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Intestinal Calcium-Binding Protein (CaBP) and Bone Calcium Mobilization in Response to 1,24(R),25-$(OH)_3D_3$

Comparative Effects of 1,25-$(OH)_2D_3$ and 24(R),25-$(OH)_2D_3$ in Rats

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SUMMARY

THOMASSET, M., P. CUISINIER-GLEIZES, H. MATHIEU AND H. F. DELUCA. Intestinal calcium-binding protein (CaBP) and bone calcium mobilization in response to 1,24(R),25-$(OH)_3D_3$: Comparative effects of 1,25-$(OH)_2D_3$ and 24(R),25-$(OH)_2D_3$ in rats. *Mol. Pharmacol.* **17**: 362–366 (1980).

The response of intestinal calcium-binding protein (CaBP) synthesis and the rise in the serum calcium level to vitamin D metabolites were measured in vitamin D-deficient rats fed a low-calcium diet. Our results showed a linear relationship between the amount of intestinal CaBP and the logarithm of the dose for all the compounds. 1,24(R),25-$(OH)_3D_3$ was approximately four times less effective than 1,25-$(OH)_2D_3$ in increasing the CaBP level and about eight times more effective than 24(R),25-$(OH)_2D_3$. The bone calcium mobilization response expressed as the rise in serum calcium was also dose related. The relative potencies of the metabolites in increasing the serum calcium level were approximately the same as those elevating CaBP synthesis. Time course studies showed that 1,24(R),25-$(OH)_3D_3$ was as rapidly active as 1,25-$(OH)_2D_3$ in inducing intestinal CaBP synthesis and increasing serum calcium. The duration of its calcemic response was shorter and the magnitude less than that of 1,25-$(OH)_2D_3$. A longer time lag was necessary to obtain intestinal and calcemic responses after 24(R),25-$(OH)_2D_3$ injection. 1,24(R),25-$(OH)_3D_3$ and 1,25-$(OH)_2D_3$ were still active in promoting intestinal CaBP synthesis in nephrectomized rats, whereas a large dose of 24(R),25-$(OH)_2D_3$ was inactive. Thus C-1 hydroxylation is essential for the stimulation of both the intestinal CaBP production and the rise in the serum calcium level, whereas C-24 hydroxylation diminishes the magnitude of the intestinal and calcemic responses. These results strongly suggest that 1,24(R),25-$(OH)_3D_3$ represents an inactivation rather than an activation of the hormonal form of vitamin D_3.

INTRODUCTION

Vitamin D_3 or cholecalciferol must be hydroxylated to 25-hydroxycholecalciferol (25-OH-D_3) in the liver and subsequently to 1,25-dihydroxycholecalciferol (1,25-$(OH)_2D_3$) in the kidney before it carries out its function in the intestine and bone (1). The 1,25-$(OH)_2D_3$ presently known as the most potent and rapidly acting metabolite of vitamin D_3 is regarded as its hormonal form. Intestinal calcium-binding protein (CaBP) synthesis can be considered as a molecular expression of the hormonal action of 1,25-$(OH)_2D_3$ on the enterocyte (2, 3) and consequently as a sensitive index of the intestinal response to various vitamin D_3 metabolites (4, 5). Other dihydroxy metabolites are synthesized *in vivo*. Thus 25-OH-D_3 can be hydroxylated on carbon 24 to yield 24(R),25-dihydroxycholecalciferol (24(R),25-$(OH)_2D_3$) (1). In vitamin D-deficient rats fed a low-calcium diet, 24(R),25-$(OH)_2D_3$ has significant biological activity in stimulating intestinal calcium transport (6), bone calcium mobilization (4, 6), and intestinal calcium-binding protein synthesis (4) but lacks biological activity on the intestine in nephrectomized rats (4, 6). This indicates that 24(R),25-$(OH)_2D_3$ must be further metabolized in the kidney to become active in vitamin D- and calcium-deficient rats. Such a trihydroxy vitamin D_3 metabolite has been isolated and identified as 1,24(R),25-trihydroxycholecalciferol (1,24(R),25-$(OH)_3D_3$) (7). In the rat, the biological activity of 1,24(R),25-$(OH)_3D_3$ has been reported in terms of

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intestinal calcium transport (8), bone calcium mobilization (8, 9), and epiphyseal plate calcification (8).

The purpose of the present study was to investigate the effect of 1,24(R),25-(OH)$_3$D$_3$ in inducing intestinal CaBP production and increasing the serum calcium level in intact and nephrectomized rats. As will be shown, time course and dose–response data of both intestinal CaBP and calcemic responses to 1,24,25-(OH)$_3$D$_3$, 1,25-(OH)$_2$D$_3$, and 24,25-(OH)$_2$D$_3$ administration attempt to elucidate the relative importance of C-24 and C-1 hydroxylations.

MATERIALS AND METHODS

Compounds

The vitamin D metabolites used were all synthetic compounds. 1,24(R),25-(OH)$_3$D$_3$, 24(R),25-(OH)$_2$D$_3$, and 1,25-(OH)$_2$D$_3$ were gifts from Dr. M. Uskokovic, Hoffmann–La Roche, Nutley, New Jersey.

Animals and Diets

Male weanling rats (40–50 g) of the Sprague–Dawley strain were fed ad libitum a vitamin D-deficient diet containing (w/w) 0.50% calcium and 0.36% phosphorus for 4 weeks. Deionized water was supplied ad libitum throughout the experiment. Serum calcium was measured at weekly intervals. For 1 additional week, the hypocalcemic rats (6.5 mg Ca/100 ml serum) considered as vitamin D-deficient animals were kept on a low-calcium, vitamin D-deficient diet containing (w/w) 0.03% calcium and 0.36% phosphorus. In the experiments on nephrectomized rats, the animals were anesthetized with ether and subjected to either a sham operation or a total bilateral nephrectomy through a dorsal incision over both flanks.

Protocol

In dose–response experiments, all rats were killed 48 h after the injection of different doses of each vitamin D$_3$ metabolite. In time course experiments, rats received a single dose, determined from dose–response studies, and were sacrificed at various intervals following the injection. Immediately after surgery, nephrectomized and sham-operated rats received a dose of vitamin D metabolite active in intact animals. The rats were sacrificed 30 h later, a period compatible with the survival period of anephric rats. All the vitamin D$_3$ metabolites were dissolved in ethanol and administered intravenously. All control animals received the appropriate volume of ethanol.

Analytical Procedures

Preparation of tissue. The proximal 10 cm of intestine was removed and everted. All subsequent procedures were performed at 4°C. The mucosal tissue, harvested by scraping with a glass slide, was homogenized in 4× the volume of Tris buffer (0.013 M Tris–HCl, 0.12 M NaCl, 3 mM KCl, pH 7.4), then centrifuged at 100,000*g* for 1 h, and the supernatant fraction (S-100) was stored at −30°C.

Intestinal CaBP. CaBP was quantitated either directly by radioimmunoassay according to Marche *et al.* (10) or by its calcium-binding activity according to Freund and Bronner (11). For radioimmunoassay, the supernatant S-100 from each rat was analyzed for immunoreactive CaBP using a final antiserum dilution of 1/15,000 and for protein by a modified Lowry procedure (12). CaBP was expressed as micrograms per milligram of protein in S-100. For calcium-binding capacity determination, the lyophilized supernatant S-100 was generally taken from six rats and fractionated by elution on a Sephadex G-75 column. Fractions were tested for calcium-binding activity with a competitive binding assay using ^{45}Ca and Chelex resin. The material (peak B, V_e/V_o = 2.0) was pooled and the CaBP content evaluated by its calcium-binding activity determined as a function of the calcium concentration in the assay; the amount of added calcium was 0–100 μg/ml and the final free calcium concentration varied from 0.2 to 15 μM. The binding data were analyzed by saturation kinetics. The calcium-binding capacity was calculated by the method of Scatchard and expressed as nanomoles of calcium bound per milligram of protein in peak B. There is a good correlation between the CaBP concentrations determined by radioimmunoassay and by Chelex assay (13).

Calcemic response. The increase in serum calcium after the injection of a vitamin D compound to vitamin D-deficient rats fed a low-calcium diet is regarded as resulting from the mobilization of bone calcium. Serum calcium was determined by atomic absorption spectrophotometry.

Statistical Analysis

The time course study of calcemic response was analyzed by variance analysis. When statistical significance was reached, the groups were then compared by means of Student's *t* test using the residual variance derived from the variance analysis. Student's *t* test was used in other comparisons. The regression equations were calculated with the linear least-squares method using the logarithmic values of the doses. After testing the parallelism of the lines, the logarithm of the relative potency of one metabolite versus another was represented by the horizontal distance between the regression lines.

RESULTS

Time Course Studies

Intestinal CaBP. Figure 1 shows the time course of the intestinal CaBP production in response to the administration of 130 pmol 1,24(R),25-(OH)$_3$D$_3$ or 1,25-(OH)$_2$D$_3$ or 520 pmol 24(R),25-(OH)$_2$D$_3$. The administration of 130 pmol 1,24(R),25-(OH)$_3$D$_3$ or 1,25(OH)$_2$D$_3$ resulted in a similar onset and duration of the CaBP response, but the trihydroxy derivative was less active than 1,25-(OH)$_2$D$_3$. Intestinal CaBP production following 1,25-(OH)$_2$D$_3$ or 1,24,25-(OH)$_3$D$_3$ injections was detectable at as early as 6 h, was significantly elevated at 16 h, and reached a plateau from 24 to 48 h. In contrast, intestinal CaBP synthesis was undetectable 12 h after the injection of 520 pmol 24(R),25-(OH)$_2$D$_3$ but significantly increased at 24 and at 48 h.

Calcemic response. Figure 2 shows the time course of the calcemic response to 130 pmol 1,24(R),25-(OH)$_3$D$_3$ or

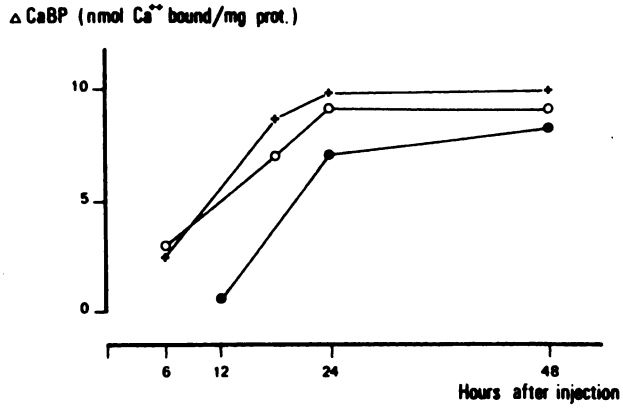

FIG. 1. *Time course of the intestinal CaBP response to 130 pmol (56 ng) of 1,24(R),25-(OH)$_3$D$_3$* (○), *130 pmol (54 ng) of 1,25-(OH)$_2$D$_3$* (+), *or 520 pmol (216 ng) of 24(R),25-(OH)$_2$D$_3$* (●) *in vitamin D-deficient rats fed a low-calcium diet*

ΔCaBP = CaBP of experimental group of six rats − CaBP of control group of six rats.

1,25-(OH)$_2$D$_3$ and to 260 pmol 24(R),25-(OH)$_2$D$_3$. The rise in the serum calcium level was significant ($P < 0.01$) as early as 6 h after the intravenous injection of 130 pmol 1,24(R),25-(OH)$_3$D$_3$, remained elevated at 16, 24, and 48 h, but was not significantly different from that of the controls at 72 h. By comparison, an identical dose of 1,25-(OH)$_2$D$_3$ showed a similar rapid onset of the calcemic response ($P < 0.01$ at 6 h), a marked rise in serum calcium with a maximal response observed at 16 and 24 h ($P < 0.001$), and also a longer duration of the activity throughout the experiment (72 h). When 1,24(R),25-(OH)$_3$D$_3$ and 1,25-(OH)$_2$D$_3$ were compared at the time of maximal response (24 and 16 h, respectively) and at the same dose level (130 pmol), the trihydroxy derivative was half as active as 1,25-(OH)$_2$D$_3$ in raising the serum calcium level.

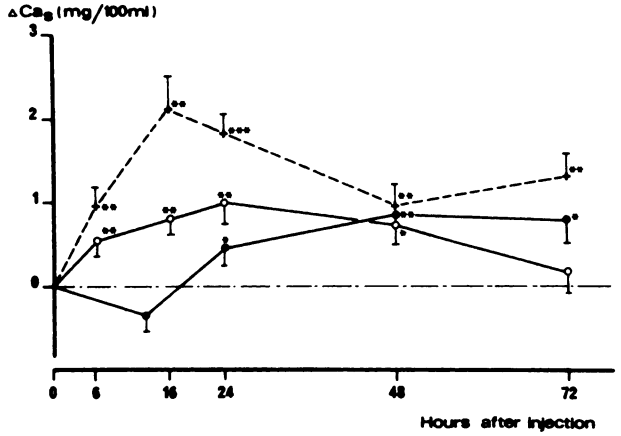

FIG. 2. *Time course of the response of bone calcium mobilization (increment of serum calcium) to 130 pmol (56 ng) of 1,24(R),25-(OH)$_3$D$_3$* (○), *130 pmol (54 ng) of 1,25-(OH)$_2$D$_3$* (+), *or 260 pmol (108 ng) of 24(R),25-(OH)$_2$D$_3$* (●) *in vitamin D-deficient rats fed a low-calcium diet*

Serum calcium was measured before the intravenous injection of steroid and at the time of assay. Each value and vertical bar represent the mean ± SEM of six rats. Significance vs $t = 0$: $^*P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$. The dashed line (-·-) represents the nonsignificant change in serum calcium levels in vitamin D-deficient rats fed a low-calcium diet and receiving only the vehicle (5.8 ± 0.3, 5.7 ± 0.3, and 5.6 ± 0.2 mg/100 ml at 24, 48, and 72 h, respectively).

In contrast, 260 pmol 24(R),25-(OH)$_2$D$_3$ was ineffective in elevating the serum calcium level 12 h after its intravenous injection but significantly active at 24, 48, and 72 h. The calcemic response reached a plateau from 16 to 72 h. At the time of the peak responses (48, 24, and 16 h respectively), 260 pmol 24(R),25-(OH)$_2$D$_3$ was as effective as 130 pmol 1,24(R),25-(OH)$_3$D$_3$ and half as active as 130 pmol 1,25-(OH)$_2$D$_3$ in elevating serum calcium.

Dose–Response Studies

Detailed dose–response studies were carried out 48 h after 1,24(R),25-(OH)$_3$D$_3$, 1,25-(OH)$_2$D$_3$, and 24(R),25-(OH)$_2$D$_3$ treatment. This time was chosen since it was compatible with the detection of both significant calcemic and significant CaBP responses according to the aforementioned time course data. From variance analysis of the time course data (Fig. 2), there is no significant difference between the serum calcium peak and the 48-h value except for 1,25-(OH)$_2$D$_3$ treatment ($P < 0.01$). Table 1 displays the data.

Intestinal CaBP response. The minimal dose required to induce a significant increase in the CaBP level was 16, 16, and 130 pmol 1,24(R),25-(OH)$_3$D$_3$, 1,25-(OH)$_2$D$_3$, and 24(R),25-(OH)$_2$D$_3$, respectively. The CaBP dose-related response can be described as a linear function of the

TABLE 1

Response of intestinal calcium-binding protein and bone calcium mobilization (increment of serum calcium) to increasing doses of 1,24(R),25-(OH)$_3$D$_3$, 1,25-(OH)$_2$D$_3$, and 24(R),25-(OH)$_2$D$_3$ 48 h after intravenous administration to vitamin D-deficient rats fed a low-calcium diet

The values in parentheses represent the number of rats. Administration of 1,24(R),25-(OH)$_3$D$_3$, 1,25-(OH)$_2$D$_3$, and 24(R),25-(OH)$_2$D$_3$ induces a significant increase in CaBP level (a, $P < 0.05$; b, $P < 0.01$; c, $P < 0.001$) vs rat receiving vehicle and a significant increase in Cas at 48 h vs Cas at 0 h (d, $P < 0.05$; e, $P < 0.01$; f, $P < 0.001$).

Doses		Body weight	Intestinal CaBP	Cas, hours after injection	
				0	48
pmol		*g*	*μg/mg Pr S-100*	*mg/100 ml*	
1,24(R),25-(OH)$_3$D$_3$					
16.2	(5)	103 ± 9	16.8 ± 1.4^b	5.5 ± 0.2	6.1 ± 0.4
32.5	(5)	93 ± 7	17.8 ± 1.4^b	5.7 ± 0.3	6.5 ± 0.2^d
65	(5)	102 ± 5	22.6 ± 3.2^b	5.8 ± 0.1	6.5 ± 0.1^d
130	(5)	99 ± 5	23.6 ± 2.1^c	5.6 ± 0.2	6.6 ± 0.1^e
260	(5)	101 ± 5	24.4 ± 2.9^b	5.7 ± 0.2	7.0 ± 0.4^e
Vehicle	(5)	98 ± 4	9.9 ± 1.3	5.3 ± 0.1	5.7 ± 0.2
			μg/mg Pr S-100		
1,25-(OH)$_2$D$_3$					
16.2	(6)	110 ± 5.5	16.4 ± 2.0^b	6.4 ± 0.3	7.1 ± 0.1
32.5	(6)	93 ± 7.2	20.7 ± 1.6^c	6.5 ± 0.2	7.6 ± 0.3^e
65	(5)	112 ± 7.5	21.2 ± 1.3^c	6.4 ± 0.3	7.6 ± 0.3^e
130	(5)	107 ± 4.6	22.5 ± 1.5	6.5 ± 0.2	7.8 ± 0.2^f
260	(5)	94 ± 6.7	22.9 ± 1.9^c	6.1 ± 0.2	7.4 ± 0.4^f
Vehicle	(5)	106 ± 8.1	5.2 ± 0.3	6.7 ± 0.2	7.0 ± 0.2
			nmol Ca bound/mg Pr peak B		
24(R), 25-(OH)$_2$D$_3$					
130	(6)	98 ± 3	10	6.1 ± 0.3	6.0 ± 0.3
260	(6)	106 ± 7	13^a	6.3 ± 0.2	6.5 ± 0.2^d
520	(6)	99 ± 6	14^a	6.2 ± 0.2	6.8 ± 0.3^e
1040	(6)	107 ± 5	20^b	6.3 ± 0.8	8.0 ± 0.4^f
2080	(6)	108 ± 7	27^b	6.3 ± 0.3	8.3 ± 0.2^f
Vehicle	(6)	99 ± 7	5	6.3 ± 0.3	6.3 ± 0.4

logarithm of the administered dose for these three steroids over a 16- to 260-pmol range of either 1,24(R),25-$(OH)_3D_3$ or 1,25-$(OH)_2D_3$ and a 130- to 1040-pmol range of 24(R),25-$(OH)_2D_3$. The equations of the regression lines are:

1,24(R),25-$(OH)_3D_3$:ΔCaBP = 11.1 (±1.1) + 7.0 (±1.3) log dose, $r = 0.96$, $P < 0.02$; 1,25-$(OH)_2D_3$: ΔCaBP = 15.5 (±1.1) + 4.9 (±1.9) log dose, $r = 0.92$, $P < 0.05$; 24(R),25-$(OH)_2D_3$:ΔCaBP = 1.50 (±1.3) + 10.3 (±2.3) log dose, $r = 0.96$, $P < 0.05$.

Dose is units (1 I.U. = 65 pmol or 28, 27, and 27 ng of 1,24(R),25-$(OH)_3D_3$, 1,25-$(OH)_2D_3$, and 24(R),25-$(OH)_2D_3$, respectively), and ΔCaBP (μg/mg protein) represents CaBP values in experimental groups − CaBP values in controls (vehicle). The values in parentheses are SD. ΔCaBP is nanomoles of Ca bound per milligram of protein in peak B. Comparison of the three regression lines showed that 1,24(R),25-$(OH)_3D_3$ was approximately four times less active than 1,25-$(OH)_2D_3$ and about eight times more effective than 24(R),25-$(OH)_2D_3$ in promoting intestinal CaBP production.

Calcemic response. The minimal dose required to induce a significant increase in the serum calcium level was 32, 16, and 260 pmol 1,24(R),25-$(OH)_3D_3$, 1,25-$(OH)_2D_3$, and 24(R),25-$(OH)_2D_3$, respectively. The saturation of the calcemic response was evident with 260 pmol 1,25-$(OH)_2D_3$, 2080 pmol 24(R),25-$(OH)_2D_3$, and possibly 260 pmol 1,24(R),25-$(OH)_3D_3$. The calcemic dose-related response was a linear function of the logarithm of the administered dose over the range of 16–260, 16–130, and 130–580 pmol 1,24(R),25-$(OH)_3D_3$, 1,25-$(OH)_2D_3$, and 24(R),25-$(OH)_2D_3$, respectively. The equations of the regression lines are:

1,24(R),25-$(OH)_3D_3$:ΔCas = 0.84 (±0.07) + 0.54 (±0.09) log dose, $r = 0.97$, $P < 0.01$; 1,25-$(OH)_2D_3$: ΔCas = 1.24 (±0.03) + 0.77 (±0.06) log dose, $r = 0.99$, $P < 0.01$; 24R,25-$(OH)_2D_3$:ΔCas = −0.46 (±0.01) + (1.14) (±0.09) log dose, $r = 0.999$, $P < 0.02$.

Dose is units and ΔCas (mg/100 ml) represents Cas 48 h − Cas 0 h. The values in parentheses are SD. Comparison of the regression lines showed that 1,24(R),25-$(OH)_3D_3$ was about three to four times less active than 1,25$(OH)_2D_3$ in elevating the serum calcium level and that 1,24(R),25-$(OH)_3D_3$ was about eight times more effective than 24(R),25-$(OH)_2D_3$ in increasing the calcemic response.

Importance of the Renal C-1 Hydroxylation

Since the C-1 hydroxylation of vitamin D metabolites in the kidney mitochondrion is essential to induce biological activity, the intestinal CaBP response has been tested after a single intravenous injection of 1,24(R),25-$(OH)_3D_3$, 1,25-$(OH)_2D_3$, or 24(R),25-$(OH)_2D_3$ in intact and nephrectomized rats (Table 2). As already reported (5), nephrectomy depressed the serum calcium level. 1,24(R),25-$(OH)_3D_3$ and 1,25-$(OH)_2D_3$ had an identical potency in stimulating the intestinal CaBP response in sham-operated and nephrectomized rats, whereas 24(R),25-$(OH)_2D_3$ lost its potency in anephric rats despite the administration of a large dose (1040 pmol) inducing a maximal intestinal response in control rats.

TABLE 2

Response of duodenal calcium-binding protein (CaBP) and bone calcium mobilization (increment of serum calcium) to 1,24(R),25-$(OH)_3D_3$, 1,25-$(OH)_2D_3$, and 24(R),25-$(OH)_2D_3$ 30 h after intravenous administration in sham-operated and nephrectomized vitamin D-deficient rats fed a low-calcium diet

Results are the mean ± SEM. The values in parentheses represent the number of rats. b, c, and d significantly different from a, $P < 0.001$. b′ and c′ significantly different from a′, $P < 0.001$. f significantly different from Cas at 0 h, $P < 0.01$.

Rats		CaBP	Serum calcium (Cas), hours after injection	
			0	30
		μg/mg protein	*mg/100 ml*	
Sham operated				
Vehicle	(5)	7.1 ± 1.2[a]	5.7 ± 0.2	6.1 ± 0.3
1,24(R),25-$(OH)_3D_3$, 260 pmol	(4)	27.5 ± 2.9[b]	6.5 ± 0.1	7.2 ± 0.3[f]
1,25-$(OH)_2D_3$, 130 pmol	(5)	21.3 ± 2.1[c]	6.1 ± 0.3	7.9 ± 0.4[f]
24(R),25-$(OH)_2D_3$, 520 pmol	(4)	16.3 ± 2.0[d]	6.2 ± 0.1	6.9 ± 0.2[f]
Nephrectomized				
Vehicle	(5)	5.1 ± 0.8[a′]	6.4 ± 0.3	4.2 ± 0.4[f]
1,24(R),25-$(OH)_3D_3$, 260 pmol	(4)	23.1 ± 2.9[b′]	6.5 ± 0.1	3.7 ± 0.3[f]
1,25-$(OH)_2D_3$, 130 pmol	(5)	20.8 ± 2.7[c′]	6.1 ± 0.2	5.1 ± 0.4[f]
24(R),25-$(OH)_2D_3$, 1040 pmol	(4)	4.7 ± 0.9	6.3 ± 0.1	4.4 ± 0.2[f]

DISCUSSION

In terms of bone calcium mobilization, the comparison of the activity of the C-1-hydroxylated vitamin D_3 metabolites indicates that 1,24(R),25-$(OH)_3D_3$ is as rapidly active as 1,25-$(OH)_2D_3$ but exhibits a shorter duration of action (48 instead of 72 h) and a lower magnitude of the response (Fig. 2). These data are in agreement with previous reports (8, 14). Moreover, it has been recently established that 1,24(R),25-$(OH)_3D_3$ arises primarily by the 24-hydroxylation of 1,25-$(OH)_2D_3$ rather than by the 1-hydroxylation of 24,25$(OH)_2D_3$ (7). Thus the 24-hydroxylation of the hormonal form of vitamin D_3 represents an inactivation step. It is noteworthy that the true effectiveness of 1,25-$(OH)_2D_3$ is superior to its apparent effectiveness from the regression lines since at sacrifice time in the log-dose studies (48 h) the magnitude of the calcemic response to 1,25-$(OH)_2D_3$ is equal to half of its maximal response (16 h) (Fig. 2), whereas the response to 1,24(R),25-$(OH)_3D_3$ is not significantly different from 16 to 48 h after its administration. Therefore detailed time course and extensive dose–response studies reveal that 1,24(R),25-$(OH)_3D_3$ is about three to four times less active than 1,25-$(OH)_2D_3$ in the calcemic response. These observations are consistent with *in vitro* studies where organ cultures of fetal rat bone were used to test the bone-resorbing activity of the vitamin D_3 metabolites (9). The onset of the calcemic response to 24(R),25-$(OH)_2D_3$ requires a longer time lag (Fig. 2). This protracted lag may represent the delay required for renal C-1 hydroxylation of 24(R),25-$(OH)_2D_3$ to be active (Fig. 2). Such an observation has also been reported with 25(R),26-$(OH)_2D_3$ and 25(S),26-$(OH)_2D_3$ in our earlier study (5). Thus the calcemic effect observed *in vivo* is probably produced by C-1-hydroxylated derivatives rather than by the parent compound.

In terms of intestinal CaBP synthesis, 1,24(R),25-

$(OH)_3D_3$ is as rapidly active as 1,25-$(OH)_2D_3$ (Fig. 1) and about four times less active than its precursor 1,25-$(OH)_2D_3$. Again, these results show a high potency of 1,24(R),25-$(OH)_3D_3$, although the C-24 hydroxylation of 1,25-$(OH)_2D_3$ leads to a decrease in the effectiveness on CaBP synthesis.

Both C-1 hydroxyl derivatives are as effective in intact and nephrectomized rats. This suggests that these two compounds do not undergo further metabolism before acting upon intestine *in vivo*. CaBP response to 1,24(R),25-$(OH)_3D_3$ administration has not been previously reported in the rat. Since a high degree of correlation between the vitamin D-dependent CaBP level and the process of calcium transport across the intestine is well documented (16), our data may be compared to those of intestinal calcium transport investigations. Our results are in accord with those studies which show that 1,24(R),25-$(OH)_3D_3$ is less active than 1,25-$(OH)_2D_3$, although both are very active in the stimulation of intestinal calcium transport (8). 24(R),25-$(OH)_2D_3$ has a protracted lag in the stimulation of both calcium absorption and CaBP synthesis. The intestinal CaBP response to a large dose (1040 pmol) of 24(R),25-$(OH)_2D_3$ is eliminated by bilateral nephrectomy. Consequently intestinal CaBP synthesis examined in our *in vivo* system is certainly produced by C-1-hydroxylated 24(R),25-$(OH)_2D_3$. The requirement of further renal C-1 hydroxylation to induce intestinal response has also been reported with 25(R),26-$(OH)_2D_3$ and 25(S),26-$(OH)_2D_3$ (5). These results are consistent with calcium transport data (6) which demonstrate that bilateral nephrectomy prevents the duodenal calcium transport in response to 1600 pmol of 24(R),25-$(OH)_2D_3$. The three vitamin D metabolites seem more active in the rat than in the chick (17, 18). In this respect, a daily dose of 2.6 nmol 1,24(R),25-$(OH)_3D_3$ for 1 week induced a CaBP level only one-tenth as great as that achieved by 1,25-$(OH)_2D_3$ (18), and a daily dose of 320 pmol 24(R),25-$(OH)_2D_3$ for 4 weeks from hatching is unable to induce CaBP synthesis (17). The different potency of 1,24(R),25-$(OH)_3D_3$, 1,25-$(OH)_2D_3$, and 24(R),25-$(OH)_2D_3$ in the stimulation of intestinal CaBP synthesis may suggest a different receptor affinity. Indeed studies of the competition of vitamin D_3 analogs with 1,25-$(OH)_2D_3$ for the chick intestinal receptor show that 1,24(R),25-$(OH)_3D_3$ was the nearest competitor (15) and was eightfold (15) or threefold (18) less effective in this assay than 1,25-$(OH)_2D_3$. 24(R),25-$(OH)_2D_3$ competed for the cytosol receptor only when present at about a 5000-fold concentration (15, 19). Such data correspond reasonably well with the relative activities found in our current study.

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