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CHARACTERIZATION AND REGULATION OF THE VITAMIN D HYDROXYLASES

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Summary—The metabolism of vitamin D is regulated by three major cytochrome *P*450-containing hydroxylases—the hepatic 25-hydroxylase, the renal 1α-hydroxylase, and the renal and intestinal 24-hydroxylase. In the liver, the 25-hydroxylation reaction is catalyzed by microsomal and mitochondrial cytochromes *P*450cc25. The microsomal *P*450 accepts electrons from the NADPH–cytochrome *P*450 reductase, and the mitochondrial *P*450 accepts electrons from NADPH–ferredoxin reductase and ferredoxin. In the kidney, the 1α- and 24-hydroxylation reactions are catalyzed by mitochondrial cytochromes *P*450cc1α and *P*450cc24, respectively. The 24-hydroxylase is also found in vitamin D target tissues such as the intestine. The rat hepatic mitochondrial *P*450cc25 and the rat renal mitochondrial *P*450cc24 have been purified, and their cDNAs have been cloned and sequenced. 1,25-Dihydroxyvitamin D, the active metabolite of vitamin D, markedly stimulates renal *P*450cc24 mRNA and 24-hydroxylase activity in the intact animal and in renal cell lines. This stimulation occurs via a receptor-mediated mechanism requiring new protein synthesis. Despite the availability of a clone, no studies have yet been reported of the regulation of hepatic *P*450cc25 at the mRNA level. The study of one of the most important enzymes in vitamin D metabolism, the renal 1α-hydroxylase which produces the active metabolite, awaits the definitive cloning of the cDNA for the *P*450cc1α.

OUTLINE

1. INTRODUCTION

Vitamin D (or cholecalciferol as the vitamin D_3 form is called) is obtained in the diet or by the action of ultraviolet light on the skin to form vitamin D_3 from 7-dehydrocholesterol. Vitamin D is metabolized in the liver to form 25-hydroxyvitamin D [25(OH)D], and 25(OH)D is further metabolized by the kidney to form 1,25-dihydroxyvitamin D [1,25$(OH)_2$D] and other metabolites [1, 2]. 1,25$(OH)_2$D is the metabolite of vitamin D with the greatest biological activity in target organs. It acts on the intestine to stimulate absorption of dietary calcium and phosphorus, and it acts on bone in concert with parathyroid hormone (PTH) to stimulate

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resorption of calcium and phosphorus. Vitamin D metabolites also regulate differentiation in the immune system and skin.

In the liver, the 25-hydroxylation reaction is catalyzed by microsomal and mitochondrial cytochromes *P*450cc25. The microsomal *P*450 accepts electrons from the NADPH–cytochrome *P*450 reductase, and the mitochondrial *P*450 accepts electrons from NADPH–ferredoxin reductase and ferredoxin. In the kidney, 1α- and 24-hydroxylation reactions are catalyzed by cytochrome *P*450cc1α and *P*450cc24, respectively. The 24-hydroxylase (24-OHase) is also found in vitamin D target tissues such as the intestine. Other vitamin D hydroxylases, such as the 23-OHase [3] and the 26-OHase [4], have been described, but the physiological importance of these is not known.

2. REGULATION OF VITAMIN D METABOLISM *IN VIVO*

The major regulation of vitamin D metabolism occurs at the conversion of 25(OH)D to $1,25(OH)_2D$ by the 1-OHase [1, 2]. However, the production of 25(OH)D by the liver may also be regulated [5]. In animals, the renal $1,25(OH)_2D$ production has been shown to be regulated by many factors [6]. Using renal slices and cultured renal cells, *in vitro* experiments have shown that PTH [7, 8, 10] and low media calcium [8, 9] act directly on the renal cells to stimulate $1,25(OH)_2D$ production. On the other hand, $1,25(OH)_2D$ production is inhibited by high media calcium [8, 9], $1,25(OH)_2D$ itself [9], and phorbol esters [10, 11].

Degradation of $1,25(OH)_2D$ is also regulated in target tissues [12], in most cases by 24-hydroxylation of $1,25(OH)_2D$ by the 24-OHase. The 1,24,25-trihydroxyvitamin D has less biological activity [13]. This metabolite may be further degraded to $1,25(OH)_2D$-26,23-lactone and excreted. In the kidney, the 24-OHase may also function to divert 25(OH)D substrate from the 1-OHase by competing for it.

3. THE HEPATIC VITAMIN D–25-OHase

3.1. Characteristics of the 25-OHase

The major location of the 25-OHase is in the liver [1], although 25-OHase activity has also been reported in the kidney and intestine [14]. There are two distinct forms of *P*450cc25 in the liver, a mitochondrial form [15, 16] and a microsomal form [17–19]. The mitochondrial form is more abundant in the female liver, and the microsomal form predominates in the male liver [22]. Microsomal 25-OHase activity is very low in the adult female [19], and it may actually be a different molecular species [23].

The relative physiological importance of the two forms of the 25-OHase enzyme is not well known. The microsomal form may have less physiological importance since 25-OHase activity is almost completely absent from human liver microsomes [24] and from female rat liver microsomes [19]. In the rat a correlation was found between serum 25(OH)D levels and mitochondrial 25-OHase activity but not microsomal 25-OHase activity [25].

The degree to which the hepatic 25-OHase is hormonally regulated is not clear. There appears to be little regulation of the 25-OHase, and serum 25(OH)D levels seem to only reflect vitamin D availability. However, administration of $1,25(OH)_2D$ *in vivo* has been shown to lower serum 25(OH)D levels [26]. This may be due to increased metabolic clearance rather than to decreased production. In addition, a reduction of 25-OHase by $1,25(OH)_2D$ has also been demonstrated in perfused liver [27] and isolated hepatocytes [5], suggesting a direct effect of $1,25(OH)_2D$ on the liver. This effect of $1,25(OH)_2D$ on hepatocytes may be mediated by intracellular calcium [5, 28, 29]. Testosterone decreases hepatic mitochondrial 25-OHase in female rats. Estradiol increases the same activity in male rats [22]. Little change is seen in microsomal enzyme activity.

3.2. Purification and cloning of the P450cc25

*P*450cc25 (CYP27) has been purified from rat liver mitochondria [15, 16] and microsomes [17–19] and from pig kidney microsomes [20, 21]. The molecular weight and K_m values are listed in Table 1. It has greater activity with 1α-hydroxyvitamin D as a substrate [15]. The N-terminal sequences of the three *P*450cc25s are not homologous to each other or to *P*450cc24 or *P*450cc1α (Table 2). The rat liver microsomal *P*450cc25 does have homology with other male-specific microsomal *P*450s [19].

In reconstitution assays, the microsomal *P*450cc25 demonstrates activity only in the presence of NADPH–cytochrome *P*450 reductase [17–19]. The mitochondrial form requires NADPH–ferredoxin reductase and ferredoxin for activity [15, 16]. In addition to

Table 1. Characteristics of vitamin D hydroxylases

	Tissue fraction	M_W (Da)	K_m (μM)	References
*P*450cc25				
Pig	Renal microsomes	50,500	—	20,21
Rat	Liver mitochondria	52,000[a]	10	15,16
Rat	Liver microsomes	50–51,000	4.9	17–19
*P*450cclα				
Bovine	Renal mitochondria	49–52,000	0.69	52,53
Pig	Renal mitochondria	—	0.97	54
Chick	Renal mitochondria	57–59,000	—	55,57
*P*450cc24				
Bovine	Renal mitochondria	52,000	1.3	53
Pig	Renal mitochondria	—	5.4	3
Chick	Renal mitochondria	55–59,000	—	55,57
Rat	Renal mitochondria	53,000[b]	2.8	73

[a]cDNA sequence predicts M_W of 57,182 [33].
[b]cDNA sequence predicts M_W of 55,535 [34].

activity toward vitamin D metabolites, the rat liver mitochondrial *P*450cc25 also has 27-OHase activity toward 5β-cholestane-3α,7α,12α-triol as determined by expression in mammalian cells [30] and yeast [31]. The purified rat liver microsomal *P*450cc25 has 2α- and 16α-OHase activity toward testosterone, 16α-OHase activity toward dehydroepiandrosterone, and 25-OHase activity toward 5β-cholestane-3α,7α,12α-triol in reconstitution assays [17–19]. However, studies of the expressed *P*450 in yeast suggest that these other enzymatic activities may be due to different gene products [32].

Using antibodies against the purified protein, the cDNA for *P*450cc25 from rat liver mitochondria has been cloned and sequenced [33]. The isolated cDNA insert is 1900 base pairs long and contains an open reading frame coding for 533 amino acids. The primary structure contains a leader sequence characteristic of mitochondrial enzymes. The amino acid sequence is about 30% homologous to the sequence for rat renal mitochondrial *P*450cc24 [34], but it shares no homology with other known sequences.

3.3. *Regulation of P450cc25*

Despite the availability of antibodies to *P*450cc25 [17, 21, 33] and a cDNA probe [33], there have not been any studies of the regulation of this *P*450 at the molecular level. For example, it is not known if the observed effects of 1,25(OH)$_2$D and calcium on hepatic 25-OHase activity [5, 28, 29] involve changes in the expression of the *P*450cc25 itself or of the electron transfer proteins. Since regulation can be demonstrated in isolated hepatocytes, this would appear to be a useful experimental system in which to address these questions.

3.4. *The 25-OHase and disease*

In general, there has been no disease linked directly to an alteration in 25-OHase activity. With aging, there is a decline in serum 25(OH)D levels in rats [35] and in certain human populations [36]. This may contribute to a chronic vitamin D deficiency in some elderly persons.

4. THE RENAL 25-HYDROXYVITAMIN D-1-OHase

4.1. *Characteristics of the 1-OHase*

The major location of the 1-OHase is in the inner mitochondrial membrane of proximal convoluted tubule cells of the kidney [37]. The kidney is thought to be the major, if not

Table 2. N-Terminal sequences of vitamin D *P*450 components

*P*450cc	Species/tissue	N-Terminal sequence	Ref.
25	Pig kidney micro.	Gly-Leu-Leu-Thr-Gly-Leu-Leu-Leu-Gly-Ile-	21
25	Rat liver mito.	Ala-Ile-Pro-Ala-Ala-Leu-Arg-Asp-His-Glu-[a]	33
25	Rat liver micro.	Met-Asp-Leu-Pro-Val-Leu-Val-Leu-Val-	17
1α	Chick kidney mito.	Ala-X-Leu-Ala-Ala-Val-Asn-Thr-Pro-Ala-	56
1α	Chick kidney mito.	Thr-Leu-Glu-Arg-Pro-Ser-Gln-Val-Leu-Asp-	57
24	Chick kidney mito.	Ala-X-Leu-Ala-Ala-Val-Asn-Thr-Pro-Ala-	56
24	Chick kidney mito.	Thr-Leu-Glu-Leu-Pro-Ser-Gln-Val-Leu-Asp-	57
24	Rat kidney mito.	Arg-Ala-Pro-Lys-Glu-Val-Pro-Leu-Cys-Pro-[a]	34

[a]Verified by cDNA cloning.

the only, contributor to circulating serum $1,25(OH)_2D$ levels [1]. There have been reports of 1-OHase activity in placenta [38], bone [39], skin [40], and activated macrophages [41], but the physiological significance of this local $1,25(OH)_2D$ synthesis is unclear. Renal 1-OHase activity is regulated by a number of factors, including PTH, $1,25(OH)_2D$ itself, calcitonin, insulin, calcium and phosphorus, estrogens, and pituitary hormones [6]. The stimulation of 1-OHase activity by PTH and the inhibition of activity by $1,25(OH)_2D$ have been the most extensively studied. Feeding a low calcium diet or a vitamin D-deficient diet to rats markedly stimulates 1-OHase activity [42, 43]. This is probably mediated by increased serum PTH levels.

PTH markedly stimulates renal 1-OHase activity in young animals. This has been demonstrated by PTH administration to intact rats [44] and *in vitro* in renal slices [45], isolated tubules [46], and cultured renal cells [47, 48]. PTH has both short- and long-term actions on the renal 1-OHase enzyme. In short-term experiments, PTH has been shown to increase renal $1,25(OH)_2D$ production within 15 min. PTH acts rapidly by stimulating adenylate cyclase activity, increasing intracellular levels of cAMP, stimulating cAMP-dependent protein kinase activity, and phosphorylating specific proteins. An important target of PTH-mediated phosphorylation changes may be renal ferredoxin. The phosphorylation of ferredoxin is modulated by PTH in renal slices [49]. Reconstitution studies have shown a relationship between 1-OHase activity and the phosphorylation state of ferredoxin [50]. PTH also has long-term effects on the 1-OHase enzyme. In many *in vitro* systems [45, 47, 48], PTH takes 3–4 h to significantly stimulate renal $1,25(OH)_2D$ production. These chronic actions are inhibited by cycloheximide and actinomycin D, suggesting that new protein synthesis is involved [51].

The effect of $1,25(OH)_2D$ on inhibiting renal 1-OHase activity has also been demonstrated *in vivo* [43] and *in vitro* in slices[45] and cultured renal cells [47]. Inhibition takes several hours to occur, requires new protein synthesis, and appears to be mediated by the vitamin D receptor. Interestingly, $1,25(OH)_2D$ blocks the PTH-stimulated increase in 1-OHase activity when both are added simultaneously to renal slices [45].

4.2. *Purification of the P450cc1α*

There are reports of the purification or partial purification of the 1-OHase (*P*450cc1α) from cow [52, 53], pig [54], and chick [55, 57] (Table 1). The *P*450s associated with 1- and 24-OHase activity appear to comprise only a small fraction of the total mitochondrial *P*450 in the kidney [52, 54]. The purified *P*450cc1α from each species requires ferredoxin reductase and ferredoxin to exhibit enzymatic activity in reconstitution studies. The reconstituted enzyme preparations do not exhibit 24-OHase activity, indicating that 1- and 24-OHase are different enzymes. The molecular weight of the *P*450cc1α from mammals is significantly lower than that of the chick (Table 1). Monoclonal antibodies against chick renal hydroxylases do not cross-react with mitochondrial *P*450 preparations from rat and cow [57]. The cloning of a *P*450cc1α cDNA from any species has not yet been reported.

Monoclonal antibodies have been used to immuno-isolate chick kidney proteins which may be *P*450cc1α and *P*450cc24 [56, 57]. The first ten amino acids of the *P*450cc1α and *P*450cc24 amino terminals were found to be identical (Table 2). The amino acid composition of the two proteins were very similar [56]. In a different study, the *P*450cc1α and *P*450cc24 amino terminal sequences were found to differ in the 4th and 17th positions (Table 2), and the amino acid compositions were significantly different [57]. The amino terminal sequences of the chick *P*450cc1α proteins from these two studies showed no homology with each other (Table 2). The reason for this is not known, but it is possible that the chick kidney contains more than one form of *P*450cc1α [57].

4.3. *Regulation of the P450cc1α*

The regulation of *P*450cc1α has been studied only indirectly, since the cDNA has not yet been cloned. The effect of dietary calcium has been studied in pigs [54]. The 1-OHase activity of the renal mitochondrial *P*450 fraction isolated from pigs fed either a low calcium or normal calcium diet was almost identical. This is surprising since the serum $1,25(OH)_2D$ levels were twice as high in the pigs fed the low calcium diet, presumably in response to elevated serum PTH. These findings would suggest a post-translational regulation of the hydroxylase enzyme complex.

Two mechanisms which have been proposed for the post-translational regulation of the 1-

OHase are cleavage of the cytochrome *P*450 and phosphorylation of ferredoxin. In the chick, monoclonal antiboides against *P*450cc1α immunoprecipitate two proteins which differ only by 2000 Da [56]. This led to the suggestion that conversion from 1- to 24-OHase activity involves the cleavage of the C-terminal of the *P*450cc1α to produce the *P*450cc24 [58]. Phosphorylation of ferredoxin results in a decrease in 1-OHase activity and an increase in 24-OHase activity in a reconstituted system [49, 50]. Both of these post-translational mechanisms may work together to regulate renal hydroxylase activity [58].

4.4. The 1-OHase and disease

The activity of the 1-OHase has been observed to change in certain animal models of disease. In diabetic rats, renal 1-OHase activity is decreased compared to non-diabetic rats, and administration of insulin significantly increases 1-OHase activity [59]. In hypophosphatemic mice, renal 1-OHase activity is decreased compared to control animals with the same degree of hypophosphatemia [60]. 1-OHase activity decreases with age, as measured in both renal slices [61] and isolated renal mitochondria [62].

In addition to a change in 1-OHase activity, there are also changes in the hormonal regulation of the 1-OHase in certain diseases. The capacity of PTH to stimulate 1-OHase activity is diminished in diabetic [63], hypophosphatemic [64], and old [44] rodents. In the case of diabetic and old animals, this is not due to decreased PTH-stimulated adenylate cyclase or cAMP-dependent protein kinase activity [65, 66]. In diabetic animals, PTH does not alter the phosphorylation state of ferredoxin as it does in young animals [65]. Interestingly, calcitonin stimulates 1-OHase activity in diabetic [67], hypophosphatemic [68], and old [69] rodents. It is possible that calcitonin is acting on a different renal cell type or via a different (cAMP-independent) pathway to stimulate 1-OHase activity in these disease states.

5. THE RENAL AND INTESTINAL 25-HYDROXYVITAMIN D-24-OHase

5.1. Characteristics of the 24-OHase

Compared to the 1-OHase, the 24-OHase has a wide tissue distribution. It is found in kidney [42], intestine [70], lymphocytes[71], fibroblasts [72], bone [39], skin [40], macrophages [41], and other tissues which may be target tissues for $1,25(OH)_2D$ action. The renal 24-OHase is found on the inner mitochondrial membrane of the proximal convoluted tubule (like the 1-OHase) but also in the proximal straight tubule, which does not have 1-OHase activity [46]. An mRNA similar to the renal 24-OHase *P*450 is also expressed in the intestine [74].

In the kidney, the main hormonal regulators of the 24-OHase are PTH and $1,25(OH)_2D$. *In vivo* administration of PTH to thyroparathyroidectomized rats decreases renal $24,25(OH)_2D$ production, and administration of $1,25(OH)_2D$ increases $24,25(OH)_2D$ production. These effects have also been demonstrated *in vitro* in renal slices [45], isolated tubules [46] and renal cell lines [75]. This suggests that PTH and $1,25(OH)_2D$ act directly on renal cells to modulate $24,25(OH)_2D$ production. The mechanism of action of these hormones on the 24-OHase seems to be similar to their action on the 1-OHase. PTH acts through cAMP as a second messenger, and $1,25(OH)_2D$ action requires new protein synthesis. Feeding a low calcium diet or a vitamin D-deficient diet to rats decreases 24-OHase activity [42, 43]. As with 1-OHase regulation, this is probably the result of increased serum PTH levels.

In the intestine, the major regulator of the 24-OHase is $1,25(OH)_2D$. *In vivo* administration of $1,25(OH)_2D$ to rats increases intestinal 24-OHase activity. Preliminary studies suggest that PTH has no effect on intestinal 24-OHase activity [76], which is consistent with the fact that the intestine lacks PTH receptors. In intestinal cell lines, $1,25(OH)_2D$ also increases 24-OHase activity through a mechanism requiring new protein synthesis [77]. In the kidney, the affinity of the 24-OHase for $1,25(OH)_2D$ is about 10-fold greater than the affinity for 25(OH)D, suggesting that inactivation of $1,25(OH)_2D$ may be an important function of the enzyme [78].

5.2. Purification and cloning of the renal P450cc24

The renal *P*450cc24 has been purified from the bovine [53], pig [3], chick [55, 57], and rat [73] (Table 1). The mammalian *P*450cc24 has a lower molecular weight than the chick. *P*450cc24 was isolated from the kidneys of rats treated with vitamin D [73], and its cDNA has been cloned and sequenced [34]. In a reconstituted system, the purified *P*450cc24 hydroxylates 25(OH)D and $1,25(OH)_2D$ at the 24

position but not at the 1α position, indicating that the 24-OHase enzyme is distinct from the 1-OHase.

The cloned cDNA has an open reading frame of 1542 base pairs which encodes 514 amino acids [34]. The deduced amino acid sequence shows the highest homology (30%) to the rat hepatic mitochondrial *P*450cc24 [33]. When the cDNA is expressed in COS cells, only 24-OHase activity toward the 25(OH)D substrate is observed in the solubilized mitochondrial fractions [34]. The amino terminal sequence of the rat *P*450cc24 has no homology with the chick *P*450cc24 sequences (Table 2).

5.3. Regulation of the P450cc24

As with the 1-OHase enzyme, the molecular mechanism by which the 24-OHase enzyme is regulated is unknown. The enzyme activity may be regulated by the expression of the *P*450, by the post-translational modification of the *P*450 [58], or by phosphorylation/dephosphorylation of the renal ferredoxin [79].

Administration of a single dose of $1,25(OH)_2D$ to rats markedly increases the *P*450 mRNA in the kidney and intestine [74]. In the kidney, the mRNA is first increased at 3 h, peaks at 16 h, and is still elevated at 48 h after $1,25(OH)_2D$ injection. In the intestine, the response is similar in magnitude but more transient. The mRNA level peaks at 3 h and is undetectable by 16 h. *P*450cc24 mRNA is not induced by $1,25(OH)_2D$ in the liver, which does not express 24-OHase activity. The rapid induction of mRNA by $1,25(OH)_2D$ in kidney and intestine is consistent with the effect of $1,25(OH)_2D$ on 24-OHase activity. $1,25(OH)_2D$ increases renal and intestinal 24-OHase activity 6–17 h after administration to mice [80].

The induction of *P*450cc24 mRNA by $1,25(OH)_2D$ has been further studied in primary cultures of rat renal tubular cells (Armbrecht *et al.*, unpublished studies). Addition of $1,25(OH)_2D$ to the serum-free culture medium increased *P*450cc24 mRNA content about 25-fold over basal levels. A significant increase was seen at 2 h with the peak at 24 h and a return to near baseline by 48 h. This increase was blocked by actinomycin D and cycloheximide, indicating that new mRNA and protein synthesis is required for induction (Table 3). Of the other vitamin D metabolites tested, only 1,24,25-trihydroxyvitamin D significantly increased mRNA content, indicating the importance of the 1-hydroxyl group for biological activity (Table 3).

These studies are consistent with $1,25(OH)_2D$ acting through its intracellular receptor to increase mRNA levels via a mechanism requiring new protein synthesis. The rise in mRNA is rapid enough to account for the increase in 24-OHase activity after 3–6 h which has been reported in renal cell lines [75]. Thus, the changes in *P*450 mRNA can account for the reported changes in 24-OHase activity, although more systematic time and dose–response studies are needed to confirm this.

5.4. The 24-OHase and disease

Alterations in the activity and regulation of the 24-OHase have been observed in several disease states. In diabetic rats, renal 24-OHase activity is increased compared to non-diabetic rats, and the increased activity is reduced by treatment with insulin [59]. In hypophosphatemic mice, renal 24-OHase activity has been reported to be increased compared to control animals [81]. Finally, in old animals renal 24-OHase activity is increased compared to young animals [42]. Thus, in diabetes, hypophosphatemia, and aging, the renal 24-OHase activity increases while the 1-OHase activity decreases. This suggests that similar mechanisms may be regulating both hydroxylases.

The cDNA probe has been used to study the effect of diabetes on expression of the *P*450cc24 mRNA. Diabetes increases renal 24-OHase enzyme activity [59]. Diabetes also significantly increases the *P*450cc24 mRNA in the kidney (Table 4). Diabetes had no effect on the mRNA levels for ferredoxin and ferredoxin reductase

Table 3. Effect of vitamin D metabolites and inhibitors on *P*450cc24 mRNA levels in cultured renal cells

Metabolites	% Maximal response
Control	2 ± 1
$1,25(OH)_2D$	100 ± 2
$1,24,25(OH)_3D$	43 ± 6
$24,25(OH)_2D$	5 ± 1
25(OH)D	9 ± 2
Vitamin D	7 ± 1
Inhibitors	
Control	100 ± 7
Cycloheximide	7 ± 2
Actinomycin D	7 ± 1

Table entries are the mean ± SE of 3 determinations. Renal tubular cells were isolated from young rats and cultured for 5 days. In vitamin D metabolite experiments, indicated metabolites (10^{-7} M) were incubated for 24 h. Inhibitors were added for 30 min before addition of $1,25(OH)_2D$ (10^{-7} M), and incubation was then continued for 6 h. *P*450cc24 mRNA levels were determined as described previously [74].

Table 4. Effect of diabetes on vitamin D metabolism

	Control	Diabetic
Renal $1,25(OH)_2D$ production (pg/min/g)	0.69 ± 0.11	0.20 ± 0.01*
Renal $24,25(OH)_2D$ production (pg/min/g)	0.23 ± 0.04	0.53 ± 0.05*
*P*450cc24 mRNA (% of control)	100 ± 4	809 ± 68*
Ferredoxin mRNA (% of control)	100 ± 30	148 ± 37
Ferredoxin reductase mRNA (% of control)	100 ± 23	106 ± 23

Table entries are the mean ± SE of 4–6 rats. Asterisk indicates significantly different from Control ($P < 0.05$, *t*-test). Values for renal vitamin D production taken from Ref. [59]. *P*450cc24, ferredoxin, and ferredoxin reductase mRNA levels were determined as described previously [74].

mRNA in the same experiment. The mechanism responsible for this modulation of 24-OHase activity and mRNA is not known. However, $1,25(OH)_2D$ would not appear to be involved, since serum $1,25(OH)_2D$ levels decrease in diabetic rats.

6. CONCLUSIONS

Vitamin D is metabolized by three major cytochrome *P*450-containing enzyme systems—the liver 25-OHase, the renal 1α-OHase, and the renal and intestinal 24-OHase. The rat liver mitochondrial *P*450cc25 and the rat kidney mitochondrial *P*450cc24 have been purified, and their cDNAs have been cloned and sequenced. The study of the regulation of one of the most important enzymes in vitamin D metabolism, the renal 1α-OHase, awaits the cloning of the cDNA for the *P*450cc1α.

The isolation and cloning of the cDNA for the *P*450cc24 have made it possible to study the regulation of the 24-OHase, which in general is opposite to that of the 1α-OHase. Initial studies have shown that $1,25(OH)_2D$ increases *P*450cc24 mRNA by a receptor-mediated mechanism requiring new protein synthesis. This may account for the stimulation of 24-OHase activity by $1,25(OH)_2D$. Preliminary data suggest that PTH does not markedly alter 24-OHase *P*450 mRNA levels in the intact animal or in cultured cells (Armbrecht *et al.*, unpublished studies). It may be that PTH acts at a translational or post-translational step to alter 24-OHase activity. Thus, the vitamin D-metabolizing *P*450s, like the adrenal steroidogenic *P*450s, may be regulated by a number of factors using multiple signaling pathways.

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