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Is calcitonin an active hormone in the onset and prevention of hypocalcemia in dairy cattle?

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ABSTRACT

The objective of this study was to assess the potential importance of calcitonin (CALC) in the onset of subclinical hypocalcemia (experiment 1) and in the physiological mechanisms underlying the prevention of bovine hypocalcemia under metabolic acidosis (experiments 2 and 3). In experiment 1, 15 Holstein cows naturally incurring subclinical hypocalcemia during the first 5 d postpartum were classified as low subclinical hypocalcemia (LSH) when blood Ca concentrations were between 7.5 and 8.5 mg/dL, or as high subclinical hypocalcemia (HSH) when blood Ca concentrations were between 6.0 and 7.6 mg/dL. Blood samples were taken daily from d −5 to 5 relative to parturition to determine concentrations of parathyroid hormone (PTH), CALC, and $1,25(OH)_2D_3$. In experiment 2, 24 Holstein bulls (497 ± 69 kg of body weight and 342 ± 10.5 d of age) were assigned to 2 treatments (metabolic acidosis or control). Metabolic acidosis was induced by an oral administration of ammonium chloride (2.5 mEq/d) during 10 d, and animals were slaughtered thereafter. Blood samples were collected before slaughter to determine CALC, PTH, $1,25(OH)_2D_3$, and samples of urine, kidney, parathyroid, and thyroid glands were obtained immediately after slaughter to determine expression of several genes in these tissues. Last, in experiment 3, we tested the activity of CALC under metabolic acidosis in vitro using breast cancer cell (T47D) cultures. Although PTH tended to be greater in HSH than in LSH, the levels of $1,25(OH)_2D_3$ were lower in HSH cows (experiment 1). Blood CALC concentration was not affected by the severity of subclinical hypocalcemia, but it was influenced by days from calving (experiment 1). The expression of PTH receptor (PTHR) in the kidney was increased under metabolic acidosis (experiment 2). Furthermore, the activity of CALC was impaired under acidic blood pH (experiment 3). In conclusion, the CALC rise in HSH cows after calving impaired the recovery of blood Ca concentrations because the PTHR response was not sufficient to activate $1,25(OH)_2D_3$ and compensate for the CALC effect. Metabolic acidosis prevents hypocalcemia because the expression of PTHR is upregulated in the kidney, resulting in an increased PTH activity and a subsequent increase in $1,25(OH)_2D_3$ serum concentrations. Moreover, an impairment of CALC activity at low pH enhances the hypercalcemic role of PTH.

Key words: calcitonin, hypocalcemia, metabolic acidosis, parathyroid hormone, $1,25(OH)_2D_3$

INTRODUCTION

Parturient paresis is a common disease following calving in dairy cattle resulting from an inability of homeostatic mechanisms to regulate calcemia during a high demand for Ca when the lactating period begins. Hypocalcemia appears not only in clinical cases, but also in a subclinical form in 45% of cows. In both clinical and subclinical cases, the longevity and productivity of the cow are impaired (Goff, 2008; Murray et al., 2008), resulting in important economic losses for the dairy cattle industry. Under physiological conditions, the actions of calcitonin (**CALC**), parathyroid hormone (**PTH**), and $1,25(OH)_2D_3$ control blood Ca concentrations. A decrease in blood Ca triggers the parathyroid gland to secrete PTH, which increases renal Ca reabsorption and induces the expression of 1-α-hydroxylase in the kidney, responsible for producing $1,25(OH)_2D_3$ (Wasserman and Fullmer, 1995). One of the most important effects of $1,25(OH)_2D_3$ is to stimulate Ca absorption through the active transport across intestinal epithelial cells (Pérez et al., 2008). In addition, $1,25(OH)_2D_3$ stimulates, in conjunction with PTH, osteoclastic resorption activity of bone Ca (Horst et al., 2003). By contrast, CALC inhibits the resorption of bone Ca and increases urinary Ca loss to lower blood Ca concentration (Murray et al., 2008). Blood CALC concentrations increase in response

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to hypercalcemia (Austin and Heath, 1981; Findlay and Sexton, 2004) and studies with nonruminant animals also demonstrate an increase of CALC in response (or even in advance) to feed intake (Roos et al., 1980).

Thus far, no consensus exists about the triggering hormonal cause of the sudden drop of blood Ca. It is known that PTH and $1,25(OH)_2D_3$ increase after a decrease in blood Ca around parturition (Wasserman and Fullmer, 1995). Because cow hypocalcemia is a disease related to low levels of Ca in blood, it could be argued that CALC may be involved in the onset of this affliction. However, evidence supporting a role of CALC in hypocalcemia in the cow is inconsistent. Capen and Young (1967) first proposed an etiological role of CALC in the incidence of parturient paresis. They found diminished CALC content in thyroid glands of cows with parturient paresis and suggested that an abrupt release of CALC near parturition may be related to the development of hypocalcemia. Barlet (1967) induced hypocalcemia in young and mature cows by intravenous administration of porcine CALC and also concluded that CALC release contributed to hypocalcemia or parturient paresis. In contrast, Mayer et al. (1975) suggested that the development of hypocalcemia at parturition may be associated with a diminished prepartal secretion of CALC. Similarly, Hollis et al. (1981) did not observe changes in blood CALC concentration along the periparturient period, but found that paretic animals had lower blood concentrations of CALC than normocalcemic animals. Lastly, Shappell et al. (1987) detected fluctuations in serum CALC between parturition and the fifth day postpartum in contrast to the response reported by Hollis et al. (1981). According to the Mayer et al. (1975) and Hollis et al. (1981) experiments, cows with normal or subclinical concentrations of blood Ca showed the highest peak of CALC at postpartum, whereas the lowest concentrations of serum CALC were found in clinical hypocalcemic cows.

Conversely, the diet of the cows before parturition may be involved in the incidence of periparturient paresis and hypocalcemia. Most prepartal diets based on forages are high in cations, such as Na and especially K. These diets may induce metabolic alkalosis and promote hypocalcemia because renal production of $1,25(OH)_2D_3$ and osteoclastic bone resorption are decreased in contrast to anionic diets (Goff et al., 2008). Hence, the induction of metabolic acidosis through the supplementation of anionic salts creates a negative metabolic balance of Ca and prevents hypocalcemia at calving (Goff and Horst, 1998; Goff, 2008). Understanding the mechanisms that prevent a decrease in blood Ca concentrations at calving could also help to elucidate the key factors involved in the onset of hypocalcemia and to assess whether CALC has a relevant role in this process. However, the mechanism by which addition of anions to counteract cations in the diet of a cow enhances Ca homeostasis is not well understood. Leclerc and Block (1989), Goff et al. (1991), and Phillippo et al. (1994) provided indirect evidence that physiological functions stimulated by PTH, such as bone resorption and production of $1,25(OH)_2D_3$, were enhanced in cows fed diets containing added anions. Also, Goff et al. (2014) recently demonstrated that metabolic alkalosis reduces tissue sensitivity to PTH injections in comparison to cows under metabolic acidosis. Previous studies have also reported that metabolic acidosis increases the expression of CALC receptor (**CALCR**) in mouse osteoclasts (Biskobing and Fan, 2000) and upregulates the expression of PTH receptor (**PTHR**) along with PTH in rat osteoblast-like cells (Disthabanchong et al., 2002). Nevertheless, to our knowledge, no study has been conducted in cattle to assess the role of PTHR and CALCR in Ca homeostasis during metabolic acidosis. Furthermore, we know of no previous study that has evaluated whether CALC activity is affected under metabolic acidosis. The objective of the current study was to assess the role of CALC during subclinical hypocalcemia by comparing the hormonal levels at the onset of subclinical hypocalcemia and assessing the potential involvement of CALC in the physiological mechanisms participating in the prevention of bovine hypocalcemia under metabolic acidosis.

MATERIALS AND METHODS

Experiment 1

Animals and Sample Collection. Fifteen Holstein cows (mean ± SD; 4.01 ± 1.1 yr old, average lactation number = 1.6 ± 0.7, lactation length = 362 ± 86.1 d, average milk production/yr = 8,550 ± 2,503.5 kg, average BW of 674 ± 22.8 kg, and with a milk production peak in previous lactations above 30 kg/d) experiencing subclinical hypocalcemia (blood Ca between 6 and 8.5 mg/dL) during the first 5 d postpartum were classified in 2 groups depending on the levels of blood Ca. Eight animals were categorized as undergoing a low subclinical hypocalcemia (**LSH**), with blood Ca levels ranging between 7.5 and 8.5 mg/dL, and 7 animals were classified as high subclinical hypocalcemia (**HSH**), with blood Ca levels between 6 and 7.4 mg/dL. Blood samples were taken daily from d −5 to 5 relative to parturition to perform subsequent hormonal analyses.

Blood Ca and Hormone Analysis. Plasma Ca was determined by atomic absorption spectrophotometry. Plasma PTH and $1,25(OH)_2D_3$ concentrations were analyzed using commercial bovine ELISA kits from Immunotopics (San Clemente, CA) and Immunodiagnostic