Recurring Hypocalcemia of Bovine Parturient Paresis Is Associated with Failure to Produce 1,25-Dihydroxyvitamin D

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Recurring Hypocalcemia of Bovine Parturient Paresis Is Associated with Failure to Produce 1,25-Dihydroxyvitamin D

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ABSTRACT. Parturient paresis (milk fever) is a hypocalcemic disorder caused by the onset of lactation in the dairy cow. In most cows a complete recovery follows a single iv calcium treatment to correct the acute hypocalcemia. However, about 20% of cows treated for parturient paresis experience recurring episodes of hypocalcemia (relapses) requiring further treatment. Analysis of plasma from 8 nonrelapsing parturient paretic and 11 relapsing parturient paretic cows revealed differences in plasma 1,25-dihydroxyvitamin D [1,25-$(OH)_2$D] concentrations before and during the development of hypocalcemia. In nonrelapsing cows, plasma 1,25-$(OH)_2$D increased to 4- to 5-fold as plasma calcium concentrations declined during the first stage of parturient paresis. In relapsing cows, decreases in plasma calcium concentrations during the first stage of parturient paresis were accompanied by just a 2- to 2.5-fold increase in plasma 1,25-$(OH)_2$D. Plasma 1,25-$(OH)_2$D eventually increased 4- to 5-fold in the relapsing cows, but this response was delayed 24–48 h compared with the response in the nonrelapsing cows. Plasma PTH concentration profiles were similar in relapsing and nonrelapsing cows, suggesting that renal 25-hydroxyvitamin D 1α-hydroxylase was temporarily refractory to stimulation by PTH in the relapsing cows. In both groups of cows recovery from parturient paresis began about 12–24 h after plasma 1,25-$(OH)_2$D concentrations had increased 4- to 5-fold. These data imply that lack of production of 1,25-$(OH)_2$D is an important factor in predisposing the cow to relapses of parturient paresis and is critical for recovery from the hypocalcemia associated with the onset of lactation. (*Endocrinology* **125:** 49–53, 1989)

PARTURIENT paresis (milk fever, periparturient hypocalcemia) is an acute hypocalcemic disorder associated with the onset of lactation in the cow. On the day of parturition, dairy cows commonly produce 10 liters or more of colostrum containing 23 g or more of calcium, approximately 6 times as much calcium as the extracellular calcium pool contains (1). Most animals adapt to the onset of lactation by rapidly increasing intestinal calcium absorption and bone calcium resorption mechanism activity, permitting replacement of extracellular calcium lost to lactation. However, in some cows the calcium homeostatic mechanisms of the body fail to adequately replace calcium lost from the extracellular calcium pool as a result of lactation. These animals become severely hypocalcemic, which disrupts nerve and muscle function, resulting in recumbency and the clinical syndrome referred to as parturient paresis. Without veterinary attention (consisting primarily of iv administration of calcium salts), many of these animals would die. Most cows recover after a single treatment. However, approximately 20% of parturient paretic cows will suffer one or more relapses to treatment and require repeated injections of calcium (2).

As assays for measuring PTH and 1,25-dihydroxyvitamin D [1,25-$(OH)_2$D] in blood were developed, it was shown that both PTH (3) and 1,25-$(OH)_2$D (4) were present in very high concentrations in the blood of cows with parturient paresis. Thus, failure to produce and secrete either hormone in response to hypocalcemia was not believed to contribute greatly to the development of or clinical recovery from parturient paresis. We have identified a number of parturient paretic cows in which the plasma 1,25-$(OH)_2$D concentration does not increase in response to the hypocalcemia occurring as a result of the onset of lactation. All of these cows had suffered relapses after initial treatment of parturient paresis. Therefore, although a primary hormone deficiency does not seem to be the cause of parturient paresis in most cases, there exists a subtype of parturient paresis in which lack of production of 1,25-$(OH)_2$D is a major factor in the development and persistence of severe hypocalcemia.

Materials and Methods

The National Animal Disease Center maintains a herd of mature Jersey cows to study the pathogenesis of parturient

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paresis. A retrospective analysis of data obtained from 19 parturient paretic cows will be presented. All cows used in this study had at least 3 previous lactations and had a history of parturient paresis at a previous calving. To maintain a high incidence of parturient paresis, the cows were fed a high calcium diet before parturition (5). Beginning 6 weeks prepartum, cows were fed a diet of alfalfa hay *ad libitum* plus 4.0 kg 0.93% calcium concentrate feed. The dietary ingredients supplied each animal with approximately 100–120 g calcium and 30–40 g phosphorus daily. Approximately 2 weeks prepartum, the cows received an additional 6.5 kg 1.25% calcium concentrate daily so that their diet supplied about 140–170 g calcium and 75–80 g phosphorus/animal·day in the last days of gestation and for the first 2 weeks of lactation (6). Approximately 85% of the cows in the herd developed parturient paresis under these conditions.

Daily plasma samples were obtained from all cows between the 10th day prepartum and the 14th day postpartum. Around the time of parturition, sampling was more frequent, usually every 12 h for 1 or 2 days before and after parturition. Cows that developed clinical parturient paresis (recumbent and hypocalcemic) were sampled again just before treatment with 500 ml 23% calcium borogluconate solution (Norcalciphos, Norden Labs, Lincoln, NE). Eight of the cows in this study recovered uneventfully after a single treatment and are referred to as nonrelapsing cows. Relapsing cows suffered additional periods of hypocalcemia and recumbency and required additional iv calcium therapy to effect a recovery.

The plasma calcium concentration was determined by atomic absorption spectrophotometry (7). The plasma phosphorus concentration was determined by a colorimetric assay (8). The plasma 1,25-$(OH)_2$D concentration was determined by the method of Reinhardt *et al.* (9). Plasma concentrations of intact PTH were determined by immunoradiometric assay (10) (Incstar Corp., Stillwater, MN), using human PTH standards. Serially diluted hyperparathyroid plasma obtained from a cow with parturient paresis exhibited parallelism compared to human PTH standards in this assay (data not shown). To further validate the assay for the cow, plasma PTH determined in a severely hypocalcemic cow (plasma calcium, 4.8 mg/dl) decreased from 943 to 41 pg/ml within 20 min of treatment with iv calcium salts (plasma calcium, 15.1 mg/dl). Therefore, the immunoradiometric assay is able to detect acute changes in PTH status in the cow. Plasma from four nonrelapsing and eight relapsing parturient paretic cows was available for PTH analysis. Unfortunately, there was not enough plasma collected and stored to determine intact PTH concentrations in all cows involved in the study.

Student's t test was used to test the hypothesis that the mean difference between relapsing and nonrelapsing values was equal to zero at daily intervals for each parameter. Results were considered significant when the probability of a larger t was less than 0.05. (11). Student's t test was used to test for differences in the slope of lines determined by the least squares criterion method for the regressions of plasma concentrations of 1,25-$(OH)_2$D or PTH on calcium (11).

Results

Plasma calcium and 1,25-$(OH)_2$D concentration profiles from 11 relapsing cows are contrasted with those obtained from 8 nonrelapsing cows in Figs. 1 and 2. All cows were severely hypocalcemic at parturition, and all cows became recumbent within the first 24 h after calving. Nonrelapsing cows were treated with iv calcium and returned to normocalcemia about 3 days after parturition. Relapsing cows required 2 or more iv calcium treatments and did not become normocalcemic until about 5 days after parturition. Plasma phosphorus concentrations did not differ between relapsing and nonrelapsing cows. Both groups became moderately hypophosphatemic as they developed hypocalcemia. Relapsing cows remained hypophosphatemic for 24–48 h longer than the nonrelapsing cows.

Plasma 1,25-$(OH)_2$D concentrations during the week before parturition were lower in the relapsing cows than in the nonrelapsing cows. Plasma 1,25-$(OH)_2$D in nonrelapsing cows increased 4- to 5-fold as the hypocalcemia

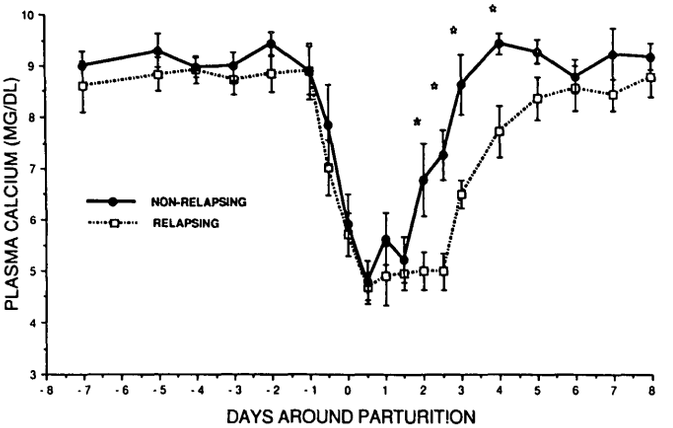

FIG. 1. Mean (± SE) plasma calcium concentrations in relapsing and nonrelapsing parturient paretic cows around the time of parturition (day 0). ★ Significantly different from relapsing parturient paretic cows ($P < 0.05$).

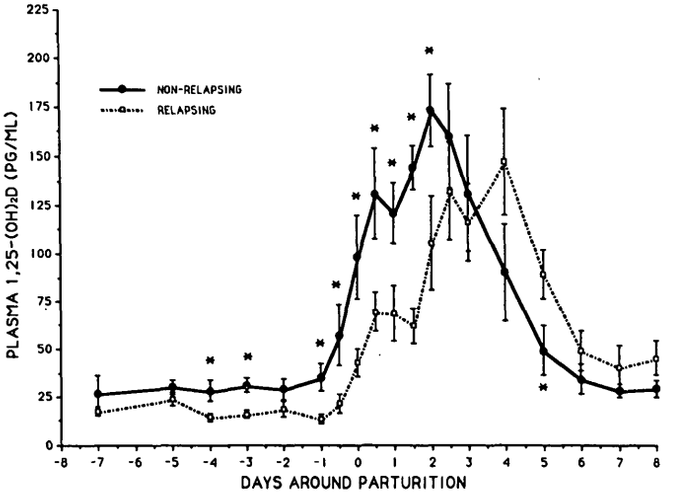

FIG. 2. Mean (± SE) plasma 1,25-$(OH)_2$D concentrations in relapsing and nonrelapsing parturient paretic cows around the time of parturition (day 0). * Significantly different from relapsing parturient paretic cows ($P < 0.05$).

associated with the onset of parturient paresis developed. However, despite the fact that all cows suffered similar degrees of hypocalcemia, plasma 1,25-$(OH)_2D$ in relapsing cows did not exhibit the same increase as in nonrelapsing cows. A strong inverse relationship was found between the plasma concentration of 1,25-$(OH)_2D$ and the plasma calcium concentration during the period encompassing 3 days before parturition and the development of parturient paresis in the nonrelapsing cows, but not in the relapsing cows (Fig. 3). The slope of the line describing the regression of plasma 1,25-$(OH)_2D$ on the plasma calcium concentration was significantly smaller ($P < 0.02$) in the relapsing cows than in the nonrelapsing cows. Relapsing cows eventually exhibited a 5- to 6-fold increase in plasma 1,25-$(OH)_2D$ concentration, but this increase was delayed 24–48 h compared to that in nonrelapsing cows.

Plasma PTH concentrations were determined in eight relapsing cows and four nonrelapsing cows. Plasma PTH began to increase in both relapsing and nonrelapsing cows 12 h before parturition and was about 5–7 times prepartum PTH levels at the time of parturition in both groups (Fig. 4). Plasma PTH returned to prepartal levels 5 days postpartum in both groups of cows. Plasma PTH was inversely correlated with plasma calcium concentration in both the relapsing and the nonrelapsing cows during the period encompassing 3 days before parturition and the development of parturient paresis. The slopes of the lines describing the regression of plasma PTH on plasma calcium concentration in relapsing and nonrelapsing cows were not statistically different ($P > 0.05$).

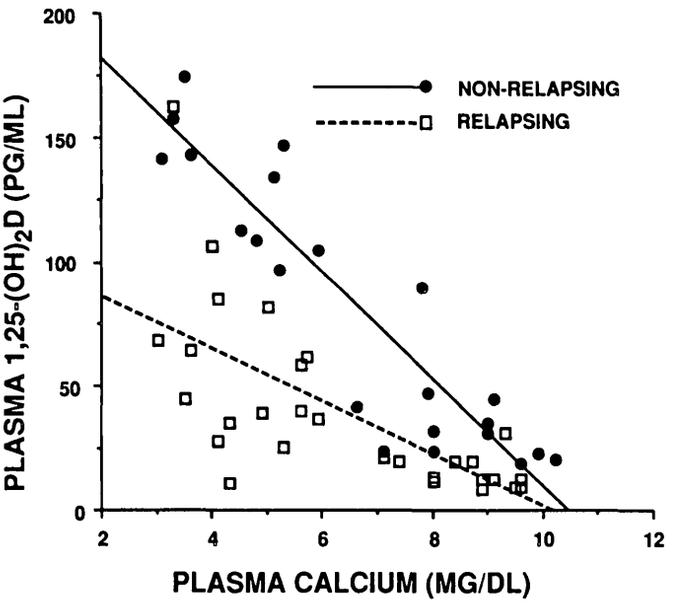

FIG. 3. Plasma concentration of 1,25-$(OH)_2D$ plotted against plasma calcium concentration 3 days before parturition and just before first treatment for parturient paresis was administered. The equation of the regression line was $y = 21.53\ x + 225$ ($r^2 = 0.819$) for the nonrelapsing parturient paretic cows and $y = 10.52\ x + 107$ ($r^2 = 0.465$) for the relapsing parturient paretic cows.

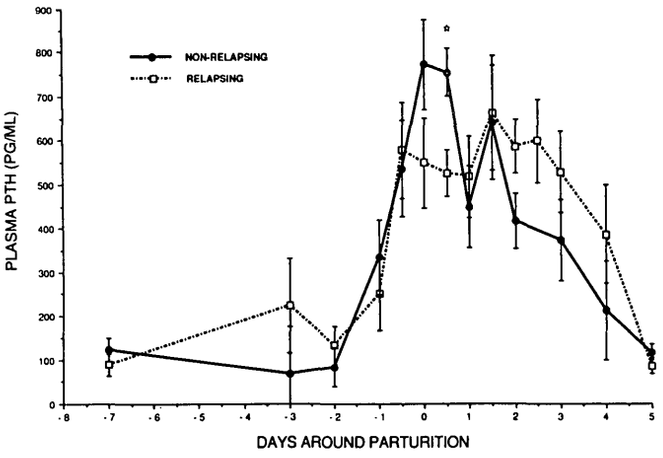

FIG. 4. Mean (± SE) plasma intact PTH concentrations in relapsing and nonrelapsing parturient paretic cows around the time of parturition (day 0). ★ Significantly different from relapsing parturient paretic cows ($P < 0.05$).

Discussion

Cows developing parturient paresis can be classified as relapsers or nonrelapsers based on the ability of a single calcium treatment to effect a complete recovery. The present data suggest that the differences in clinical response of the cows result from differences in plasma 1,25-$(OH)_2D$ concentrations observed as the animals develop severe hypocalcemia. Both relapsing and nonrelapsing cows develop the same degree of initial hypocalcemia and secondary hyperparathyroidism, but production of 1,25-$(OH)_2D$ is about 2-fold greater in nonrelapsing than in relapsing cows. Reduced and delayed production of 1,25-$(OH)_2D$ in response to severe hypocalcemia seems to be an important factor in predisposing the cow to relapses of parturient paresis.

Bovine parturient paresis occurs in three stages. In the first stage, the plasma calcium concentration decreases rapidly as calcium leaves the extracellular fluid and enters the mammary gland. In most cows (nonrelapsers), plasma PTH and 1,25-$(OH)_2D$ inversely increase as the plasma calcium level decreases. However, about 16–24 h of 1,25-$(OH)_2D$ stimulation are required before intestinal calcium transport is increased significantly above basal prepartal rates (12, 13). Similarly, bone calcium resorption (recruitment and activation of osteoclasts) is not significantly increased until after 48 h of PTH and 1,25-$(OH)_2D$ stimulation (14). Thus, the increased plasma PTH and 1,25-$(OH)_2D$ levels are not immediately capable of reversing the declining plasma calcium concentrations. In the second stage of parturient paresis, plasma calcium concentration decreases to the point where nerve and muscle function are disrupted, resulting in clinical

recumbency and eventually coma. Rumen motility and intestinal peristalsis cease, preventing utilization of dietary calcium. Unless treated with iv calcium salts, most of these cows die. Treatment temporarily restores the plasma calcium concentration to levels that will permit normal nerve and muscle function for several hours. The nonrelapsing cow now begins the third stage of parturient paresis, during which calcium homeostasis is recovered. With rumen and intestinal motility restored, the intestinal absorption mechanisms, now activated by 1,25-$(OH)_2D$, can more efficiently use dietary calcium. Slowly over the next 3–4 days, the plasma calcium concentration will return to normal levels.

In contrast to the 4- to 5-fold increases in plasma 1,25-$(OH)_2D$ observed in nonrelapsing parturient paretic cows, plasma 1,25-$(OH)_2D$ concentrations in cows that suffer relapses of parturient paresis increase just 2-fold during the decline in plasma calcium that occurs during the first stage of parturient paresis. The relapsing parturient paretic cow enters the second stage of parturient paresis without adequate 1,25-$(OH)_2D$ stimulation to activate efficient intestinal calcium absorption mechanisms. Therefore, even when rumen and intestinal motility are restored temporarily by iv calcium salt injection, hypocalcemia and clinical paresis are likely to recur, since the extracellular calcium flow to the mammary gland will continue to be much greater than the entry of calcium via the intestine. Some cows remain in this stage (stage II) of the disease for several days, dependent on repeated injections of calcium salts to sustain life. After anywhere from 12–48 h of severe hypocalcemia, plasma 1,25-$(OH)_2D$ eventually increases 4- to 5-fold in these cows. Shortly thereafter, these cows also begin their recovery and enter stage III of parturient paresis.

Since the plasma PTH concentration responses to declining plasma calcium concentration were similar in relapsing and nonrelapsing cows, failure of relapsing cows to produce 1,25-$(OH)_2D$ indicates end-organ resistance to PTH stimulation. These cows could then be considered to be temporarily pseudohypoparathyroid, as PTH normally stimulates renal 1α-hydroxylase activity (15). A current hypothesis on the etiology of parturient paresis suggests that bone and intestine of the parturient paretic cow are temporarily refractory to stimulation by PTH and/or 1,25-$(OH)_2D$ (16, 17). The relapsing cow may simply represent a more exaggerated form of the PTH refractoriness common to all parturient paretic cows in that renal 25-hydroxyvitamin D-1α-hydroxylase activity, in addition to bone, is affected.

Regardless of the underlying defect in the parturient paretic cow, it seems clear that recovery from the hypocalcemia associated with the onset of lactation occurs only after there is a significant increase in the plasma concentration of 1,25-$(OH)_2D$. The dairy cow relies more on intestinal calcium absorption than bone calcium resorption to meet the lactational demands for calcium during the first weeks after parturition (18). Since 1,25-$(OH)_2D$ stimulates efficient transport of calcium across the intestine, it seems reasonable to assume that cows that fail to produce adequate 1,25-$(OH)_2D$ after parturition will not be able to maintain normal plasma calcium concentrations. These data suggest that therapeutic intervention with 1,25-$(OH)_2D$ may be of some aid in preventing relapses of parturient paresis. A trial involving the use of prepartal injections of a synthetic analog of 1,25-$(OH)_2D$ to prevent parturient paresis supports this concept (6). In this trial cows receiving 24-F-1,1,25-$(OH)_2D_3$ that developed parturient paresis (generally cows treated <24 h before calving) suffered fewer relapses than untreated cows that developed parturient paresis.

Earlier studies have indicated that prepartal plasma 1,25-$(OH)_2D$ concentrations in cows developing parturient paresis and those that do not develop parturient paresis do not differ (4). However, these studies did not separate the parturient paretic cows into relapsing and nonrelapsing groups for examination. In the present study prepartal plasma 1,25-$(OH)_2D$ concentrations of relapsing parturient paretic cows were generally lower than those of nonrelapsing cows, which may indicate that intestinal calcium absorption mechanisms are more severely depressed in relapse-prone cows than in the nonrelapsers. The plasma 1,25-$(OH)_2D$ concentration 1 week prepartum may, therefore, help identify those cows at risk of developing the relapsing parturient paretic syndrome and which therapies would be most appropriate.

In summary, we have identified a subset of dairy cows in which the inverse relationship between plasma concentrations of calcium and 1,25-$(OH)_2D$ observed in most cattle is temporarily suspended. These cows fail to produce increased amounts of 1,25-$(OH)_2D$ despite severe hypocalcemia and very high circulating PTH levels in the blood. Further, recovery from the hypocalcemia associated with parturient paresis seems to occur only after there is a significant increase in the plasma concentration of 1,25-$(OH)_2D$ in both relapsing and nonrelapsing parturient paretic cows.

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