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Expression of 25-hydroxyvitamin D 24-hydroxylase cytochrome P450 in kidney and intestine

Effect of 1,25-dihydroxyvitamin D and age

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To study the mechanism of hormonal regulation of the 25-hydroxyvitamin D 24-hydroxylase, a DNA probe complementary to the published sequence of the recently cloned P450 component [(1991) FEBS Lett. 278, 195] was employed. Young (2 month) and adult (12 month) F344 rats, deficient in 1,25-dihydroxyvitamin D, were given a single dose of 1,25-dihydroxyvitamin D. In young rats, 1,25-dihydroxyvitamin D markedly increased P450 mRNA levels within 3 h in both kidney and intestine, and maximal levels were attained at 16 and 3 h, respectively. In adult animals, maximal induction of mRNA was diminished in the kidney, and the decline was slower in the intestine. Time of maximal induction did not change with age. These studies demonstrate for the first time regulation of the 24-hydroxylase enzyme by 1,25-dihydroxyvitamin D at the level of the mRNA for the cytochrome P450. They also demonstrate that this regulation may change with age.

25-Hydroxyvitamin D; 1,25-Dihydroxyvitamin D; 24-Hydroxylase; Cytochrome P450; Age; Intestine; Kidney; Rat

1. INTRODUCTION

The kidney hydroxylates 25-hydroxyvitamin D in the 1-position to form 1,25-dihydroxyvitamin D ($1,125(OH)_2D$), the most biologically active form of vitamin D, or in the 24-position to form 24,25-dihydroxyvitamin D, whose biological function is unknown [1]. Both of these hydroxylations are accomplished by mixed function oxidases of the cytochrome P450 family, which are part of a multi-protein complex found on the inner mitochondrial membrane [2]. Although the 1-hydroxylation of 25(OH)D takes place almost exclusively in the kidney, 24-hydroxylase activity is found in intestine, bone, skin fibroblasts and lymphocytes, in addition to kidney. The presence of 24-hydroxylase in these tissues, which are target tissues for $1,25(OH)_2D$ action, has led to the suggestion that the 24-hydroxylation plays a role in the metabolic inactivation of $1,25(OH)_2D$ [3].

In the kidney, both the 1-hydroxylase (1-OHase) and the 24-hydroxylase (24-OHase) enzymatic activities are highly regulated by parathyroid hormone (PTH), $1,25(OH)_2D$ itself, and other factors [2]. The hydroxylase activities tend to be regulated in a reciprocal fashion. PTH stimulates 1-OHase activity and inhibits 24-OHase activity [4], and $1,25(OH)_2D$ inhibits 1-OHase activity and stimulates 24-OHase activity [5]. The precise pathways by which PTH and $1,25(OH)_2D$ regulate these hydroxylase activities are not known, but several mechanisms have been proposed. These mechanisms include the regulation of transcription and translation of components of the cytochrome P450 complex [6,7], the post-translational cleavage of synthesized components [8], and the post-translational phosphorylation of components [9].

With the recent isolation [10,11] and cloning [12] of the rat renal cytochrome P450 component of the 24-OHase, it is now possible to study the hormonal regulation of these hydroxylases in more detail. The experiments reported here were designed to determine whether the induction of the 24-OHase enzyme by $1,25(OH)_2D$ may involve an increase in mRNA levels for the P450 component. This was examined in both kidney and intestine using strontium-fed rats as a model for vitamin D deficiency [13]. In addition, the action of $1,25(OH)_2D$ was studied in adult as well as young rats, since the action of $1,25(OH)_2D$ in its target tissues has been reported to decline with age [14].

2. MATERIALS AND METHODS

Experiments were performed using male virgin Fischer 344 rats (F344/NNIA), aged 2 (young) and 12 months (adult) of age. Animals were obtained from the National Institute on Aging colony maintained by Harlan Industries (Indianapolis, IN). Rats were initially fed a

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semisynthetic diet containing 1.2% calcium, 0.8% phosphorus, and 3.3 IU/g vitamin D3 (Purina rodent chow, Ralston-Purina, St. Louis, MO). Animal studies were conducted in accord with the NIH Guidelines for the Care and Use of Laboratory Animals.

To make the rats deficient in $1,25(OH)_2D$, they were fed a low calcium diet (Teklad Test Diet no. 170120) containing 0.8% strontium for 6 days [14]. This high strontium diet has been shown to markedly reduce serum 1,25-dihydroxyvitamin D, intestinal calcium transport, and intestinal calbindin content [14]. To determine the effect of $1,25(OH)_2D$ on 24-OHase P450 mRNA, rats were given a single intraperitoneal injection of $1,25(OH)_2D$ (300 ng/100 g body weight) dissolved in ethanol. Control rats (0 hour) were given vehicle only. Rats were killed at the indicated times after dosing with $1,25(OH)_2D$, and the abdominal cavity was exposed by midline incision. The proximal duodenum (0–10 cm distal to the pylorus) was removed, slit lengthwise, and scraped with glass microscope slides to remove mucosa. Kidneys were also removed. Kidneys and intestinal mucosa were quick-frozen for later isolation of mRNA.

The amount of 24-OHase P450 mRNA in the intestine and kidney was determined by Northern and dot blot as described previously [15]. Total RNA was isolated using guanidium isothiocyanate [16]. The RNA was probed using a synthetic cDNA probe based on the first 42 bases of the published sequence for rat renal 24-OHase P450 [12]. The probe was 3′ end labeled with deoxyadenosine monophosphate tails containing radiolabeled phosphorus [17]. Northern blotting was used to assess the integrity of the isolated RNA and the validity of the probe. Dot blots were used to quantitate the amount of 24-OHase P450 mRNA [15]. After hybridization, the blots were autoradiographed, and the X-ray films were quantitated by densitometric scanning. Dot blots were quantitated in the linear range of RNA application. mRNA content was expressed per μg of total RNA, which was determined by optical density (OD). In addition, blots were stripped and rehybridized with a probe to β-actin (OnCor, Gaithersburg, MD), which was nick translated. β-Actin, which is not affected by 1,25-dihydroxyvitamin D treatment [15], was used to verify the amount of RNA on the dot blots.

The data from these experiments are reported as the mean±SE of the number of animals indicated. Statistical analyses were performed using two-tailed Student's t-test [18]. A confidence level of 95% or greater was considered significant.

3. RESULTS

To determine tissue specificity, Northern analysis was performed on total RNA isolated from kidney, intestine, and liver of rats dosed with 1,25-dihydroxyvitamin D 6 h previously. The oligonucleotide probe for the 24-OHase P450 was found to hybridize strongly with RNA from the rat kidney and intestine but not with RNA from the liver (Fig. 1). A major band was seen at 3.4 kb in the kidney, as has been reported previously using the full-length clone [12]. An identical band was found in the intestine, suggesting that the same mRNA may encode the 24-OHase P450 present in both tissues. Similar results were found using poly A RNA from these tissues (not shown).

A single injection of $1,25(OH)_2D$ produced a marked increase with time in 24-OHase P450 mRNA in the kidney (Fig. 2). In young rats, the renal mRNA levels were significantly elevated by 3 h, peaked at 16 h, and declined by 24–48 h. The adult rats showed a similar time course in response to $1,25(OH)_2D$. However, the amount of P450 mRNA was less than that seen in the young at each time point. This was particularly evident at 3–6 h, suggesting a lag in the action of $1,25(OH)_2D$ in the adult relative to the young.

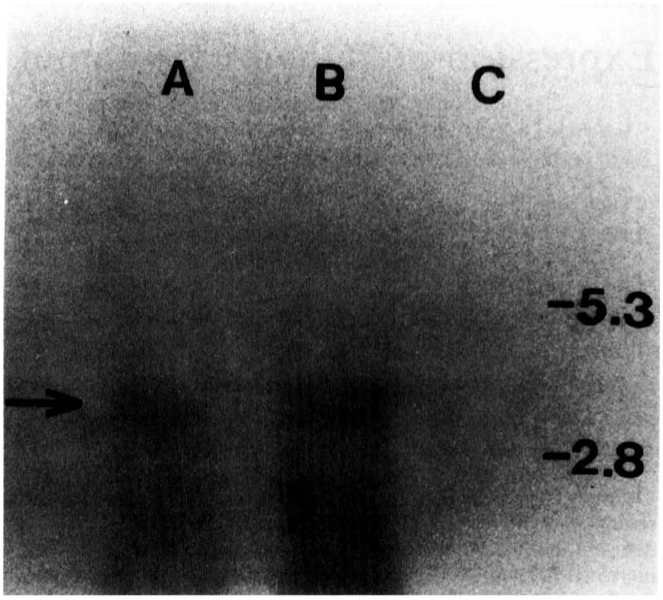

Fig. 1. Expression of mRNA for 24-hydroxylase P450 in kidney (A), intestine (B), and liver (C). Total RNA from each tissue was isolated from young rats treated with 1,25-dihydroxyvitamin D for 6 h. Northern blotting of RNA was performed by electrophoresing in agarose gel (21, 26 and 23 μg in lanes A, B and C, respectively), blotting onto nitrocellulose, and incubating with the radiolabeled synthetic probe for the renal 24-OHase P450. The blot was autoradiographed, and message size was determined by co-electrophoresis of size markers. Arrow indicates major band with a calculated size of 3.4 kb.

In the intestine, $1,25(OH)_2D$ also produced a rise in message for the 24-OHase P450 enzyme (Fig. 3). In the young animals, the maximal increase was seen at 3 h with a sharp decline thereafter. No message was detectable in the intestine at 16 h, which was the time of maximal message concentration in the kidney. The adult animals showed a equally rapid rise in intestinal message, but the decline was much more gradual. The maximal levels of P450 message seen in the intestine did not change with age and were similar in magnitude to those seen in the kidney (Fig. 2).

4. DISCUSSION

These studies demonstrate the presence of 24-OHase cytochrome P450 mRNA in both the kidney and intestine and its stimulation by $1,25(OH)_2D$ administration in vivo. The fact that the P450 mRNA is found in the kidney and intestine but not in the liver correlates with the reported tissue distribution of 24-OHase activity [19]. Induction of the mRNA for the 24-OHase P450 by $1,25(OH)_2D$ has not been reported in the intestine and kidney. An increase in renal 24-OHase P450 mRNA in response to chronic vitamin D administration has been previously reported [12]. However, in view of the poor

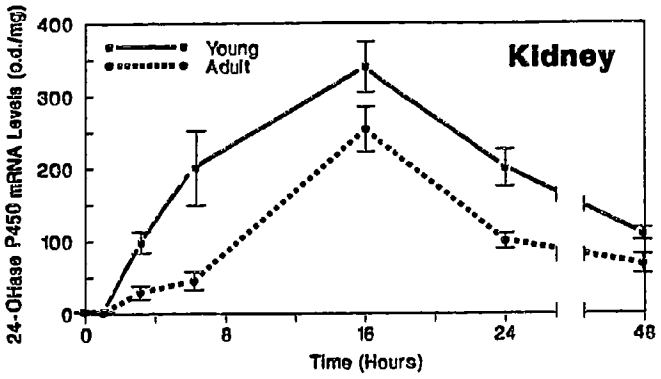

Fig. 2. Effect of 1,25-dihydroxyvitamin D and age on expression of 24-hydroxylase P450 mRNA in kidney. Total RNA was isolated from kidney at indicated times after dosing with 1,25-dihydroxyvitamin D. mRNA for the 24-hydroxylase P450 was determined by dot blot using the radiolabeled synthetic probe for the renal 24-hydroxylase P450. Dot blots were quantitated by measuring the optical density of the autoradiographed dots. Each data point represents the mean±SE of 6 rats and is expressed as optical density (o.d.) per mg total RNA.

affinity of the vitamin D receptor for vitamin D compared to 1,25D [1], this increase was probably mediated by $1,25(OH)_2D$ itself.

In general, the induction of 24-OHase mRNA by $1,25(OH)_2D$ appears to be rapid enough and of sufficient magnitude to account for the increases in 24-OHase enzyme activity reported in previous studies. In the experiments reported here, $1,25(OH)_2D$ increased 24-OHase mRNA to 60% of maximum by 6 h in the kidney (Fig. 2) and to maximal levels by 3 h in the intestine (Fig. 3) in young animals. This is consistent with the fact that renal and intestinal 24-OHase activity has been reported to be elevated 6–17 h after $1,25(OH)_2D$ administration in the intact animal [20]. A similar time course is seen in experiments with cultured cells. In JTC-12 cells, a monkey kidney cell line, 24-OHase activity peaks at 4–8 h after $1,25(OH)_2D$ exposure [21]. In Caco-2 cells, a human colon adenocarcinoma cell line, 24-OHase activity peaks at 6–17 h after $1,25(OH)_2D$ administration [22].

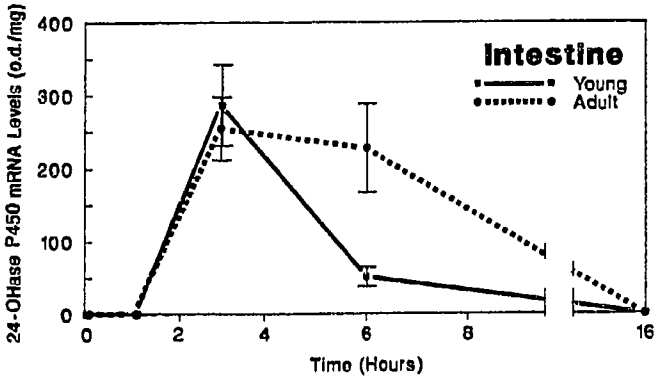

Fig. 3. Effect of 1,25-dihydroxyvitamin D and age on expression of 24-hydroxylase P450 mRNA in intestine. Total RNA was isolated from intestine at indicated times, and the 24-hydroxylase P450 mRNA quantitated by dot blot. See Fig. 2 for experimental details.

These experiments also demonstrate age differences in the response to $1,25(OH)_2D$. In the adult kidney, the response to $1,25(OH)_2D$ was decreased at all time points compared to the young kidney (Fig. 2). This was especially apparent at 3 and 6 h, where 24-OHase mRNA P450 levels in the adult were less than 30% of the young. However, the decline in mRNA levels from the peak at 16 h was similar in both age groups. In the intestine, other differences were seen. In both the young and adult animals, there was a rapid increase in mRNA levels at 3 h which was almost identical (Fig. 2). However, the decline in mRNA levels was much slower in the adult compared to the young. These findings suggest that there may be age-related changes in the initiation of gene transcription in the kidney and in the stabilization of mRNA in the intestine in response to $1,25(OH)_2D$. These age-differences were not due to changes in the metabolism of the injected $1,25(OH)_2D$, since serum $1,25(OH)_2D$ levels in young and adult rats were virtually identical at all time points (data not shown).

In summary, these studies demonstrate for the first time that 1,25-dihydroxyvitamin D, which stimulates 24-OHase activity, also increases the mRNA levels for the cytochrome P450 component of the enzyme complex. This is consistent with previous studies which demonstrated that stimulation of activity by $1,25(OH)_2D$ was sensitive to transcription inhibitors [7]. The hormonal regulation of other mitochondrial steroidogenic systems also involves increases in the mRNA for the appropriate P450. Adrenocorticotropin markedly increases the mRNA for the side chain cleavage cytochrome P450 in the adrenal cortex [23], and chorionic gonadotropin has a similar effect in the ovary [24]. The relative contribution of regulation at the mRNA level to the overall regulation of 24-hydroxylase activity remains to be determined.

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