Effect of Treatment of Dairy Cows with Slow-Release Bovine Somatotropin During the Periparturient Period on Minerals in Plasma and Milk and on Parathyroid Hormone-Related Protein in Milk

F.M.K. LAW, D. D. LEAVER, and T. J. MARTIN
St. Vincent's Institute of Medical Research
41 Victoria Parade
Fitzroy, Victoria 3065, Australia

K. SELLECK and J. J. CLARKE
Prince Henry's Institute of Medical Research
Box 152
Clayton, Victoria 3168, Australia

P. J. MOATE
Department of Agriculture
Ellinbank Dairy Research Institute Warragul
Victoria 3820, Australia

ABSTRACT

Slow-release bST was given to dairy cows as a single injection prior to calving to determine whether such treatment prevented parturient hypocalcemia or modified the concentrations of Ca and parathyroid hormone-related protein in milk during the periparturient period. Cows were treated about 1 wk prepertum, and serial blood and milk samples were taken from these and similar prepertum control cows over a 3-wk period. Plasma growth hormone concentrations in the bST-treated group reached a peak 2 d after administration and then declined linearly to control concentrations over a 14-d period. Plasma Ca was unaffected by bST treatment 1 d prior to parturition, on the day of parturition, and at 1 and 6 to 9 d postpartum. Plasma P was higher, and plasma Mg was lower, in the bST-treated group on the day of parturition and 1 d postpartum. Concentrations of Ca, P, Mg, and protein in milk were lower in bST-treated cows than in controls at parturition. Milk production of the bST-treated and control groups was similar when measured at d 6 to 9 postpartum. Concentrations of parathyroid hormone-related protein in milk were substantial at parturition and remained high thereafter, although at parturition the concentration in the milk of the bST-treated group was lower than that of the control group.

(Key words: somatotropin, dairy cows, parturient hypocalcemia, parathyroid hormone-related protein)

Abbreviation key: GH = growth hormone, PTHrP = parathyroid hormone-related protein.

INTRODUCTION

During the periparturient period, many dairy cows develop hypocalcemia, which, if sufficiently severe, results in parturient paresis (milk fever). Some 75% of all cases of milk fever occur within 24 h of calving (16) when the onset of lactation places a sudden demand for Ca on the cow. This metabolic disorder is more common in older than in younger cows, probably because the basal rates of intestinal absorption and bone resorption of Ca are lower in older cows (20). Nevertheless, the endocrine response to the hypocalcemia appears to be adequate (7), except for a group of cases that relapse subsequent to treatment with Ca. Consequently, the reason for the failure to mobilize sufficient systemic Ca to meet the demand of lactation has yet to be established.

Parturient hypocalcemia and milk fever may be prevented by the injection of vitamin D or
its more active analogs just prior to parturition (7), but the response to a single injection is transitory, and one aim of this study was to examine the possibility that injection of cows with a slow-release preparation of bST prior to parturition would prevent the development of parturient hypocalcemia. The rationale for undertaking this experiment was that administration of bST or growth hormone (GH), in addition to increasing milk production in cows (18), also results in net retention of Ca in other species (9), presumably because it raises the serum concentration of 1,25-dihydroxyvitamin D3 (23).

A second aim was to measure the secretion of the novel hormone potentially regulating Ca of PTHrP, in milk during the periparturient period; mRNA for PTHrP is produced in lactating mammary tissue (25), and PTHrP is secreted into milk (12). To explain the changes in maternal Ca metabolism associated with lactation in the rat, a hormone in addition to 1,25-dihydroxyvitamin D3 and parathyroid hormone appears to be required. Thus, loss of bone mineral during lactation was independent of dietary P content, plasma P concentration, and the vitamin D status of the rats. Also, rats fed a high Ca diet lost the same amount of bone mineral as controls even though their mean plasma Ca concentration was 12 mg/dl. Under those conditions, secretion of PTH was minimal and therefore was unlikely to have been responsible for the bone loss. Originally the factor responsible for humoral hypercalcemia of malignancy, subsequently shown to be PTHrP, was proposed to be this additional hormone (2). We have recently demonstrated a linear relationship between the concentrations of PTHrP and Ca in bovine milk (12); thus, although there is no direct evidence, PTHrP produced in mammary tissue may be involved in the transport of Ca from blood to milk. If this is the case, then differences in the production of PTHrP by the mammary gland of individual cows may explain the susceptibility of particular cows to parturient hypocalcemia and milk fever.

MATERIALS AND METHODS

Thirty-four Friesian and Friesian-Jersey cross dairy cows from farms at Ellinbank Dairy Research Institute were used. All cows were over 5 yr of age, calved in spring, had mean production for the previous lactation of 4012 ± 608 (SD) kg and grazed on perennial ryegrass (Lolium perrenne L.) plus white clover (Trifolium repens L.) pasture throughout the experiment. Fourteen cows were given a single subcutaneous injection of 640 mg of slow-release bST (somidobove; Elanco Products Co., West Ryde, New South Wales, Australia) 7 d prior to their expected calving date. Each treated cow was matched with an untreated control cow on the basis of calving date. To ensure that sufficient control cows calved at the appropriate times, more control than treated cows were sampled, and, as a result, the final control group contained 6 more cows than the treated group. The results from all control cows were included in the analyses.

Blood samples were obtained by jugular venipuncture from the treated cows prior to bST administration and then every 2nd to 3rd d until parturition (d 0). Blood was taken on d 0, on d 1, and between d 6 and 9 postpartum. Milk samples were collected manually on d 0, d 1, or both, and between d 6 and 9 postpartum by hand stripping the cows after they were machine milked. Starting at d 8 prior to the estimated calving date, blood samples were taken from control cows, and then blood and milk samples were taken at the same time as for the treated group. Cows that developed milk fever were treated with 350 ml of Ca borogluconate intravenously and the same amount subcutaneously; blood and milk samples were taken prior to treatment. Plasma was obtained by centrifugation of blood samples at 2600 × g at 4°C for 15 min immediately after collection, and aliquots were stored at −20°C until further analysis. Milk samples were frozen and stored at −20°C shortly after collection until analysis.

Concentration of GH in bovine plasma was measured using a radioimmunoassay for ovine GH (having crossreactivity with bovine GH) as described by Thomas et al. (26). The standard ovine GH I-4 and rabbit antibody GH-2 were supplied by the National Institute of Diabetes and Digestive and Kidney Diseases (Washington, DC). The second antibody was a goat anti-rabbit batch 10 prepared at Monash University (Melbourne, Australia). The rabbit antibody GH-2 and the second antibody were used at dilutions of 1:25,000 and 1:60, respectively.

Journal of Dairy Science Vol. 77, No. 8, 1994
Plasma Ca and Mg concentrations were determined using atomic absorption spectrophotometry (19), and P was determined by the colorimetric method of King (11) modified for an autoanalyzer. Milk samples (5 ml) were ashed, dissolved in 10 ml of 20% (vol/vol) HCl, and then diluted to a total volume of 50 ml. This diluted solution was used for the measurement of Ca, Mg, and P using the same methods described for plasma. Milk fat, protein, and lactose concentrations were determined using a Dairylab 2 infrared milk analyzer (Multispec, York, England). The concentration of PTHrP in milk was measured by radioimmunoassay (12).

The data were analyzed using ANOVA with the Genstat 5 computer software (Lawes Agricultural Trust, Rothamsted Experimental Station, Hertfordshire, England). Means were considered different at \( P < .05 \) unless otherwise stated.

RESULTS

For the bST-treated cows, the day of parturition ranged from d 1 to 12 after treatment; 1 cow that calved later than d 13 after treatment was excluded from the analysis. Four cows calved on d 1; 2 each on d 5 and 7; and 1 each on d 2, 3, 8, 10, and 12 after bST administration. The mean time of calving after treatment was 7.2 d. For the control cows, the mean day of calving after the commencement of blood sampling was 6.6 d (range 0 to 18 d).

The mean plasma GH concentrations in both the bST and control groups are shown in Figure 1. For the untreated cows, plasma GH concentrations fluctuated periodically, and the absolute mean concentration varied between 3 and 15 ng/ml. For the cows given the slow-release bST, the plasma GH concentration increased rapidly to a peak of 50 ng/ml 2 d after administration and then gradually declined in a linear manner over the ensuing 14 d to a

![Figure 1. Plasma growth hormone (GH) concentrations in control cows (n = 20; *) and cows (n = 13; ○) treated with a single subcutaneous injection of 640 g of slow-release bST. The 1st d of blood sampling in control cows is d 0. Vertical bars are standard errors of the mean.](image-url)
<table>
<thead>
<tr>
<th>Day</th>
<th>Ca (mM)</th>
<th>Mg</th>
<th>P</th>
<th>Fat (g/L)</th>
<th>Protein</th>
<th>Lactose</th>
<th>PTHrP</th>
<th>PTHrP/Protein</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>X</td>
<td>SD</td>
<td>X</td>
<td>SD</td>
<td>X</td>
<td>SD</td>
<td>X</td>
<td>SD</td>
</tr>
<tr>
<td>0</td>
<td>Control (n = 14)</td>
<td>46.6</td>
<td>4.9</td>
<td>12.0</td>
<td>1.4</td>
<td>55.8</td>
<td>7.0</td>
<td>35.7</td>
</tr>
<tr>
<td></td>
<td>bST (n = 9)</td>
<td>41.1</td>
<td>5.6</td>
<td>9.7</td>
<td>2.5</td>
<td>47.2</td>
<td>4.5</td>
<td>50.4</td>
</tr>
<tr>
<td>1</td>
<td>Control (n = 16)</td>
<td>39.2</td>
<td>5.3</td>
<td>8.6</td>
<td>2.2</td>
<td>44.4</td>
<td>5.9</td>
<td>70.5</td>
</tr>
<tr>
<td></td>
<td>bST (n = 10)</td>
<td>36.0</td>
<td>5.3</td>
<td>6.6</td>
<td>2.4</td>
<td>40.8</td>
<td>5.6</td>
<td>59.3</td>
</tr>
<tr>
<td>6-9</td>
<td>Control (n = 20)</td>
<td>32.8</td>
<td>2.6</td>
<td>5.2</td>
<td>.9</td>
<td>33.8</td>
<td>3.7</td>
<td>97.3</td>
</tr>
<tr>
<td></td>
<td>bST (n = 13)</td>
<td>32.7</td>
<td>2.3</td>
<td>5.4</td>
<td>1.0</td>
<td>31.5</td>
<td>4.6</td>
<td>87.4</td>
</tr>
</tbody>
</table>

*aMeans within the same time period are significantly different (P < .05).

*aMeans within the same time period are significantly different (P < .01).

*PTHrP = Parathyroid-hormone-related protein.
concentration that was similar to that in untreated cows. Treatment of the cows with BST had no influence on plasma Ca concentrations (Figure 2).

The concentrations of plasma GH, Ca, Mg, and P in the BST-treated and control cows during the periparturient period are shown in Table 1. The only significant difference between the groups was for plasma P, which was higher in the BST-treated group on the day of parturition and on d 1 postpartum. Plasma Mg tended to be lower in the BST-treated group, but the difference was not significant. The concentrations of the various constituents of colostrum or milk collected on the day of parturition and on d 1 and 6 to 9 postpartum are shown in Table 2. The concentrations of mineral, protein, and PTHrP in colostrum collected on the day of parturition were significantly lower in the BST-treated group than in control cows, but, thereafter, no significant differences occurred between the two groups.

**DISCUSSION**

The absolute concentrations of plasma GH in the control cows were in agreement with those reported previously (3, 27). For the pregnant cows treated with BST, the pattern of change in plasma GH concentrations was also consistent with the pattern reported previously in lactating cows treated with the same preparation (22). An important problem associated with the application of conventional treatments for milk fever is the maintenance of the elevated plasma Ca concentration over the period between initiation of treatment and some 3 to 4 d after actual calving (15). Consequently, a significant feature of the response to the BST treatment was that plasma GH concentrations were elevated for at least 10 d following treatment. Hence, if BST treatment elevated plasma Ca, the duration of the response would be sufficiently prolonged to avoid the necessity for accurate prediction of the time of calving. Despite the substantial increase in plasma GH concentrations in the treated cows, the plasma Ca concentrations remained within the normal range, and the only significant difference in the plasma constituents between control and treated cows during the periparturient period was an increase in plasma P concentration; this increase was likely due to enhanced intestinal absorption of P (1).

Surprisingly, no significant hypocalcemia occurred in the untreated cows on the day following calving. Although a decline in plasma Ca may have been identified if blood samples had been taken more frequently during the periparturient period, the study was designed on the assumption that hypocalcemia and milk fever normally occur within 24 h of calving (16). Because the cows were on pasture during the experimental period, more detailed metabolic studies to examine whether the turnover of Ca in the treated cows was different from that of the untreated cows were not possible. Nevertheless, if BST treatment modified Ca metabolism through increasing plasma 1,25-dihydroxyvitamin D3 concentrations, then Ca concentration in plasma should have increased (6, 10). Hence, it appeared to be unlikely that a single injection of slow-release BST could substitute for either 1α-hydroxyvitamin D3 or 1,25-dihydroxyvitamin D3 therapy (7, 10) for the prevention of parturient hypocalcemia and milk fever. Before conclusive proof can be obtained, experiments need to be undertaken with parturient cows in which the untreated cows develop hypocalcemia; taking into consideration the changes in milk composition associated with treatment, it is still possible that BST could prevent the decline in plasma Ca that usually follows parturition.

In contrast to the response in plasma mineral concentrations, BST had a significant
effect on the composition of the colostrum obtained on the day of calving, although, except for Mg, these differences disappeared by the following day. The protein content in colostrum was lower, and the fat content higher, in the bST-treated cows than the untreated cows. Similar, although much less substantial, changes in the composition of the milk of multiparous cows treated with bST at 6 mo of lactation (5) apparently resulted from the negative energy and protein balance induced by the treatment. The more substantial changes observed in this study may be due to a direct effect of bST on body tissue because exogenous bST treatment has been shown to elevate blood concentrations of FFA and ketones in some studies (17). Because of these effects on fat metabolism, bST may increase the risk of ketosis, especially during the periparturient period (13). Although this study was not specifically designed to examine ketosis risk, none of the bST-treated cows developed any signs of ketosis during the experimental period.

The concentrations of Ca and Mg in the colostrum of bST-treated cows were significantly lower than in untreated cows. The lower Ca is presumably due to the lower concentration of protein in the milk because two-thirds of Ca in milk is bound to protein. The lower concentration of Mg in colostrum could be because the treated cows had a slightly lower plasma Mg concentration, which was presumably due to the increased lipolysis normally associated with bST treatment (14). Lipolysis may be associated with hypomagnesemia (21) as mobilization of fat results in the redistribution of Mg into adipocytes (4). Periparturient hypocalcemia arises from the sudden sequestration of Ca in the udder and its loss in colostrum on initial suckling or milking. Because the mineral concentration in the colostrum of the bST-treated group was lower than that in the control group, less Ca would be transferred from blood to colostrum of the bST-treated group, provided the total amount of colostrum produced by the two groups was similar. Although the total milk production in the two groups could not be measured until between 6 to 9 d after calving, at that time, no difference existed between the two groups. The failure of bST to increase milk production may have been due to the decline in the amount of bST released from the slow-release preparation; by 6 to 9 d after calving, the concentration of GH in the plasma of the bST-treated cows was the same as in the controls. Alternatively, because endogenous GH secretion is elevated during the periparturient period (3), the mammary gland may be less sensitive to bST during this stage of lactation.

In relation to the secretion of PTHrP in the milk, a striking finding was that significant amounts of PTHrP were present in the colostrum; the concentration in the colostrum of the bST-treated group was lower than that in the untreated group. When the concentration of PTHrP was expressed in relation to the protein content, this difference between the groups tended to disappear and was no longer significant. However, if PTHrP is involved in mobilization of maternal Ca, as it appears to be in humans (8), then parturient hypocalcemia and milk fever cannot be attributed to a lag in production of PTHrP by the mammary gland because PTHrP was present at the same concentration in colostrum and milk collected some 6 to 9 d after calving. If PTHrP is involved in the transfer of Ca from blood to milk, then the lower quantities of PTHrP in the bST-treated group would suggest that this group should be less likely to develop hypocalcemia during the periparturient period. The resolution of these questions depends in part on the measurement of concentrations of the various fragments of PTHrP in mammary vein and systemic venous blood.

ACKNOWLEDGMENTS

We are grateful for the help of Anne Bingham, Veterinary School, University of Melbourne, and Stuart Tracey, the Department of Agriculture, La Trobe University, Melbourne, for carrying out some of the mineral measurements. We also thank Elanco for providing the slow-release bST and the National Health and Medical Research Council and Dairy Research and Development Corporation for financial support.

REFERENCES