein kinase C activity through two phospholipid-dependent pathways involving phospholipase A2 and phospholipase C in growth zone chondrocytes. *Journal of Bone and Mineral Research* **13** 559–569.

Takeyama K, Kitanaka S, Sato T, Kobori M, Yanagisawa J & Kato S 1997 25-Hydroxyvitamin D_3 1α-hydroxylase and vitamin D synthesis. *Science* **277** 1827–1830.

Wang JT, Lin CJ, Burridge SM, Fu GK, Labuda M, Portale AA & Miller WL 1998 Genetics of vitamin D 1 alpha-hydroxylase deficiency in 17 families. *American Journal of Human Genetics* **63** 1694–1702.

Weisman Y, Harell A, Edlestein S, David M, Spirer Z & Golander A 1979 1α,25-Dihydroxyvitamin D_3 and 24,25-dihydroxyvitamin D_3 *in vitro* synthesis by human decidua and placenta. *Nature* **281** 317–319.

Yoshida T, Monkawa T, Tenenhouse HS, Goodyer P, Shinki T, Suda T, Wakino S, Hayashi M & Saruta T 1998 Two novel 1α-hydroxylase mutations in French-Canadians with

vitamin D dependency rickets. *Kidney International* **54** 1437–1443.

You Q, Claveau D, Hilal G, Leclerc M & Brunette MG 1997 Effect of calcitonin on calcium transport by the luminal and basolateral membranes of the rabbit nephron. *Kidney International* **51** 1991–1999.

Zehnder D, Bland R, Walker EA, Bradwell AR, Howie AJ, Hewison M & Stewart PM 1999 Expression of 25-hydroxyvitamin D_3-1α-hydroxylase in the human kidney. *Journal of the American Society for Nephrology* **10** 2465–2473.

Zehnder D, Bland R, Stewart PM & Hewison M 2000 Analysis of the tissue distribution of 1α-hydroxylase identifies novel extra-renal sites for the synthesis of 1,25-dihydroxyvitamin D_3. *Journal of Endocrinology* **164** (S) P1.

RECEIVED 21 March 2000