Regulation of the immunoglobulin G₁ receptor: effect of prolactin on *in vivo* expression of the bovine mammary immunoglobulin G₁ receptor

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Abstract

Induction of colostrogenesis in non-pregnant cows was used to evaluate the relationship between prolactin (PRL) and mammary immunoglobulin G₁ (IgG₁) receptor expression. Six of eleven non-pregnant, non-lactating Holstein cattle responded to a standard lactation induction protocol by development of elevated IgG₁ concentrations in mammary secretions. In order to increase the diversity in PRL concentrations, two of the six cattle were treated with bromocriptine, and two others were treated with recombinant bovine PRL. Serum α -lactalbumin, serum PRL and mammary secretion IgG₁ concentrations were measured throughout the experiment. Biopsies of mammary tissue were collected after induction of lactation,

and after treatments to alter serum PRL. Immunohistochemistry was used to evaluate IgG₁ receptor expression. Administration of recombinant bovine (rbPRL) was associated with increased lactogenic activity, decreased secretion IgG₁ concentrations, and decreased IgG₁ receptor expression. Decreased serum PRL, due to bromocriptine, was associated with decreased lactogenic activity and maintenance of IgG₁ receptor expression. Results of this experiment are consistent with an effect of PRL in decreasing the expression of the bovine mammary IgG₁ receptor at the onset of lactogenesis.

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Introduction

Immunoglobulin G₁ (IgG₁) is the principal immunoglobulin of bovine colostrum and is derived from maternal circulation (Butler 1969). It is transported into colostrum by an IgG₁-specific receptor located on the surface of alveolar epithelial cells (Hammer *et al.* 1969, Kemler *et al.* 1975). Prepartum transfer of immunoglobulins into mammary secretions, termed colostrogenesis, is a distinct stage of mammary development. During mammary development, colostrogenesis succeeds growth and differentiation of mammary ductular and alveolar tissue (mammatogenesis) and precedes the periparturient onset of copious milk secretion (lactogenesis stage II) (Dembinski & Shiu 1987). Despite broad knowledge regarding regulation of many stages of mammary development, relatively little is known about the regulation of colostrogenesis.

Transfer of IgG₁ into colostrum is initiated during the last weeks of gestation and rapidly declines prior to the onset of lactation. The relationship between lactogenesis

and IgG₁ transport suggests that the hormones which initiate lactation may also suppress colostrogenesis. Hormonal mechanisms regulating lactogenesis have been reviewed (Topper & Freeman 1980, Tucker 1981, Ostrom 1990). During colostrogenesis, serum estrogen increases approximately 4 weeks prepartum; serum corticosteroids, growth hormone, and prolactin (PRL) concentrations increase approximately 1 week prepartum; and serum progesterone decreases 1–2 days prepartum (Tucker 1985). Minimum *in vitro* hormonal requirements for lactogenesis include corticosteroids, PRL and insulin; however, PRL is considered to be the principal lactogenic hormone (Denamur 1971, Ostrom 1990). Indeed, the periparturient secretion of PRL is necessary for the complete structural and biochemical differentiation of bovine mammary epithelial cells (Akers *et al.* 1981a,b). PRL also stimulates *de novo* synthesis and release of α -lactalbumin, the regulatory subunit of lactose synthetase (Goodman *et al.* 1983).

Because of the critical importance of PRL in differentiation of bovine mammary epithelial cells, we initially

investigated the effects of PRL on *in vitro* expression of the bovine mammary IgG₁ receptor (Barrington *et al.* 1997). Explants of mammary tissue from cows forming colostrum were cultured in serum-free, hormonally defined media. α -Lactalbumin production was used as an indicator of lactogenesis and IgG₁ receptor expression was evaluated using immunohistochemistry. Supplementation of culture medium with PRL resulted in decreased binding of IgG₁ to mammary epithelial cells and increased α -lactalbumin production. Results from the *in vitro* study suggest that in addition to its positive lactogenic effect, PRL decreases expression of the bovine mammary IgG₁ receptor.

The purpose of the present study was to investigate the effects of PRL on *in vivo* expression of the bovine mammary IgG₁ receptor. A model of induced lactation was used to initiate IgG₁ transport and receptor expression by treating cows for 7 days with subcutaneous norgestomet ear implants and injections of estradiol 17 β . After induction, cows were administered either; 1) recombinant bovine PRL (rbPRL), to increase serum PRL levels, 2) bromocriptine, to decrease serum PRL levels (Akers *et al.* 1981a), or 3) the vehicle control. Biopsies of mammary tissue were collected to evaluate IgG₁ receptor expression using immunohistochemistry to measure IgG₁ bound to receptors. Serum and mammary secretion samples were obtained to measure serum PRL, serum α -lactalbumin, and secretion IgG₁ concentration.

Materials and Methods

Animals

Eleven non-pregnant, multiparous, non-lactating Holstein cows were obtained at public auction for use in this study. Rectal palpation was performed to determine pregnancy status and identify abnormal ovarian structures (cystic ovaries, retained corpora lutea). No ovarian abnormalities were detected. Bacteriologic cultures of individual glands were obtained and glands yielding positive cultures were omitted from the experiment. The cows were housed in an open sided barn and were given free access to cubed alfalfa hay, fresh water, and trace mineral-supplemented salt blocks. Animal care and procedures were conducted in accordance with established protocols as set forth by the University Animal Care and Protection Committee.

Hormones

Estradiol-17 β (Sigma Chemical Co., St Louis, MO, USA) was solubilized in absolute ethanol to give a stock solution containing 20 mg/ml. The solution was stored at 25 °C until used. Norgestomet ear implants (SYNCHROMATE-B, Sanofi Animal Health, Overland Park, KS, USA 6 mg/implant) were obtained commercially.

rbPRL (lot number 921110–10, Monsanto Co., St Louis, MO, USA) was solubilized in sterile water to give

a concentration of 40 mg/ml and then sterilized by filtration (0.22 μ m). A total of 1.54 g rbPRL was available for the experiment. Aliquots for daily treatments (9.6 ml) were divided and stored at –20 °C until used.

Bromocriptine (Parlodel, Sandoz Research Institute, East Hanover, NJ, USA) was solubilized at a concentration of 40 mg/ml in absolute ethanol:methanol (1:1) immediately prior to use.

Experimental groups

The study was performed during July when the mean temperature was 15.5 °C and the approximate duration of daylight is 15.5 h. To induce lactation, all cows received two subcutaneous norgestomet implants (12 mg total) placed in the left ear on day 1, and daily subcutaneous injections (between 0800 and 0900 h) of estradiol-17 β (0.05 mg/kg) on days 1–7, as previously described with some modifications (Venkatramiah & Narasimha Rao 1992, Byatt *et al.* 1994). Norgestomet implants were removed on day 7 (1800 h). Treatments (rbPRL, bromocriptine, or control) were administered (between 0800 and 0900 h) once daily on days 8–11, as previously described with some modifications (Byatt *et al.* 1994). Three cows received subcutaneous injections of rbPRL (0.19 mg/kg); three cows received subcutaneous injections of bromocriptine (0.11 mg/kg); and five cows received subcutaneous injections of the vehicle control (2 ml, 1:1 ethanol:methanol).

Sample collection

Blood samples were collected every 48 h beginning the day before induction of lactation (day 0) through day 14 of the experiment. Samples (10 ml) were obtained from the coccygeal artery or vein immediately prior to injection of the steroid or treatments. Blood was allowed to clot at 5 °C for 12 h prior to harvest of serum, and serum was stored at –20 °C until assayed.

Mammary secretions were collected every 48 h beginning on day 0 through day 14 of the experiment. At each collection period, samples (2–10 ml) from separate glands were pooled. Samples were stored at –20 °C until assayed.

Between 1 and 2 g samples mammary tissue were obtained via biopsy of either the right or left rear gland using a previously described technique (Farr *et al.* 1996) on days 8 and 12. For anesthesia, cows were administered xylazine (0.05 mg/kg in 5 ml 0.9% saline) in the epidural space (Zaugg & Nussbaum 1990). Biopsies were immediately covered with embedding medium (Tissue-Tek II O.C.T. compound; Miles Inc., Elkhart, IN, USA), frozen over liquid nitrogen, then stored in liquid nitrogen until immunohistochemistry procedures.

Assays

α -lactalbumin assay Serum α -lactalbumin was measured by radioimmunoassay, as previously described (Akers *et al.* 1986). Purified α -lactalbumin was iodinated with ¹²⁵I, as described by Bolt (1981). Serum α -lactalbumin was expressed as ng/ml (mean of triplicates). Intra-assay coefficient of variation on control serum was 11.9%.

Prolactin assay Serum PRL was measured by radioimmunoassay, as previously described (Barnes *et al.* 1985). Serum PRL was expressed as ng/ml (mean of triplicates). Two assays were conducted (high pool and low pool) due to differences in PRL levels from samples. Intra-assay coefficient of variation on control serum was 11.1 and 6.5% for the low pool and high pool respectively.

IgG₁ assay Mammary secretion IgG₁ concentration was measured by radial immunodiffusion, as previously described (Besser *et al.* 1985).

Immunohistochemistry for expression of the IgG₁ receptor

Tissues were placed at -86°C overnight prior to sectioning. Sections were cut to a thickness of $4\text{ }\mu\text{m}$, placed on glass slides (ProbeOn Plus; Microprobe, Fisher Scientific, Kent, WA, USA) and fixed in acetone for 10 min at 25°C . Endogenous peroxide was inhibited by 5 min incubation in 3% hydrogen peroxide in absolute ethanol. Sections were brought to water through graded alcohol, washed in water for 5 min, transferred to a capillary action system (Microprobe), blocked in 0.1% gelatin in Tris-buffered saline for 30 min at 37°C , and incubated with bovine IgG₁ ($10\text{ }\mu\text{g/ml}$) in Tris-buffered saline for 30 min at 37°C . Throughout the remainder of the procedure, slides were rinsed and blotted 15 times in Tris-buffered saline between each incubation. Sections were incubated with mouse anti-bovine IgG₁ for 30 min at 37°C (15 g/ml ; BIG715A, W C Davis, Pullman, WA, USA) and then with biotinylated goat anti-mouse IgG plus IgM (1:500) for 20 min at 37°C (Caltag, South San Francisco, CA, USA). Sections were incubated in ABC reagent (Avidin, biotinylated horseradish peroxidase; Vector Laboratories, Burlingame, CA, USA) for 20 min at 37°C and in 3-amino-9-ethyl-carbazole in N,N-dimethylformamide, diluted 1:100 in hydrogen peroxide in acetate buffer (Biogenex Labs, San Ramon, CA, USA) for 5 min at 21°C . Following rinsing in double distilled water, sections were counterstained for 2 min with Mayer's hematoxylin and mounted. Controls included positive and negative tissue controls, incubation with isotype matched, mouse monoclonal antibody to lipopolysaccharide of *Escherichia coli* J5 (Aydintug *et al.* 1989), substitution of mouse anti-bovine IgG₁ and goat anti-mouse IgG plus IgM with blocking agent.

A 100 unit ($10 \times 10\text{ mm}^2$), ocular micrometer grid was used to quantify IgG₁ expression of receptors in biopsy sections. Slides were randomized and examined using a microscope ($10\times$ objective) by an observer blind to treatments. An IgG₁ receptor score (scale 1–100) was obtained by placing the micrometer grid over each section and counting the number of grid squares out of 100 in which positive 3-amino-9-ethyl-carbazole staining was seen.

Analysis

Data from cows that responded positively to induction of lactation were included in the analysis. Because the study was designed to evaluate effects of PRL on IgG₁ receptor function, criteria for inclusion was based on mammary secretion IgG₁ concentrations at the end of hormonal induction. In an earlier study, mammary secretion IgG₁ concentrations in cows successfully induced into lactation were increased by the end of a 7-day hormonal induction period (Winger *et al.* 1995). In the present study, six of eleven cows (two cows/treatment group) produced mammary secretions with $>20\text{ mg/ml}$ IgG₁ by day 8. Data from these six cows was subjected to analysis of variance using the General Linear Models procedure of the statistical analysis system (SAS). Data from histologic assessment of IgG₁ receptors was subjected to one-way analysis of variance. Data from sequential measurements of blood and secretion samples were analyzed as a split-plot in time. The among-animal error term was used to test for significance of treatment effects. Effects of sample day and the treatment by day interaction were tested using the residual error term. Data were also analyzed within sample day to determine significance of treatment effects on each sample day. For each variable, differences between treatment means were detected by least significant difference. In addition, Pearson correlation coefficients were obtained using the Correlation procedure of SAS.

The use of larger experimental groups was precluded by several factors including the limited availability of rbPRL, the cost of and availability of cows, and the invasive nature of the biopsy procedure.

Results

Biopsy of the two cows in the bromocriptine treatment group on day 8 failed to obtain mammary parenchymal tissue for analysis of IgG₁ receptor activity. However, IgG₁ concentrations in mammary secretions were similar between cows that received rbPRL ($33 \pm 8.0\text{ mg/ml}$, mean $\pm$ S.E.M.), bromocriptine ($36.2 \pm 7.2\text{ mg/ml}$), and treatment control ($31.5 \pm 3.5\text{ mg/ml}$).

The overall effects of treatment ($P=0.24$), day ($P=0.8$), and treatment by day interaction ($P=0.19$) on serum PRL were not significant. However, treatment effects were

Figure 1 Least squares mean concentrations of PRL in serum of cows treated with rbPRL, bromocriptine, or vehicle (control). Data are least squares means $\pm$ S.E.M. of samples collected on even days 0–14. Asterisks indicate treatment means that differ from the control mean on the same day (* $P<0.10$; ** $P<0.05$).

observed when data were analyzed within day. On day 12, the day after the final administration of the treatments, serum PRL was reduced in cows that received bromocriptine (10 ± 0 ng/ml), compared with control cows (80.5 ± 29.5 ng/ml; $P<0.07$) or cows that received rbPRL (49 ± 8 ng/ml), although the latter difference was not significant. On day 14, serum PRL was greater in cows administered rbPRL compared with cows treated with bromocriptine ($P<0.003$) or controls ($P=0.01$). Cows treated with bromocriptine had lower serum PRL compared with control cows ($P=0.04$) (Fig. 1).

The overall effects of treatment ($P=0.02$), day ($P<0.0001$), and the treatment by day interaction ($P<0.0003$) on serum α -lactalbumin were significant. On day 12, serum α -lactalbumin was increased in cows that received rbPRL (825.5 ± 76.5 ng/ml) compared with cows that received bromocriptine (107.5 ± 71.5 ng/ml; $P<0.05$) or control cows (300 ± 148 ng/ml; $P<0.05$). On day 14, cows treated with rbPRL had higher serum α -lactalbumin compared with control cows ($P<0.08$) and cows treated with bromocriptine ($P<0.04$) (Fig. 2).

The overall effect of treatment on mammary secretion IgG₁ concentration was not significant ($P=0.24$). However, day of treatment ($P<0.01$) and the treatment by day interaction ($P<0.06$) significantly affected mammary secretion IgG₁ concentrations. On day 12, mammary secretion IgG₁ concentrations were increased in cows that received bromocriptine (62.6 ± 12.4 mg/ml) compared with cows that received rbPRL (7.7 ± 3.5 mg/ml, $P<0.05$) or control cows (11.3 ± 7.7 mg/ml; $P<0.05$) (Fig. 3).

On day 12, IgG₁ receptor score was higher in cows that received bromocriptine (67.5 ± 2.5) compared with cows that received rbPRL (1.5 ± 1.5 ; $P<0.001$) and control

Figure 2 Least squares mean concentrations of α -lactalbumin in serum of cows treated with rbPRL, bromocriptine, or vehicle (control). Data are least squares means $\pm$ S.E.M. of samples collected on even days 0–14. Asterisks indicate treatment means that differ from the control mean on the same day (* $P<0.10$; ** $P<0.05$).

Figure 3 Least squares mean concentrations of IgG₁ in mammary secretions of cows treated with rbPRL, bromocriptine, or vehicle (control). Data are least squares means $\pm$ S.E.M. of samples collected on even days 0–14. Asterisk indicates the treatment mean differs from the control mean on the same day (* $P<0.05$).

cows (0 ± 0 ; $P<0.001$) (Fig. 4). Immunohistochemistry of mammary tissue biopsies obtained on day 12 demonstrated that IgG₁ staining was maintained in cows treated with bromocriptine compared with cows treated with rbPRL or controls (Fig. 5).

Pearson correlation coefficients for serum PRL, serum α -lactalbumin, secretion IgG₁ concentration and IgG₁ receptor score were calculated for samples collected at the end of treatments (day 12). Serum PRL levels were negatively correlated to mammary secretion IgG₁ concentrations ($r = -0.79$; $P=0.06$) and IgG₁ receptor score ($r = -0.77$; $P=0.07$). Serum α -lactalbumin concentration tended to be negatively correlated with secretion IgG₁

Figure 4 Least squares mean IgG₁ receptor scores in mammary tissue of cows treated with rbPRL, bromocriptine, or vehicle (control). Data are least squares means $\pm$ S.E.M. of biopsy samples obtained on day 12. The mean IgG₁ receptor score was significantly higher in bromocriptine-treated cows relative to rbPRL-treated or control cows. Asterisk differs from rbPRL-treated and control (* $P < 0.001$).

concentrations ($r = -0.69$; $P = 0.13$) and IgG₁ receptor score ($r = -0.65$; $P = 0.16$). Mammary secretion IgG₁ concentrations were highly correlated with IgG₁ receptor scores ($r = 0.96$; $P < 0.005$) (Table 1).

Discussion

The proportion of cows successfully induced into lactation (6 of 11) was similar to previous studies where initiation of lactation occurred in approximately 60% of cows after one attempt at hormonal induction (Smith & Schanbacher 1973). A definite cause for the variation in response to induction has not been identified. Factors that may contribute to the variability in response include differences in absorption of hormones from injection or implantation sites, and differences in the functional status of existing endocrine glands (ovary, pituitary, adrenal, etc.).

Results suggest that treatment with bromocriptine successfully decreased basal serum PRL levels, similar to previous reports (Akers *et al.* 1981a). The finding that serum PRL levels were not increased in cows treated with rbPRL compared with control cows may have been due to a combination of factors including the short half life of PRL, its daily variation, time of sampling in relation to treatments, and animal variation. The half life of PRL in blood is approximately 30 min (Akers *et al.* 1980, Hart *et al.* 1980), and release is episodic and responsive to stress (Ostrom 1990). Because samples were obtained immediately before daily treatments, approximately 24 h had lapsed after treatment of cows with rbPRL. This period could allow sufficient time for serum PRL to return to basal levels or to be affected by daily variations or stress. Therefore, it was not surprising to find that serum PRL

Figure 5 Photomicrographs of bovine mammary biopsies collected on day 12. The avidin-biotin-peroxidase complex was used to identify receptor-bound IgG₁ on alveolar epithelial cells. Positive staining was revealed with 3-amino-9-ethyl-carbazole. Sections were counterstained with Mayer's hematoxylin. A) Biopsy from cow treated with rbPRL, B) biopsy from cow treated with bromocriptine, and C) biopsy from control cow. $\times 200$.

levels in the rbPRL-treated cows were generally similar to controls. Alternate sampling times or multiple samplings over time may have elucidated differences in serum PRL between rbPRL-treated cows and controls.

Although serum PRL levels were generally similar in cows receiving rbPRL and controls, serum α -lactalbumin

Table 1 Day 12, correlation coefficients

	Serum PRL	Serum α-lactalbumin	Secretion [IgG ₁]	IgG ₁ receptor
Serum PRL	1.0			
Serum α-lactalbumin	0.4091	1.0		
Secretion [IgG ₁]	0.7937	−0.6886	1.0	
IgG ₁	−0.7733	−0.6524	0.9554	1.0

levels were increased in cows that received rbPRL. This confirms that treatment with rbPRL was sufficient to increase lactogenic activity, despite the fact that elevated serum PRL levels were not maintained.

IgG₁ receptor score and mammary secretion IgG₁ concentrations remained elevated in bromocriptine treated cows but not rbPRL treated cows or controls. This indicates that the reduction in serum PRL concentrations due to bromocriptine treatment was sufficient to maintain IgG₁ receptor activity and concurrently suppress lactogenic activity. The finding that control cows exhibited less lactogenic activity than rbPRL-treated cows, but had similarly low concentrations of IgG₁ in secretions and decreased IgG₁ receptor expression, may indicate that different thresholds exist for the effect of PRL on lactogenic activity and IgG₁ receptor expression. It is possible that basal levels of serum PRL in control cows were not sufficient to trigger full lactogenic activity but were able to decrease expression of IgG₁ receptors and subsequent IgG₁ transfer into secretions.

The ability to regulate induction of α-lactalbumin expression by manipulating PRL status is reflective of the key role of PRL in the lactogenic complex of dairy cattle (Akers *et al.* 1981a). The significant negative correlation between serum PRL and IgG₁ receptor expression substantiates our earlier finding that bovine mammary IgG₁ receptor expression is reduced by exposure to PRL, *in vitro* (Barrington *et al.* 1997). These results suggest that, in addition to promoting lactogenesis, PRL may also serve to signal the cessation of colostrum formation. Such a role would appear to be critical for coordinating the profound functional changes that embody the transition from colostrogenesis to lactation.

Conclusion

This study shows that *in vivo* serum PRL levels are negatively correlated to expression of the bovine mammary IgG₁ receptor. This corroborates an earlier *in vitro* study which showed that, in addition to its positive lactogenic effects, PRL decreases expression of the bovine mammary IgG₁ receptor.

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