ABSTRACT

Parathyroid hormone and 1,25-dihydroxyvitamin D$_3$ had opposite effects on calf renal 25-hydroxyvitamin D$_3$ 24-, 23-, and 1α-hydroxylase activities. Parathyroid hormone administration increased renal 25-hydroxyvitamin D$_3$-1α-hydroxylase activity 7-fold while 25-hydroxyvitamin D$_3$-23- and 24-hydroxylase activities were essentially the same as controls. Administration of 1,25-dihydroxyvitamin D$_3$ increased 25-hydroxyvitamin D$_3$-23-hydroxylase and 24-hydroxylase activities 4-fold and decreased 25-hydroxyvitamin D$_3$-1α-hydroxylase activity to undetectable concentrations. Vitamin D deficiency increased 25-hydroxyvitamin D$_3$-1α-hydroxylase activity 13-fold, and 25-hydroxyvitamin D$_3$-24-hydroxylase and 1α-hydroxylase activities were undetectable. These results confirm previous reports with regard to control of renal 25-hydroxyvitamin D$_3$-24-hydroxylase and 1α-hydroxylase in other species and represent new findings relative to the control of 25-hydroxyvitamin D$_3$-23-hydroxylase. Plasma P was lower and 1,25-dihydroxyvitamin D$_3$ higher in calves treated with parathyroid hormone, and Ca and 1,25-dihydroxyvitamin D$_3$ were lower in the vitamin D-deficient calves. 1,25-Dihydroxyvitamin D$_3$-treated calves had higher plasma P and lower Mg than controls. Further studies using this calf model should lead to better understanding of Ca-regulating hormones control of vitamin D metabolism.

INTRODUCTION

The most active form of vitamin D is 1,25-dihydroxyvitamin D$_3$ [1,25-(OH)$_2$D$_3$], which is produced by successive hydroxylation of vitamin D at C-25 by a liver enzyme, vitamin D 25-hydroxylase (24), and at C-1 by a kidney enzyme, 25-hydroxyvitamin D$_3$-1α-hydroxylase (1α-hydroxylase) (11). The biological activity of 1,25-(OH)$_2$D$_3$ includes increased Ca and P absorption from the intestine (20) and resorption from bone (27). 1α-Hydroxylase activity is regulated by the vitamin D status of the animal (4, 14), dietary Ca (2, 21), dietary P (5, 10), parathyroid hormone (PTH) (8), insulin (28), growth hormone (9), and prolactin (26). Control of 1α-hydroxylase has been studied in the rat (8) and chick (23). Little has been reported about control of 1α-hydroxylase in large domestic animals. The high cost of animals and radiolabeled metabolites have precluded study of vitamin D metabolism in the cow. This report presents results of a study on the effect of vitamin D deficiency and of 1,25-(OH)$_2$D$_3$ and PTH treatment on 1α-hydroxylase, 25-hydroxyvitamin D-24-hydroxylase and 1α-hydroxylase in other species and represent new findings relative to the control of 25-hydroxyvitamin D$_3$-23-hydroxylase. Plasma P was lower and 1,25-dihydroxyvitamin D$_3$ higher in calves treated with parathyroid hormone, and Ca and 1,25-dihydroxyvitamin D$_3$ were lower in the vitamin D-deficient calves. 1,25-Dihydroxyvitamin D$_3$-treated calves had higher plasma P and lower Mg than controls.
tritiated methyl magnesium iodide reduction of 17-nor-26-keto vitamin D₃ (18). 23S,25-Dihydroxy[26,27-³H]D₃ (90 Ci/mmol) was synthesized in vitro from 25-OH[26,27-³H]D₃ (5 mCi/15 ml of reaction mixture) by use of kidney homogenates from 1,25-(OH)₂D₃-treated chicks (15). Crystalline 23S,25-(OH)₂D₃ was chemically synthesized (22).

Animals and Diets
Jersey bull calves, born at the National Animal Disease Center, were fed their dam's milk for the first 3 to 4 d and then switched to a commercial milk replacer diet (Maxi-Care with glymaxene; Land-O-Lakes, Inc., Fort Dodge, IA). This was supplemented with Calf Manna (Carnation Company Milling Division, Los Angeles, CA) until sacrifice (age range 2 to 4 mo). Eight calves were used as controls.

Nine calves received injections intramuscularly (i.m.) of 25 µg of 1,25-(OH)₂D₃ in 1.0 ml ethanol at 24 and 2 h before sacrifice. Five calves received i.m. injections of 7 mg of synthetic N-terminal 1-34 fragment of bovine PTH (Peninsula Laboratories, Inc., San Carlos, CA) dissolved in 2% bovine serum albumin - .15 M NaCl - .01 N acetic acid solution at 16 and 4 h prior to sacrifice. Three calves received an injection of 25 µg of 1,25-(OH)₂D₃ in 1.0 ml ethanol i.m. 24 h before sacrifice, and an i.m. injection of 7 mg of PTH 10 h before sacrifice. The vitamin D-deficient basal diet (without added Ca or P) was purchased from Teklad Test Diet Company, Madison, WI (TD #81127). Calcium carbonate and CaHPO₄ were added to the basal mix to give a final concentration of .69% Ca and .54% P. This diet met minimum requirements for all nutrients except vitamin D (19). Four calves were fed their dam’s milk for the first 3 to 4 d, then switched to the vitamin D-deficient diet. They were fed the vitamin D-deficient diet for 50 to 57 d prior to sacrifice.

Methods
Preparation of kidney homogenate and assay of 1α-, 24- and 23-hydroxylases were done according to the method of Engstrom et al. (4). Vitamin D metabolites determined by the method of Horst et al. (16), Ca and Mg by atomic absorption spectrophotometry (23), and P by the method of Fiske and Subbarow (7).

RESULTS
Table 1 shows 25-OHD₃-1α-, 24- and 23-hydroxylase activities of calves treated with 1,25-(OH)₂D₃, PTH, 1,25-(OH)₂D₃ and PTH, or fed a vitamin D-deficient or normal diet. The 1α-hydroxylase activity of calves treated with 1,25-(OH)₂D₃ was reduced to below detectable limits and both 24- and 23-hydroxylase activities were increased 4-fold over that of their respective controls (Table 1). In contrast, calves fed a vitamin D-deficient diet for 50 to 57 d increased their renal 1α-hydroxylase activity (479 ± 53 pmol/min per g tissue) 13-fold rela-
tive to the control 1α-hydroxylase of 37 ± 11 pmol/min per g. The 24-hydroxylase and 23-hydroxylase activities decreased to below the detectable concentrations. Calves treated with PTH had a 7-fold increase (254 ± 56 pmol/min per g) in 1α-hydroxylase activity. Parathyroid hormone had no significant effect on 24-hydroxylase and 23-hydroxylase activities (Table 1). Renal 1α-hydroxylase activity of calves treated first with 1,25-(OH)2D3 and 12 h later with PTH was lower than the formal control calves. Renal 24-hydroxylase and 23-hydroxylase activities of these same calves were higher than for calves treated with 1,25-(OH)2D3 alone.

Plasma Ca, P, Mg, and 1,25-(OH)2D3 concentrations in relation to treatment are shown in Table 2. Plasma Ca was not significantly increased in 1,25-(OH)2D3-treated calves. In contrast, plasma P was increased (P<.01) and plasma Mg was decreased (P<.01) in 1,25-(OH)2D3-treated calves. In the vitamin D-deficient calves, plasma Ca and 1,25-(OH)2D3 were reduced (P<.01) compared with that of controls. The PTH-treated calves had increased plasma 1,25-(OH)2D3 (P<.01) and decreased P (P<.01) concentrations compared with those of controls (Table 2). Plasma Ca, P, and Mg of the calves treated with 1,25-(OH)2D3-PTH were not significantly different than those of the controls (Table 2).

### DISCUSSION

Vitamin D metabolism studies usually involve assay of circulating blood concentrations of vitamin D metabolites in response to a treatment or diet variation. These analyses do not determine differences in rate of biosynthesis or degradation of the metabolites. In order to understand the biochemical mechanism for regulation of production of 1,25-(OH)2D3, direct assays of renal 1α-hydroxylase activity are necessary. This is the first report in which bovine renal 1α-hydroxylase, 24-hydroxylase and 23-hydroxylase activities, plasma Ca, Mg, P,

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**TABLE 1. Activities** of renal 25-hydroxyvitamin D3 (25-OHD3) 1α-hydroxylase, 24-hydroxylase, and 23-hydroxylase in calves receiving different treatments.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Kidney 25-OHD3 hydroxylases</th>
<th>1α-</th>
<th>24-</th>
<th>23-</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>pmol/min/g</td>
<td>X</td>
<td>SE</td>
<td>X</td>
</tr>
<tr>
<td>Control (8)</td>
<td>37 ± 11</td>
<td>138</td>
<td>32</td>
<td>85</td>
</tr>
<tr>
<td>1,25-(OH)2D3 2 (9)</td>
<td>ND ab</td>
<td>570</td>
<td>68ab</td>
<td>357</td>
</tr>
<tr>
<td>-D3 (4)</td>
<td>479 ± 53ab</td>
<td>112</td>
<td>44</td>
<td>52</td>
</tr>
<tr>
<td>PTH6 (5)</td>
<td>254 ± 56a</td>
<td>1159</td>
<td>592ab</td>
<td>959</td>
</tr>
</tbody>
</table>

*Significantly different from control (P<.01).

*Significantly different than PTH-treated animals (P<.01).

1 Activity is expressed as picomoles of 24,25-(OH)2D3, 23,25-(OH)2D3, or 1,25-(OH)2D3 produced per minute per gram of kidney cortex tissue. The 25-OHD3 substrate concentration was 100 µg/incubation tube (50 µM). Numbers in parentheses are the animals per treatment group, and the values for hydroxylase activity are means ± SEM.

2 1,25-Dihydroxyvitamin D3-treated calves received intramuscularly (i.m.) 25 µg in 1 ml ethanol at 24 h and again 2 h before sacrifice.

3 Calves were fed a vitamin D-deficient diet 50 to 57 d prior to sacrifice.

4 Calves treated with parathyroid hormone received 3 to 4 mg in .75 ml .01 M acetic acid i.m. 16 h and again 4 h before sacrifice.

5 Calves given 25 µg 1,25-(OH)2D3 i.m. 24 h presacrifice and 7 mg PTH i.m. 12 h presacrifice.

6 Enzyme activity was below the detectable limits of the assay.
and 1,25-(OH)₂D₃ were all determined in the same experiment. The results provide an insight to vitamin D metabolism that has not been possible using traditional methods.

Calf renal 1α-hydroxylase activity was inhibited by treatment with 1,25-(OH)₂D₃. This was accompanied by large increases in renal 24-hydroxylase and 23-hydroxylase activities (6). The reciprocal relationship of renal 1α-hydroxylase and 24-hydroxylase activities was consistent with reports of these enzyme activities in rats (2), chicks (21), pigs (5), and other animals (6). Inhibition of 1α-hydroxylase activity is reported to be due to feedback inhibition by 1,25-(OH)₂D₃ (3), although it is likely that increases in plasma Ca and P also inhibit renal 1α-hydroxylase. These observations are important in regard to reports on the use of 1,25-(OH)₂D₃ for treatment of milk fever. Hove and Kristiansen (17) reported that treating cows with daily oral doses of 200 µg of 1,25-(OH)₂D₃ for 5 d before parturition reduced the incidence of milk fever at parturition but induced a delayed severe hypocalcemia at 9 to 13 d after parturition. They suggested that this was due to the action of 1,25-(OH)₂D₃ in suppressing renal 1α-hydroxylase.

Kidneys of vitamin D-deficient calves had the greatest 1α-hydroxylase activity and the lowest 24-hydroxylase and 23-hydroxylase activities, in agreement with previous reports (2, 4). The PTH treatment stimulated 1α-hydroxylase activity, but the increase was less than that obtained in vitamin D-deficient calves. It seems likely that the higher blood Ca concentration of the PTH-treated animals prevented 1α-hydroxylase activity from reaching the extremely high activity seen in vitamin D deficiency.

Our study demonstrated that PTH stimulated renal 1α-hydroxylase activity in young calves 7-fold relative to activity in the untreated controls. The PTH has also been reported to stimulate renal 1α-hydroxylase activity in rat kidneys (1), chick kidney slices (25), and cultured kidney cells (13). Although PTH treatment stimulated 1α-hydroxylase activity, there was no significant reciprocal decline in 23-hydroxylase and 24-hydroxylase activity, as was seen in vitamin D-deficient calves. The 1α-hydroxylase activity of calves treated with PTH after receiving 1,25-(OH)₂D₃ was lower than that of control calves, although not as low as in 1,25-(OH)₂D₃-treated animals that did not receive PTH.


<table>
<thead>
<tr>
<th>Treatment</th>
<th>Ca</th>
<th>Mg</th>
<th>P</th>
<th>1,25-(OH)₂D₃</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>X</td>
<td>SE</td>
<td>X</td>
<td>SE</td>
</tr>
<tr>
<td>Control (8)</td>
<td>10.9</td>
<td>.7</td>
<td>2.23</td>
<td>.13</td>
</tr>
<tr>
<td>1,25-(OH)₂D₃ (9)</td>
<td>11.2</td>
<td>.4</td>
<td>1.76</td>
<td>.07ab</td>
</tr>
<tr>
<td>-D³ (4)</td>
<td>6.9</td>
<td>.8ab</td>
<td>.23</td>
<td>.14</td>
</tr>
<tr>
<td>PTH (5)</td>
<td>11.0</td>
<td>.3</td>
<td>.23</td>
<td>.14</td>
</tr>
<tr>
<td>1,25-(OH)₂D₃ + PTH (3)</td>
<td>11.8</td>
<td>.7</td>
<td>1.99</td>
<td>.11</td>
</tr>
</tbody>
</table>

aSignificantly different from control (P<.01).
bSignificantly different from PTH-treated animals (P<.01).

1 The number of animals per treatment group are shown in parentheses and the concentrations of plasma constituents is the mean ± SEM.
2 The 1,25-(OH)₂D₃-treated calves received 25 µg in 1.0 ml ethanol i.m. 24 h and again 2 h before sacrifice.
3 Calves were fed a vitamin D-deficient diet 50 to 57 d prior to sacrifice.
4 Calves treated with parathyroid hormone received 3 to 4 mg in .75 ml .01 M acetic acid i.m. 16 h and again 4 h prior to sacrifice.
5 Calves received 25 µg of 1,25-(OH)₂D₃ and 7 mg PTH 24 and 10 h prior to sacrifice, respectively.

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not receive PTH. The dosages used in this experiment were pharmacological; nevertheless, our results indicated that the inhibitory effect of 1,25-(OH)\(_2\)D\(_3\) on 1α-hydroxylase activity was greater than the stimulatory effect of PTH. Vitamin D-deficient calves had lower plasma Ca concentrations than the PTH-treated calves, an observation that suggests that 23-hydroxylase and 24-hydroxylase enzymes are regulated by plasma concentration of Ca rather than by PTH.

Recently, Goff et al. (12) reported that acute (96 h) PTH administration to cows resulted in increased plasma concentrations of both Ca and 1,25-(OH)\(_2\)D\(_3\). The PTH given to four cows prone to milk fever for at least 60 h prior to calving prevented development of milk fever; four untreated cows developed milk fever. Goff et al. (12) concluded that stimulation of 1α-hydroxylase activity by PTH increased production of 1,25-(OH)\(_2\)D\(_3\), which increased intestinal absorption of Ca and augmented bone mineral mobilization. Further experiments are necessary to determine if more prolonged hypercalcemia can completely override PTH effect on renal vitamin D hydroxylases.

REFERENCES


