Effects of Continuous Administration of 1,25-Dihydroxyvitamin D3 on Plasma Minerals and Unoccupied Colon Mucosal 1,25-Dihydroxyvitamin D3 Receptor Concentrations

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ABSTRACT

Six mature nonlactating, nonpregnant Jersey cows were implanted with Alzet mini-osmotic pumps, which delivered 50 μg of 1,25-dihydroxyvitamin D3 [1,25-(OH)2D3] each day for 7 d in an effort to mimic plasma concentrations of 1,25-dihydroxyvitamin D [1,25-(OH)2D] observed in cows at parturition. Plasma samples were obtained daily beginning 6 d prior to implantation and ending 8 d after removal of the implants. Six biopsies of the descending colon mucosa were obtained per rectum before and after implantation and assayed for unoccupied 1,25-(OH)2D3 receptor concentration. Plasma concentration of 1,25-(OH)2D increased from 37 pg/ml pretreatment to 294 pg/ml with the pumps implanted. Plasma Ca concentration increased within 2 d after implantation and remained elevated for 7 d after the pumps were removed. Unoccupied colon mucosa 1,25-(OH)2D3 receptor mean concentration prior to treatment was 14.6 fmol/mg protein and increased within 2 d following implantation to 30.5 fmol/mg protein. These data suggest that 1,25-(OH)2D3 upregulates its own receptor in the intestine of the cow.

INTRODUCTION

Bovine parturient paresis is a hypocalcemic disorder associated with a failure of the Ca homeostatic mechanisms to meet the Ca demands imposed by the onset of lactation. Intramuscular administration of 1,25-dihydroxyvitamin D3 [1,25-(OH)2D3] can be used to prevent parturient paresis (1, 10). This route of administration has two major faults that have limited the use of 1,25-(OH)2D3 in dairy cows. When a prophylactic dose of 1,25-(OH)2D3 is injected intramuscularly, blood concentrations of 1α,25-dihydroxyvitamin D [1,25-(OH)2D] will often exceed 1000 pg/ml for 24 h after injection, which is several times greater than the highest physiological concentration observed in the bovine. Thereafter, blood concentrations decline rapidly so that by 3 d after injection, exogenous 1,25-(OH)2D3 is no longer detectable in the blood (16). To be effective, 1,25-(OH)2D3 has to be administered not more than 4 d prior to parturition. Repeated injections of 1,25-(OH)2D3 are inconvenient and may result in severe hypercalcemia prior to parturition. Also, there is some evidence that very high 1,25-(OH)2D can directly affect on renal tissue, resulting in loss of renal function (11, 14). Elevating blood concentrations of 1,25-(OH)2D several times above normal prepartal plasma concentrations and maintaining that concentration for some period prior to parturition may eliminate some of the toxicity problems associated with supraphysiologic 1,25-(OH)2D in plasma and may also permit earlier administration of the compound without reducing....
EFFECTS OF 1,25-DIHYDROXYVITAMIN D3 ON ITS RECEPTOR

2937

its ability to prevent parturient paresis.

Costa and Feldman (6) reported that 1,25-(OH)2D3 administration increased intestinal unoccupied receptors for 1,25-(OH)2D in the rat. These results suggest that 1,25-(OH)2D regulates concentrations of its own receptor, which might be expected to regulate tissue responsiveness to subsequent 1,25-(OH)2D3 stimulation.

The objective of the study was to examine the effects of continuous subcutaneous delivery of 1,25-(OH)2D3 by an osmotic pump on plasma concentrations of 1,25-(OH)2D3, 25-hydroxyvitamin D3 (25-OHD3), Ca, inorganic phosphorus (Pi), and Mg in dairy cows.

MATERIALS AND METHODS

Six mature, nonlactating, nonpregnant Jersey cows weighing between 370 and 450 kg were housed in individual pens and fed 4 kg of alfalfa hay cubes and 3 kg of pelleted concentrate that supplied 9.13 Mcal net energy, .94 kg protein, 78 g Ca, 23 g Pi, and 6600 IU vitamin D/d, and met or exceeded NRC recommendations (19) for other nutrients. All cows had been fed the experimental diet for at least 1 wk prior to use in this experiment. Three hundred fifty micrograms of 1,25-(OH)2D3 were dissolved in 1932 μl of propylene glycol and inserted into an Alzet mini-osmotic pump (Model #2ML1, Alza Corp., Palo Alto, CA). Each osmotic pump was implanted subcutaneously and delivered 50 μg of 1,25-(OH)2D3 each day for 7 d in an effort to mimic plasma concentrations of 1,25-(OH)2D observed in cows at parturition. Heparinized blood samples were obtained from the jugular vein daily beginning 6 d prior to implantation and ending 8 d after removal of the implants. Plasma Ca and Mg concentrations were determined by atomic absorption spectrophotometry (20). Plasma Pi was determined colorimetrically (9). Plasma concentrations of 1,25-(OH)2D and 25-OHD3 were determined by the method of Reinhardt et al. (21).

Seven biopsies of the descending colon mucosa were obtained per rectum before and after implantation. Mucosal biopsies from the colon were obtained after restraining the cows in stanchions and administering 5 ml of a 2% lidocaine solution into the epidural space between the first and second caudal vertebrae. This prevented cows from straining and reduced the risk of rectal tears during the procedure. Approximately 15 L of warm tap water were then used to flush feces from the rectum and descending colon. One-gram samples of mucosa were scraped from the descending colon mucosa with a bent medical spoon for assay of unoccupied 1,25-(OH)2D3 receptor concentration. Colon mucosa was placed in 10 mM Tris buffered saline containing 50 IU Trasylol/ml (pH 7.4) and maintained on ice until assayed (less than 1 h). All subsequent steps were performed at 4°C. Samples were vigorously mixed and centrifuged at 3000 rpm for 10 min. The supernant, containing traces of feces and blood, was discarded, and the pellet was resuspended in 10 mM Tris, 1 mM dithiothreitol, and 1.5 mM EDTA buffer containing 200 μg/ml soybean trypsin inhibitor (pH 7.4), and was again mixed vigorously and centrifuged at 3000 rpm for 10 min. The supernatant, containing extracellular proteases and debris, was discarded and the pellet was resuspended in three volumes (weight/volume) of 600 mM KCl, 50 mM Tris, 1.5 mM EDTA, and 5 mM dithiothreitol buffer containing 200 μg/ml soybean trypsin inhibitor (pH 7.4). The mucosal cells were then disrupted using a Brinkman Instruments Polytron and the homogenate was centrifuged at 229,000 × g for 20 min in a Beckman ultracentrifuge (Beckman Instruments, Palo Alto, CA). Homogenization of the mucosal cells in a high salt buffer extracts the 1,25-(OH)2D receptor protein out of the cell nucleus into the cytosol (8, 25). The 1,25-(OH)2D3 receptor protein in the supernatant was collected and assayed for its unoccupied receptor content. Five hundred microliters of cytosol were added to three 12 × 75-mm test tubes containing an excess of [3H]1,25-(OH)2D3 [150,000 cpm/tube, specific activity = 88 Ci/mmol 1,25-(OH)2D3] and to three tubes containing 100 ng unlabeled 1,25-(OH)2D3 in addition to [3H]1,25-(OH)2D3 in order to determine total and nonspecific binding of [3H]1,25(OH)2D3 in the samples. Receptor-bound [3H]1,25-(OH)2D3 was separated from free [3H]1,25-(OH)2D3 by hydroxylapatite (26). Protein content of
the cytosol preparations was determined (2) and receptor assay results are expressed as femtomoles 1,25-(OH)₂D₃ receptor/mg protein in the cytosol preparation. Differences between the observed values at each time point during the treatment period and those observed during the control period prior to 1,25-(OH)₂D₃ administration were determined for each parameter measured. Student's paired t test (24) was used to test the hypothesis that the mean difference was equal to zero. Results were declared significant if \( P<.05 \).

RESULTS

Plasma concentration of 1,25-(OH)₂D (Figure 1) increased \((P<.01)\) from 37 ± 4 pg/ml pretreatment to 197 ± 33 pg/ml at 24 h after implantation with 1,25-(OH)₂D₃. Plasma concentration of 1,25-(OH)₂D peaked at 48 h after pump implantation at 294 ± 31 pg/ml and remained elevated until the pumps were removed on d 7.

On the 3rd and 4th d after removal of the pumps, plasma concentration of 1,25-(OH)₂D was lower \((P<.05)\) that that observed during the pretreatment period. Plasma concentration of Ca increased \((P<.05)\) from 9.82 ± .25 mg/dl pretreatment to 11.74 ± .49 mg/dl within 3 d after implantation and remained elevated for 8 d after removal of pump (Figure 1). Plasma concentrations of Pi increased \((P<.01)\) from 5.58 ± .28 mg/ml pretreatment to 7.45 ± .31 mg/dl within 3 d after implantation and remained elevated \((P<.05)\) for 8 d after removal of the implants (Figure 2). Plasma concentrations of Mg decreased \((P<.05)\) from 1.98 ± .09 mg/dl pretreatment to 1.58 ± .04, and 1.69 ± .06 mg/dl at 2 and 3 d after implantation, respectively. Plasma concentration of Mg returned to pretreatment values within 48 h of removal of the 1,25-(OH)₂D₃ implants (Figure 2). Plasma concentrations of 25-OHD₃ decreased gradually from 46.3 ± 5.1 ng/ml pretreatment to 41.1 ± 4.7 ng/ml at d 7 after implantation, and continued to decrease slowly after the implants were removed (Figure 2). The concentration of colon mucosal unoccupied receptors for 1,25-(OH)₂D₃ prior to implantation was 14.6 ± 4.8 fmol/mg protein and increased \((P<.1)\) within 2 d following implantation to 30.5 ± 12.3 fmol/mg protein. Colon mucosal unoccupied receptor concentration remained elevated for at least 5 d after implants were removed when compared with the pretreatment period (Figures 1 and 3).

DISCUSSION

The elucidation of the vitamin D endocrine system and the availability of potent vitamin D metabolites renewed the search for an agent capable of preventing parturient paresis.
Figure 3. The effects of continuous administration of 1α, 25-dihydroxyvitamin D$_3$ [1α, 25-(OH)$_2$D$_3$; 50 μg/d] on colon mucosal 1α, 25-(OH)$_2$D$_3$ receptor (●) concentrations as a percentage of pretreatment of receptor. Values are means plus standard errors of six cows.

(11, 16, 18, 23). The use of 1,25-(OH)$_2$D$_3$ has several advantages over the use of vitamin D or 25-(OH)D$_3$. It is capable of rapidly increasing plasma concentrations of Ca and, since it has a relatively well-defined duration of action, it is less likely to result in prolonged hypercalcemia and toxicity. However, the shorter biological life also requires a more accurate prediction of parturition for full effectiveness if intramuscular injection is used. When 500 μg of 1,25-(OH)$_2$D$_3$ were administered intramuscularly to nonlactating, nonpregnant Jersey cows, the plasma 1,25-(OH)$_2$D$_3$ concentration rapidly increased after injection, reached a peak of 1,050 pg/ml at 12 h, and returned to preinjection concentration within about 4 d (16). Oral administration of 500 μg of 1,25-(OH)$_2$D$_3$ increased plasma 1,25-(OH)$_2$D$_3$ concentration to about 250 pg/ml 1 d after treatment and, as with intramuscular administration, returned to preinjection concentrations within 4 d (16).

The relative degree of hypercalcemia induced by the two methods of administration was virtually the same, suggesting that the mechanisms for intestinal absorption of Ca are stimulated maximally at a concentration of plasma 1,25-(OH)$_2$D$_3$ of 250 pg/ml or less (oral administration may have had some advantages as a result of local effects on the gut). Therefore, elevating blood 1,25-(OH)$_2$D$_3$ to concentrations that stimulate maximal intestinal absorption of Ca several days prior to parturition should be capable of preventing parturient paresis. The additional benefit would be that the blood concentration of 1,25-(OH)$_2$D$_3$ required would be within the physiological range rather than the pharmacological range seen following intramuscular administration. This should make it possible to minimize the prolonged inhibition of renal 1α-hydroxylase that follows administration of the commonly used doses of most vitamin D compounds (17). An implant that released small amounts of 1,25-(OH)$_2$D$_3$ continuously prior to parturition might increase the practicality and the safety of 1,25-(OH)$_2$D$_3$ as a preventive of parturient paresis. We used mini-osmotic pumps in nonpregnant, nonlactating cows to increase plasma 1,25-(OH)$_2$D$_3$ to concentrations similar to those observed in the experiments of Hove et al. (16) and maintained those concentrations for approximately 1 wk. Mini-osmotic pumps implanted subcutaneously and delivering 50 μg 1,25-(OH)$_2$D$_3$ raised plasma 1,25-(OH)$_2$D$_3$ concentration to about 300 pg/ml, which is at the high end of the physiological range for plasma 1,25-(OH)$_2$D$_3$. The pump effectively maintained this concentration of 1,25-(OH)$_2$D$_3$ until it was removed. Plasma concentration of Ca was increased by the 1,25-(OH)$_2$D$_3$ administration. The increase in plasma concentration of Ca observed in this experiment was similar to that observed by Hove et al. (16) following oral or intramuscular administration of 500 μg of 1,25-(OH)$_2$D$_3$, except that the hypercalcemia induced was of longer duration. It has previously been shown that exogenously supplied 1,25-(OH)$_2$D$_3$ does not stimulate increased bone resorption and that hypercalcemia results from an increased rate of intestinal absorption of Ca (3, 11). The degree of hypercalcemia seen in our experiment was higher than is probably safe in the dairy cow (11). Perhaps plasma 1,25-(OH)$_2$D$_3$ can be reduced to much lower concentrations and still be capable of preventing parturient paresis. Once the implants were removed, plasma 1,25-(OH)$_2$D$_3$ concentration declined...
rapidly and fell to values slightly below those of pretreatment. This may represent some inhibition of renal 1α-hydroxylase or may be reflective of the degree of hypercalcemia present in the cows at that time. A 1,25-(OH)₂D₃ implant that supplied 1,25-(OH)₂D₃ at a reduced rate after parturition would reduce the problem of delayed parturient paresis that has been reported as a result of inhibition of endogenous 1,25-(OH)₂D₃ synthesis by exogenous hormone administration (17).

Recently, Costa and Feldman (6) reported that administering 1,25-(OH)₂D₃ to vitamin D-deficient rats significantly increased duodenal 1,25-(OH)₂D₃ receptor number to 130% of control values. These results suggested that 1,25-(OH)₂D₃ upregulates its own receptor, which would be expected to result in increased responsiveness to subsequent 1,25-(OH)₂D₃ treatment (4, 22). Repeated biopsy of duodenal mucosa for quantitation of 1,25-(OH)₂D₃ receptors was technically not feasible in this experiment. However, in previous experiments, we have found that there is a high degree of correlation between the concentration of 1,25-(OH)₂D₃ receptors in the colon and in the duodenum in rats under various physiological conditions, although concentration of unoccupied colon 1,25-(OH)₂D₃ receptor concentration is generally about 70% of that in duodenum (12). Because descending colon mucosa was readily obtained, we utilized unoccupied colon 1,25-(OH)₂D₃ receptor concentration as an indicator of receptor concentration in the higher segments of the gut. Unoccupied colon mucosa 1,25-(OH)₂D₃ receptor concentration doubled (P<.10) over pretreatment values within 2 d following implantation. These data support the results reported by Costa and Feldman (6) in rats and suggest that 1,25-(OH)₂D₃ upregulates its own receptor in the intestine of the cow. The 1,25-(OH)₂D₃ receptors did not decrease immediately upon withdrawal of the 1,25-(OH)₂D₃ implant. Unfortunately, the experiment was not designed to ascertain how long the receptors would remain upregulated. If receptor upregulation is an important aspect of 1,25-(OH)₂D₃ activity in the intestine, perhaps it is one of the means by which 1,25-(OH)₂D₃ prevents parturient paresis. Perhaps upregulation of 1,25-(OH)₂D₃ receptors can be achieved by lower doses of 1,25-(OH)₂D₃ or by compounds other than 1,25-(OH)₂D₃, such as glucocorticoids (17), which could be used in conjunction with or instead of 1,25-(OH)₂D₃ to prevent parturient paresis. Further studies are necessary to determine the physiological significance of these findings.

In the present study, plasma concentration of 25-OHD₃ after implantation of 1,25-(OH)₂D₃ tended to decrease. This is probably due to increased metabolism of 25-OHD₃ to 24- and 23-hydroxylated compounds, since 1,25-(OH)₂D₃ stimulates the activity of enzymes responsible for these reactions (5, 7). Administration of 1,25-(OH)₂D₃ caused a small but significant decline in concentration of plasma Mg and a significant increase in concentration of plasma Pi in cows similar to that observed in earlier reports (11, 16).

In conclusion, elevated plasma concentrations of 1,25-(OH)₂D₃ can be maintained by subcutaneous release of small amounts of 1,25-(OH)₂D₃ via an osmotic pump, which may improve the practicality of 1,25-(OH)₂D₃ as a means of preventing parturient paresis. These results also suggest that receptor upregulation by 1,25-(OH)₂D₃ may be an important means of enhancing intestinal Ca absorption in the cow. Further studies should concentrate on developing 1,25-(OH)₂D₃ implants to continuously deliver small amounts of 1,25-(OH)₂D₃ to prevent parturient paresis in dairy cows.

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