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POSTPARTURIENT HYPOCALCEMIA OF DAIRY COWS: A MODEL FOR THE STUDY OF THE INTERDEPENDENCE OF CA, Pi, AND MG HOMEOSTASIS

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ABSTRACT

Disorders of calcium, phosphorus and magnesium homeostasis in ruminants provide natural models for the study of the physiology and pathophysiology of these minerals. The knowledge that can be acquired with a better understanding of the pathogenesis of these diseases could give useful clues in the puzzle of human osteoporosis. In the present study, the case of parturient paresis of dairy cows is reexamined with a newly developed technique for the measurements of serum ionized magnesium concentrations (Mg^{2+}). The concentrations of total magnesium (Mg_{tot}), ionized calcium (Ca^{2+}), total calcium (Ca_{tot}), and inorganic phosphate (P_i) were also determined in the sera of seventeen 3- to 16-year-old Brown Swiss and crossed Simmental/Red Holstein cows during the periparturient period. In each animal, a transient increase of Mg^{2+} and Mg_{tot} serum concentrations was observed in association with the transient decrease after parturition of Ca^{2+}, Ca_{tot} and P_i serum concentrations. On average, throughout the study, serum Mg^{2+} concentrations were 68.5% of those of Mg_{tot} whereas serum Ca^{2+} concentrations were 52% of those of Ca_{tot}. The possible mechanisms involved in the transient increase of Mg^{2+} and Mg_{tot} serum concentrations are discussed and the relevance of this data for osteoporosis is outlined.

INTRODUCTION

Among food animal species, sheep and goats are the most adequate candidates for osteoporosis models due to their relative small size. Disorders of calcium, phosphorus and magnesium homeostasis in ruminants of larger size however provide natural models for the study of the physiology and pathophysiology of these minerals (3). Examples in dairy cows are parturient paresis (hypocalcemia), lactation tetany (hypomagnesemia) and postparturient hemoglobinuria (low dietary phosphate) which are diseases whose pathogenesis is influenced or caused by an unbalanced diet. In the case of parturient paresis, the integrated hormonal response has already been extensively studied (20,28,30,35,38). The hypocalcemia which occurs most commonly within the first 48 hours after parturition is related to the sudden drainage of calcium into milk at the onset of lactation. At that time, the cow's body is unable to mobilize enough calcium and the animals become paretic.

Magnesium deficiency causes osteoporosis (12,36,37). It has been postulated that the contribution of magnesium to bone stability occurs by activation of the magnesium-dependent adenosine triphosphatase H^+-K^+ pump in osteocytes and so by maintaining the pH of the bone extracellular fluid at a slightly higher value (13). Furthermore, in magnesium depletion the secretion of parathyroid hormone (PTH) is impaired and the serum concentrations of 1,25-vitamin D are low (1). Because magnesium concentration is marginally lower in serum and lower in erythrocytes and bone of human patients with postmenauposal or senile osteoporosis than in healthy controls, magnesium may be a contributing factor

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in the pathogenesis of osteoporosis (8,9,11,29). Magnesium malabsorption may be involved in this process. It has been suggested that supplementation with magnesium should be considered as an adjunct for the prevention and treatment of osteoporosis (39). An exact understanding of the homeostasis of magnesium is thus necessary.

In bovine plasma, about 70% of the magnesium and 50% of the calcium are in the physiologically active free form (Mg^{2+} and Ca^{2+}, respectively; 24,41). Approximately 43% of serum calcium is bound to proteins and approximately 10% is complexed with bicarbonate, phosphate, lactate and citrate (14). Similarly, serum magnesium is bound to proteins or organic and inorganic anions (40). Recently, ion-selective electrodes have been designed for determining serum Mg^{2+} concentration (40,41). For this report, the concentrations of Mg^{2+}, Ca^{2+}, total magnesium (Mg_{tot}), total calcium (Ca_{tot}), and inorganic phosphorus (P_i) were measured in serum during the periparturient period of nonparetic dairy cows.

ANIMALS, MATERIALS AND METHODS

Seventeen 3- to 16-year-old Brown Swiss and crossed Simmental/Holstein cows were studied. During the sampling period, the cows were housed indoors and fed silage, hay and a conventional concentrate supplement. Two months before parturition, they received poor quality silage and hay in order to reduce energy and calcium intake. The magnesium content of the food was measured and found to adequately cover the daily requirements. Water was provided *ad libitum*. The cows were only partially milked during the 3 days that followed parturition in order to minimize calcium drainage into milk. None of the animals exhibited signs of parturient paresis. Blood samples were obtained from one jugular vein via evacuated tubes. The first samples were taken on one occasion between 15 and 5 days before the expected calving date and once each morning during 6 days after parturition. The blood was allowed to clot, was centrifuged at 1580 g for 10 minutes at 4°C and the serum was aspirated. The serum concentrations of Mg^{2+} and Ca^{2+} were quantified within 72 hours. During this period, the samples were maintained at 4°C and both electrolytes were stable. The remaining serum was stored at -20°C for the other analyses. The serum concentrations Mg_{tot}, Ca_{tot}, P_i, total protein and albumin were determined by colorimetry with an automated analyser (Cobas Mira, F. Hoffmann-La Roche, Basle, Switzerland), using commercial kits. The analyses were based on the following methods: methylthymol blue for Ca_{tot}, phosphomolybdate without precipitation of proteins for P_i and the Biuret reaction for total protein (F. Hoffmann-La Roche), calmagite for Mg_{tot} (Biomérieux, Lyon, France) and bromocresol green for albumin (Boehringer Mannheim, Germany). Serum potassium (K) concentration was quantitated by flame photometry (Instrumentation Laboratory, Milan, Italy). Serum osmolarity was determined by depression of the freezing point (Knauer automatic semi-micro osmometer, Berlin, Germany). Serum Ca^{2+} and Mg^{2+} concentrations were quantitated using ion selective electrodes (AVL 988-4 prototype apparatus, AVL List GmbH, Graz, Austria) with internal quality and accuracy controls and were corrected for the mean physiological pH 7.4 (37°C) by linear regression. Changes with time in the parameters Mg^{2+}, Mg_{tot}, Ca^{2+}, Ca_{tot}, P_i, total protein, albumin, K and osmolarity were examined by repeated measurement analysis of variance using the version 6.08 of the main frame computer-implemented SAS procedure ANOVA (SAS Institute Inc, Cary, NC, USA). For each parameter, the values for the days postpartum were contrasted with those of the prepartum period or between adjacent days by use of the options contrast and profile, respectively. The level of significance was fixed at 0.05.

RESULTS

In each animal, a transient increase of serum Mg^{2+} and Mg_{tot} concentrations was observed in association with the transient decrease after parturition of serum Ca^{2+}, Ca_{tot}, and P_i concentrations. The situation is illustrated for one 8-year-old cow (Fig 1). Plots for individual animals indicated that the concentration profiles of serum Mg^{2+} and Mg_{tot} and of Ca^{2+} and Ca_{tot} were parallel and that the duration of the increase of Mg^{2+} and Mg_{tot} was related to the duration of the decrease of Ca^{2+} and Ca_{tot}. For both serum magnesium and calcium, the degree of complexation remained constant during the hypocalcaemic episode. On average, serum Mg^{2+} concentrations were 68.5% of those of Mg_{tot} whereas serum Ca^{2+} concentrations were 52.0% of those of Ca_{tot}. In the serum of almost all animals, the P_i concentration profiles were parallel to those of Ca^{2+} and Ca_{tot}. When compared with reference laboratory values, the serum concentrations of Ca^{2+}, Ca_{tot} and P_i of some cows dropped to values below the normal range whereas the peak increase for Mg^{2+} and Mg_{tot} always stayed within the physiological norm. When all cows were considered, the repeated measurement analysis of variance indicated that the serum concentrations of Ca^{2+}, Ca_{tot}, and P_i were significantly decreased during day 1 and day 2 postpartum, whereas those of Mg^{2+} and Mg_{tot} were significantly increased. On day 3, serum Ca_{tot} concentrations were still significantly decreased. Serum osmolarity and albumin, total protein and K serum concentrations remained unchanged throughout the course of the study.

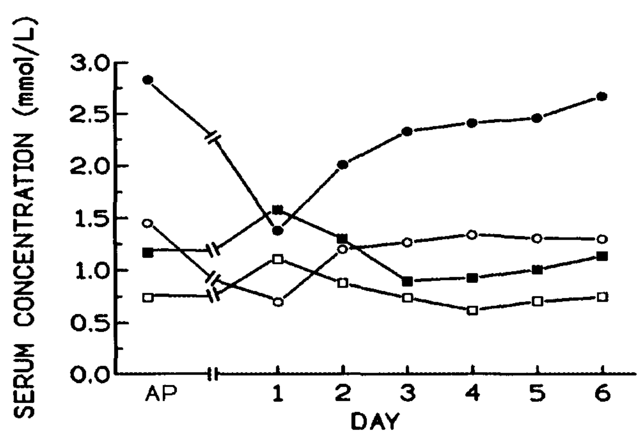

FIG. 1 Serum concentrations of Ca_{tot} (●), Ca^{2+} (O), Mg_{tot} (■) and Mg^{2+} (□) in one 8-year-old Brown Swiss cow during the periparturient period.

DISCUSSION

The parallel profiles of the serum concentrations of Mg^{2+} and Mg_{tot} during the course of the mild nonclinical hypocalcemia indicate that the free concentration may be predicted from the total concentration. In mild hypocalcemia, the concentrations of the ligands of serum magnesium are not altered and the ratio Mg^{2+}/Mg_{tot} was thus maintained. Similarly, the parallelism of the profiles of the serum concentrations of Ca^{2+} and Ca_{tot} of this report confirmed the findings of other studies which have included paretic cows (4,23,24).

Serum osmolarity and serum concentrations of albumin and total protein remained unchanged during the hypocalcemic episode indicating that no dehydration occured. During the periparturient period, water consumption has never been measured but appetite is reduced (35,38). Dehydration caused by decreased water intake was suspected because water intake is associated with food intake in healthy pigmy goats (32). Hemoconcentration has been observed in animals with clinical signs of parturient paresis (34). Because no dehydration was present in the cows of this study, an adjustment of Ca_{tot} concentration for albumin concentration was not necessary.

The milk/serum concentration ratios are 10 to 15 for Ca_{tot}, 5 for Mg_{tot} and 20 for P_i (38). The transiently increased serum concentrations of Mg^{2+} and Mg_{tot} associated with the decreased Ca^{2+} and Ca_{tot} is remarkable in light of magnesium drainage into milk. This phenomenon has been observed in several studies related with postparturient paresis (4,18,44) and during short term fasting which is characterized by decreased serum Ca_{tot} concentration (17,18). Conversely, a decreased Mg_{tot} was reported after administration of Ca_{tot}-elevating doses of vitamin D metabolites (43,44). This effect may presumably be attributable to the hypercalcemia that is induced (30). These findings strongly suggest that one or some of the mechanisms involved in the regulation of calcium and P_i homeostasis in cows also affect the serum concentrations of Mg^{2+} and Mg_{tot}. In support of this hypothesis, urinary magnesium is transiently decreased during the hypocalcemic period that follows parturition in dairy cows, suggesting increased tubular reabsorption (18). Furthermore, in the kidney of rats, the segments 1-34 of bovine PTH and synthetic PTH-related protein (PTHrP) infused via osmotic minipumps stimulate the reabsorption of magnesium (31). Also, infusion of bovine PTH for 60 minutes promotes the reabsorption of magnesium in the kidney of thyroparathyroidectomized hamsters (7). Finally, infusion of PTH in dairy cows during the last trimester of pregnancy induces an increase of serum Mg_{tot} concentration (15). Thus, the effect on the kidney of increased serum PTH induced by the hypocalcemia could be one of the factors involved in the transient increase of serum Mg^{2+} and Mg_{tot} concentrations observed in periparturient cows.

Assuming a milk magnesium concentration of 4.5 mmol/L and a milk production of 10 L, approximately 1 g of magnesium is excreted into milk. Using the data from Halse (18), it may be calculated that the magnesium loss in milk is compensated with the 1 g of magnesium reabsorbed in the kidney. However, the increase of serum Mg_{tot} concentration of 0.20 mmol/L corresponds to a magnesium increase of 1 g in the extracellular fluid volume. Magnesium must thus be mobilized from another source which may be bone, the gastro-intestinal tract, a shift of magnesium from the intracellular compartment to the extracellular compartment or a combination of the 3 factors.

As 60% of the body magnesium is stored in bone, mobilisation from bone at the time of parturition could contribute to the observed increase of Mg^{2+} and Mg_{tot} (27). Bone magnesium is present in the bone extracellular fluid, in the hydration shell of the hydroxyapatite cristals and on the cristal surfaces, but not in the cristal interior (16). PTH administration induces in a first stage and without the activation of osteoclasts the release of bone calcium from a rapidly turning over pool, presumably the bone extracellular fluid and the hydration shell on the cristal surfaces (22), and could thus also induce magnesium release. It is difficult to evaluate how much of the 150 to 200 g of magnesium of the cows' bones may be mobilized by this mechanism. In a later phase (within 24 hours), after stimulation of PTH release or PTH administration which activates osteoclasts and results in increased bone resorption (5), more magnesium may be released from bone. However, because the Ca:Mg ratio is about 50:1, a large amount of bone has to be remodeled to yield a significant amount of magnesium.

Vitamin D and its hydroxylated metabolites enhance intestinal magnesium absorption in rats and in humans (18). In the ovine and bovine rumen, an active transport has been demonstrated for the absorption of magnesium (25,26). Whether Vitamin D and its metabolites are involved in the absorption of magnesium in ruminants is unknown. As vitamin D concentration increases during postparturient hypocalcemia (30,38), it could possibly be involved in an enhancement of magnesium absorption and thus contribute to the observed transient increase of Mg^{2+} and Mg_{tot}.

A shift of magnesium from the intracellular compartment to the extracellular compartment during the course of hypocalcemia may also be involved in the observed transient increase of Mg^{2+} and Mg_{tot}. Because serum K concentration remained constant, the involuting uterus was very probably not the source of the elevation of serum Mg^{2+} and Mg_{tot}. Magnesium is only second to K as the main intracellular ion (33). A reduced affinity of magnesium for plasma proteins during hypocalcemia could also be involved.

At the onset of lactation, the portion of the large amount of the PTH-related protein produced in the mammary gland that is released in the blood stream may also play a role in magnesium homeostasis, because the biological activity of PTH-related protein is identical to that of PTH (2,21). Other hormones including some of the hormones involved in stress affect magnesium homeostasis: calcitonin, growth hormone, aldosterone, antidiuretic hormone, glucagon, ß-adrenergic agonists and insulin (6,10). However, magnesium is less rigidly regulated than calcium and a specific magnesium-regulating hormone has not been identified (42).

In conclusion, the observations on magnesium concentration during the course of hypocalcemia of parturient paresis of dairy cows raise interesting questions. A more complete understanding of the pathogenesis of this disease and other metabolic diseases of ruminants may provide clues on the homeostatic regulation of this mineral. This in turn could shed some light into some aspects of bone physiology and pathophysiology and consequently allow to design better strategies for the treatment and the prevention of osteoporosis.

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