Growth Hormone (GH) Binding and Expression of GH Receptor 1A mRNA in Hepatic Tissue of Periparturient Dairy Cows¹

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ABSTRACT

Growth hormone (GH) plays a role in metabolic adaptations that occur during lactogenesis. Liver GH receptor transcript (GHR 1A) is transiently decreased near parturition and may reduce GH-dependent signaling leading to low blood insulin-like growth factor I (IGF-I) concentrations in periparturient dairy cattle. We hypothesized that the decrease in GHR 1A mRNA at parturition was associated with decreased GH binding (i.e., GHR protein concentration) in liver. Blood and liver biopsy samples were collected from 12 Holstein cows on d -12 ± 1 , 3, and 17 relative to parturition. Total cellular RNA was isolated from a sub-sample of liver. Quantitative real-time polymerase chain reactions were used to measure GHR 1A, total GHR, IGF-I, and cyclophilin mRNA. Microsomal membranes were isolated from the remaining liver tissue and assayed for ¹²⁵I-bGH binding. Plasma was assayed for GH and IGF-I concentrations. Liver GHR 1A mRNA, specific ¹²⁵I-bGH binding to liver membranes, liver IGF-I mRNA, and plasma IGF-I concentrations were lower on d 3 relative to d -12 . The GHR 1A mRNA, ¹²⁵I-bGH binding, and plasma GH concentrations increased on d 17 but liver IGF-I mRNA and plasma IGF-I concentrations did not change between d 3 and 17. Total GHR mRNA and cyclophilin mRNA amounts were similar on d -12 , 3, and 17. Across all days, ¹²⁵I-bGH specific binding in liver was highly correlated with liver GHR 1A mRNA ($R^2 = 0.68$) but not with total GHR mRNA. Saturation binding analysis showed that GHR concentration ($B_{\max}$) in liver on d 3 had decreased to only 5% of the amount on d -12 . We conclude that decreased GHR 1A mRNA leads to decreased GHR protein concentration in liver. Reduced GHR in liver likely contributes to a decrease

in liver IGF-I production and reduced concentrations of IGF-I in blood of periparturient cows.

(**Key words:** growth hormone, receptor, parturition)

Abbreviation key: fg = femtogram, GH = growth hormone, GHR = growth hormone receptor, GHR_{tot} = total GHR, NSB = nonspecific binding, qRT-PCR = quantitative real-time polymerase chain reaction, RIA = radioimmunoassay, RT = reverse transcribed, SB = specific binding, TB = total binding.

INTRODUCTION

Growth hormone (GH) plays a central role in the adaptations that occur in nutrient metabolism during early lactation in dairy cattle (Lucy et al., 2001). Actions of GH on the liver include increased gluconeogenesis to meet glucose requirements for mammary lactose synthesis (Pocius and Herbein, 1986; Knapp et al., 1992; Bell, 1995) and increased synthesis and secretion of IGF-I; an essential growth factor for a variety of cellular processes (Hannon et al., 1991; Bichell et al., 1992; Gronowski and Rotwein, 1995; Phillips et al., 1998). Growth hormone receptors (GHR) mediate the actions of GH. Alternative splicing of exon 1 leads to multiple GHR mRNA transcripts (Edens and Talamantes, 1998; Talamantes and Ortiz, 2002). There are at least three alternative forms of GHR mRNA in cattle (GHR 1A, 1B, and 1C; Lucy et al., 2001). The GHR 1A mRNA is expressed exclusively in the adult liver (Lucy et al., 1998) and appears to play a key role in the metabolic adaptations associated with lactogenesis (Lucy et al., 2001).

The expression of liver GHR 1A mRNA during the periparturient period is dynamic. There is a decrease in liver GHR 1A mRNA at parturition that is reversed within 3 wk after calving (Kobayashi et al., 1999). The decrease in GHR 1A mRNA expression at parturition coincides with a period of liver refractoriness to GH (Lucy et al., 2001) as well as a decrease in blood IGF-I concentrations (Vicini et al., 1991). The mechanism controlling the decrease in GHR 1A around calving may be similar to those controlling the decrease in GHR

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expression during undernutrition (Renaville et al., 2002).

The decrease in GH-dependent IGF-I synthesis and secretion around parturition is indicative of lost GHR function in the liver. The GHR 1A mRNA is clearly decreased when GHR function is impaired (Kobayashi et al., 1999). Neither GH binding to liver tissue nor the relationship between GH binding and GHR 1A mRNA during this period has been investigated. The objective of the present experiment, was therefore, to collect liver tissue during the periparturient period, a time of dynamic GHR 1A mRNA expression, and to compare the expression of GHR 1A mRNA with actual binding of GH to isolated liver microsomal membranes. Periparturient mRNA and blood hormones associated with GH-dependent actions were also measured. We hypothesized that GHR 1A mRNA expression would be correlated with GH binding in liver and that GH-dependent processes in periparturient cattle would be associated with GHR 1A expression.

MATERIALS AND METHODS

Animals

Primiparous Holstein cows ($n = 12$) that calved in March 2002 at the University of Missouri Foremost Dairy farm were used. Cows were maintained on a dirt lot before parturition. The dry cow diet was a TMR based on corn silage (29% of diet DM), grass hay (16% of diet DM), soybean meal (7.5% of diet DM), soybean hulls (27.5% of diet DM), and vitamin/mineral premix in a ground corn carrier (20% of diet DM). After parturition, cows were moved to the milking herd, managed according to the farm's standard operating practices, and fed a TMR consisting of corn silage (23% of diet DM), alfalfa hay (13% of diet DM), alfalfa haylage (7.5% of diet DM), ground corn (20% of diet DM), corn gluten feed (4.7% of diet DM), soybean meal (6% of diet DM), Soy PLUS (West Central Soy, Ralston, IA) (4.7% of diet DM), soybean hulls (6% of diet DM), whole cottonseeds (9% of diet DM), and vitamin mineral premix in a ground corn carrier (5% of diet DM). Diets were balanced to meet or exceed the requirements for dry or lactating cattle (National Research Council, 1989). University of Missouri Animal Care and Use Committee approved these experimental procedures.

Liver Biopsies

Liver biopsies (0.5 to 1.5 g) were collected from cows 14 d before expected parturition (actual day relative to parturition was -12 ± 1 d) and then 3 and 17 d after parturition. Biopsies were collected according to previously published methods (Kobayashi et al., 1999) and

immediately frozen in liquid N. Samples were stored at -80°C until analysis.

Blood Samples

Immediately before each liver biopsy, a single blood sample was collected into an evacuated glass tube containing EDTA (Vacutainer, Becton Dickinson and Co., Franklin Lake, UT) via coccygeal venipuncture. Blood samples were stored on ice for transport to the laboratory and centrifuged at $2500 \times g$ for 20 min. Plasma was transferred to a polypropylene tube and frozen at -20°C until assayed.

Hormone Assays

Plasma GH concentrations were quantified using a validated homologous double antibody radioimmunoassay (RIA; Gorewit, 1981). Recombinantly derived bovine GH (SV-3001-B; Pharmacia Inc., Kalamazoo, MI) was used as the standard and as the iodinated trace (Cohick et al., 1989). Before use, the first antibody (rabbit anti-oGH2; AFP C0123080; a gift from the National Hormone and Pituitary Program, A. F. Parlow, Scientific Director) was diluted 1:20,000, and the second antibody (goat anti-rabbit; lot # 35318; Pel-Freez, Rogers, AK) was diluted 1:75. Samples were analyzed in triplicate. The minimal detectable concentration of GH was 0.7 ng/ml of standard or sample added to the assay tubes. The intraassay CV was 6.2%.

Plasma IGF-I concentrations were quantified using a validated homologous double antibody RIA (Johnson et al., 1996). The assay was modified so that the first antibody (APF-4892898) and trace were added and incubated for 24 h before addition of the second antibody (goat anti-rabbit). Recombinantly derived human IGF-I (H-5555, lot C00219; Bachem, King of Prussia, PA) was used as the standard and as the iodinated trace (Cohick et al., 1989). The ^{125}I was purchased from Perkin Elmer Life Sciences (Boston, MA; NEZ-033H). For iodination, the amount of IGF-I was reduced ($1 \mu\text{g}$) and the reaction time was increased (5 min) relative to the original procedure. Samples were analyzed in triplicate. The minimal detectable concentration of IGF-I was 0.2 ng/ml of standard or sample added to the assay tubes. The intraassay CV was 5.8%.

RNA Isolation

Total cellular RNA was isolated from a subsample of the liver biopsy by using the TRIZOL procedure (Invitrogen Life Technologies, Carlsbad, CA). After isolation, RNA was dissolved in sterile water treated with 0.1% (vol/vol) diethylpyrocarbonate. Concentrations of

RNA were determined by measuring absorbance at 260 nm and the purity of RNA was determined by calculating the ratio of absorbencies at 260 and 280 nm and by electrophoresis of an RNA aliquot (2.5 μ g) from each sample through a 1% agarose gel in Tris-borate/EDTA buffer (0.09 M Tris-borate and 0.002 M EDTA) with ethidium bromide (0.5 μ g/ml). Isolated RNA was stored at -80°C until quantitative real-time PCR (**qRT-PCR**) was performed.

Quantitative Real-Time PCR

The amounts of GHR 1A, total GHR (**GHR_{tot}**), IGF-I, and cyclophilin (a representative housekeeping gene) mRNA in liver were measured by qRT-PCR as previously described (Butler et al., 2003) with slight modifications. Total cellular RNA (2.1 μ g) was used for cDNA synthesis. Brilliant Plus quantitative PCR core reagent kit (Stratagene, La Jolla, CA) was used for qRT-PCR. An equal volume of water (no template control), internal controls (low, medium, and high), and standard curves were run in separate wells on the same plate.

Internal controls were liver samples from a previous experiment that contained low, medium, and high amounts of GHR 1A mRNA as indicated by ribonuclease protection assays (Kobayashi et al., 1999). The cDNA for standard curves were prepared as follows: a 300-bp fragment of the bovine cyclophilin gene was cloned into p-GEM-T Easy vector (Promega Corp., Madison, WI). Fragments of the bovine IGF-I (314 bp) and GHR (312 bp) genes were previously cloned into pGEM-T Easy (Kobayashi et al., 1999) and pGEM-4Z (Lucy et al., 1998) vectors, respectively. The plasmids were transformed into competent cells and grown overnight in luria broth with ampicillin. Plasmid DNA was isolated with the Qiagen midi-prep kit (Qiagen Corp, Valencia, CA). Target DNA was transcribed in vitro using the Ribomax Transcription Kit (Promega) and purified with phenol:chloroform:isoamylalcohol extraction. The RNA was quantified by measuring absorbance at 260 nm and fragments were separated on a 1% agarose gel to confirm their correct size. The RNA was serially diluted and tested with qRT-PCR probes and primers. All qRT-PCR reactions were run in triplicate, and fluorescence was quantified with the ABI PRISM 7700 Sequence Detector (Applied Biosystems, Foster City, CA). Analyses of amplification plots were performed using the Sequence Detection Software of Applied Biosystems. Standard curves were subjected to linear regression and used to estimate the amount of target RNA in the sample. The final values are reported as femtograms (**fg**) of target RNA per 25 ng of total cellular RNA assayed.

Membrane Isolation

Liver tissue (0.5 to 1.5 g) was thawed on ice, cut into 2- to 4-mm cubes, and then homogenized on ice in 10 ml of ice cold Tris-HCl buffer (0.025 M, pH 7.8) containing sucrose (0.3 M), EDTA (0.01 M), ethylene glyco-bis [β -aminoethyl ether]-N,N,N',N'-tetraacetic acid (EGTA; 0.01 M), aprotinin (100 U/ml), phenylmethyl sulfonyl fluoride (0.001 M), sodium fluoride (0.01 M), and sodium orthovanadate (0.0001 M). The homogenization lasted approximately 45 s on a Polytron tissue homogenizer (Brinkmann Instruments, Westbury, NY) using a medium speed. The homogenate was then centrifuged at 4°C at $11,000 \times g$ for 20 min to remove tissue fragments. The supernatant was transferred to a clean tube and the total volume adjusted to 30 ml with homogenization buffer and centrifuged at 4°C at $100,000 \times g$ for 2 h. The supernatant was removed and the membrane pellet was homogenized with a glass tissue homogenizer in 0.025 M Tris-HCl buffer (pH 7.8) containing 0.01 M calcium chloride. Protein concentration was determined (Bradford, 1976) using BSA as a standard. The sensitivity of the protein assay was 1.2 μ g/ml. The intraassay CV averaged 5.9% and the interassay CV was 4.9%. The membrane homogenate was frozen at -80°C until the GH binding assay.

GH Binding Assay

Liver microsomal membranes used in these experiments were not pretreated with MgCl_2 because preliminary assays failed to show a consistent increase in ^{125}I -bGH binding following treatment (data not shown). Others have shown limited value in pretreating bovine liver with MgCl_2 before binding analysis (Badinga et al., 1991).

Membranes were thawed on ice. Total binding (**TB**) reactions (1 ml) included 0.7 mg of membrane protein and 4 fmols (approx 20,000 cpm) of ^{125}I -bGH (AFP-11182B; National Hormone and Pituitary Program) in assay buffer containing 0.025 M Tris-HCl (pH 7.8), 0.01 M calcium chloride, and 0.1% bovine serum albumin. Nonspecific binding (**NSB**) was measured for each sample by adding 1 μ g of unlabeled bGH (AFP-11182B) to tubes containing 0.7 mg of membrane protein and 4 fmols ^{125}I -bGH. All reactions were done in duplicate. Three milliliters of ice-cold assay buffer was added following a 24-h room temperature (25°C) incubation, and membranes were pelleted by centrifugation at 4°C at $3000 \times g$ for 30 min. The supernatant was decanted and the radioactivity in the pellets was counted. The percentage of specific binding (**SB**) was defined as $[(\text{TB} - \text{NSB}) / \text{total counts added}] \times 100$.

Receptor Saturation Analysis

The remaining membranes were pooled by time point (–12, 3, and 17 d relative to parturition). Saturation reactions (both SB and NSB) were done in duplicate using 0.7 mg of membrane protein and increasing amounts of ^{125}I -bGH. Two separate assays were completed (duplicate assays were done on different days). The amounts of ^{125}I -bGH added were 1.0, 2.1, 4.7, 10.9, 20.5, 46.1, and 98.6 fmoles for assay 1 and 1.0, 2.0, 4.6, 10.8, 19.8, 44.0, and 96.7 fmols for assay 2. Reactions were incubated for 24 h at 25°C. Incubations were stopped with a 3-ml ice-cold assay buffer, and membranes were pelleted by centrifugation as described above. Specific binding for each dose of ^{125}I -bGH was determined as previously described. Free (unbound) ^{125}I -bGH was estimated by subtracting specifically bound ^{125}I -bGH from the total ^{125}I -bGH added to each tube. The units for the amount of bound ^{125}I -bGH were converted to fmole bGH/mg membrane protein and the units for the amount of free ^{125}I -bGH were converted to pM. The B_{max} (receptor concentration) and K_d (receptor dissociation constant) for each assay were determined by nonlinear regression (Proc NLIN; SAS, 1999) by using the equation $y = (B_{\text{max}}x)/(K_d + x)$ where x = free hormone concentration and y = bound hormone concentration at each dose of ^{125}I -bGH.

Statistical Analysis

The amounts of GHR 1A, IGF-I, GHRtot, and cyclophilin mRNA, the plasma concentrations of GH and IGF-I, and the percent SB were analyzed using Proc GLM of SAS (SAS, 1999). The model included the effects of day and cow. The Duncan's multiple range test from Proc GLM was used to separate means. Data are presented as least squares means $\pm$ the standard error of the least squares mean. Significance was declared at $P < 0.05$.

RESULTS

There were 3 missing samples for the mRNA analysis and the SB analysis (one sample on each of d –12, 3, and 17) because no liver sample could be obtained from the cows. Another 4 samples were excluded only from the SB analysis (3 samples on d 3, and 1 sample on d 17) because the samples obtained were too small for membrane isolation. The results of the Proc GLM analysis and the Duncan's multiple range test of Proc GLM are presented.

There was an effect of day on plasma GH concentrations because plasma GH increased from d –12 to 17

Figure 1. Plasma concentrations of growth hormone (GH) and IGF-I for primiparous cows on d –12, 3, and 17 relative to parturition. Each bar represents the mean of 12 observations. Bars with different letters (within hormone) represent means that differ at $P < 0.05$ (Duncan's multiple range test).

($P < 0.05$; Figure 1). There was also an effect of day on plasma IGF-I concentrations ($P < 0.001$). Plasma IGF-I concentrations decreased from d –12 to 3 and remained low on d 17. The effect of cow was not significant for plasma GH or plasma IGF-I.

The amount of liver GHR 1A mRNA declined from d –12 to 3 and then increased to prepartum levels by d 17 (effect of day, $P < 0.01$; Figure 2). The amount of liver IGF-I mRNA also decreased from d –12 to 3 (effect of day, $P < 0.001$; Figure 2). The amount of liver IGF-I mRNA did not change between d 3 and 17. There was no effect of day on the amounts of GHRtot or cyclophilin

Figure 2. Amount of growth hormone receptor (GHR) 1A, IGF-I, total GHR (GHRtot) and cyclophilin (Cyclo) mRNA in liver of primiparous cows on d –12, 3, and 17 relative to parturition. Each bar represents the mean of 11 observations. Bars with different letters (within mRNA) represent means that differ at $P < 0.05$ (Duncan's multiple range test). Cyclophilin was included in the analyses as a representative housekeeping gene.

Figure 3. Specific binding of ^{125}I -bGH to microsomal membranes isolated from liver of primiparous cows on d -12, 3, and 17 relative to parturition. There were 11 observations for d -12, 8 observations for d 3, and 10 observations for d 17. Bars with different letters represent means that differ at $P < 0.05$ (Duncan's multiple range test).

mRNA (Figure 2). The effect of cow was not significant for any of the measured mRNA.

There was an effect of day on SB of ^{125}I -bGH to microsomal membranes ($P < 0.05$). The SB decreased from d -12 to 3 and then increased to prepartum levels by d 17 (Figure 3). The effect of cow was not significant for SB. Specific binding of ^{125}I -bGH to microsomal membranes was correlated with the amount of liver GHR 1A mRNA ($R^2 = 0.68$, $P < 0.001$; Figure 4) and with the amount of liver IGF-I mRNA ($R^2 = 0.29$, $P < 0.01$). Specific binding of ^{125}I -bGH to microsomal membranes was not correlated with the amount of liver GHRtot mRNA ($P > 0.10$).

The estimates for the concentration ($B_{\max}$) and affinity (K_d) of liver GHR are presented in Table 1 and a graphical representation of the saturation analysis is presented in Figure 5. The estimates for the number and affinity of GHR were highest in prepartum liver, decreased on d 3 and then increased on d 17.

DISCUSSION

Growth hormone plays a central role in the metabolic adaptations that occur during early lactation in dairy cattle (Lucy et al., 2001). Blood GH concentrations increase shortly after parturition. The increase in blood GH concentrations coincide with a precipitous decline in blood IGF-I concentrations. Most of the IGF-I in blood arises from the liver (Sjogren et al., 1999), and the

Figure 4. Regression of liver growth hormone receptor (GHR) 1A mRNA amount on percent specific growth hormone binding in liver microsomal membranes of primiparous periparturient cows ($n = 12$). Data points represent values from membranes collected on d -12, 3, and 17 relative to parturition.

decrease in blood IGF-I concentrations is associated with a decrease in liver IGF-I mRNA (Kobayashi et al., 1999). The decrease in blood IGF-I reduces negative feedback on GH synthesis and secretion. The loss of negative feedback allows for an increase in blood GH concentrations (Veldhuis et al., 2001). The increase in blood GH promotes lipolysis and gluconeogenesis, 2 processes that are required for the transition to a high level of milk production in dairy cattle (Bell, 1995). The aforementioned changes in blood GH and IGF-I were clearly evident in the present study. Blood IGF-I concentrations decreased after calving (Figure 1). An increase in blood GH concentrations after calving coincided with the decrease in blood IGF-I. There was a decrease in liver IGF-I mRNA after calving (Figure 2), and the relative changes in liver IGF-I mRNA were similar to those observed in the plasma.

Table 1. The concentration ($B_{\max}$) and affinity (K_d) of GH binding sites in liver microsomal membranes collected from primiparous cows during the periparturient period (d -12, 3, and 17 relative to parturition). Data for assay 1 and assay 2 are from nonlinear regression and represent parameter estimates $\pm$ SEM.

Day	Assay 1	Assay 2	Mean ¹
$B_{\max}$ (fmole/mg)			
-12	4.95 $\pm$ 0.57	2.28 $\pm$ 0.60	3.62 $\pm$ 1.34
3	0.16 $\pm$ 0.03	0.18 $\pm$ 0.02	0.17 $\pm$ 0.01
17	1.38 $\pm$ 0.06	1.00 $\pm$ 0.20	1.19 $\pm$ 0.19
K_d (pM)			
-12	139.0 $\pm$ 26.2	192.7 $\pm$ 76.0	165.9 $\pm$ 26.9
3	6.1 $\pm$ 4.1	18.0 $\pm$ 5.9	12.1 $\pm$ 5.9
17	67.2 $\pm$ 6.1	79.1 $\pm$ 34.4	73.2 $\pm$ 5.9

¹Mean $\pm$ SEM for average of assay 1 and assay 2.

Figure 5. Saturation analysis for growth hormone receptor in liver microsomal membranes of periparturient dairy cows on d -12, 3, and 17 relative to parturition. The data are from assay 1. The number of binding sites asymptotically approaches $B_{\max}$ as the free hormone concentration increases. The K_d is the free hormone concentration at 50% $B_{\max}$.

The mechanisms that lead to a decrease in liver IGF-I synthesis are important because they ultimately control the increase in blood GH shortly after calving. The liver becomes refractory to GH after a cow calves and the loss of GH action leads to a decrease in GH-dependent IGF-I blood concentrations (Vicini et al., 1991; McGuire et al., 1992; McGuire et al., 1995). A dramatic decrease in GHR mRNA expression occurs during the refractory period (Kobayashi et al., 1999). One mechanism for reduced GH response, therefore, involves reduced expression of the GHR gene and a hypothetical decrease in the concentration of GHR on hepatocytes. The molecular mechanisms controlling GHR expression are complex because 3 promoters control GHR gene expression (Lucy et al., 2001). The 3 promoters drive mRNA expression of 3 different GHR mRNA transcripts. The first GHR transcript (GHR 1A) is only found in liver. The second and third GHR transcripts (GHR 1B and 1C) are ubiquitously expressed (Jiang et al., 2000). The periparturient mechanisms controlling GHR expression have a large impact on the promoter controlling GHR 1A expression, and there is a large decrease in GHR 1A mRNA shortly after calving (Kobayashi et al., 1999). Demonstrating the relative changes in GHR 1A at calving was not the principal objective of the present study. Nonetheless, the cows from the present study had the expected changes in GHR 1A mRNA. The amount of GHR 1A mRNA declined substantially by d 3 and then subsequently increased by d 17 (Figure 2). The decrease in GHR 1A was associated with a decrease in IGF-I mRNA in the liver (Figure 2).

The GHRtot mRNA is an estimate of the total pool of GHR mRNA in the liver sample. The estimate is generated by positioning qRT-PCR probe and primer sets in exons 2 and 3; regions common to all GHR transcripts (Lucy et al., 2001). We found that the GHRtot was unchanged on d -12, 3, and 17 (Figure 2). Thus, the large change in GHR 1A mRNA was not necessarily reflected in total liver GHR mRNA. A previous report had suggested that the GHR 1A mRNA comprised the bulk of liver GHR mRNA (Jiang and Lucy, 2001). It is possible that the previous analyses (based on ribonuclease protection assay) may have overestimated the amount of GHR 1A mRNA relative to other GHR mRNA. A second possibility is that alternative mRNA may be up regulated when GHR 1A mRNA is decreased shortly after calving. If alternative mRNA are increased then the mechanism does not involve GHR 1B mRNA because GHR 1B mRNA did not increase in the liver of periparturient dairy cattle (Kobayashi et al., 1999). The amount of GHR 1C (or any of the numerous other GHR transcripts; Jiang and Lucy, 2001) has not been measured during this period.

The assumption that the total amount of liver GHR mRNA is indicative of the actual amount and (or) activity of liver GHR protein (receptor concentration) is not necessarily correct. The GHR mRNA transcripts are transcribed by different promoters and their mRNA are different within the exon 1 region (Lucy et al., 2001). The exon 1 differences affect the translational efficiency of the mRNA. For example, GHR exon 1A favors translation, whereas GHR exon 1B strongly inhibits translation (Jiang and Lucy, 2001). Thus, the quantity of an mRNA is not indicative of the concentration of receptor protein on the cell surface. The primary objective of the present study was to examine GH binding in the liver and determine if the transitory period of liver GH refractoriness coincided with a decrease in liver GH binding. Furthermore, we wanted to determine if GHR 1A mRNA expression was correlated with GHR concentration.

The percentage of specific ^{125}I -bGH binding was clearly decreased on d 3 postpartum (Figure 3). The decrease in GH binding coincided with the decrease in GHR 1A mRNA and the decrease in IGF-I mRNA on d 3 (Figure 2). The GH binding increased on d 17 when the GHR 1A mRNA amount increased in liver. The specific GH binding was highly correlated with the concentration of GHR 1A mRNA (Figure 4). The large changes in GH binding were not reflected in GHRtot mRNA. The GHRtot mRNA did not decrease on d 3 (Figure 2), and we failed to detect a correlation between GHRtot mRNA and specific GH binding to liver membranes. Thus, the total amount of GHR mRNA is a poor indicator of GHR concentration in liver of peripartur-

ient dairy cows. Specific measurement of the GHR 1A mRNA is a more accurate indicator of GHR concentration. The preponderance of alternative GHR mRNA that are either not translated or whose translation products are not expressed (Jiang and Lucy, 2001) preclude the use of total GHR mRNA for estimating GHR activity in liver.

The $B_{\max}$ and K_d for a receptor cannot be determined from single point estimates of SB. Therefore, a saturation analysis was performed and analyzed by nonlinear regression to determine the $B_{\max}$ and K_d for liver membranes on d -12, 3, and 17 (Table 1 and Figure 5). Two separate assays were completed. Both assays were performed on the same pooled membrane samples. Thus, a statistical test can not be applied to those observations. The concentrations of GH binding sites on d 3 decreased to only 5% of the concentration present on d -12. Then, concentration of binding sites increased approximately 7-fold from d 3 to 17. Therefore, when actual receptor concentrations were estimated, the extent of changes in GHR in liver was greater than changes in GHR 1A mRNA. Saturation analysis predicted K_d (approximately 10^{-10} M) that were within the range of circulating GH concentrations (10^{-10} M) and consistent with values found in literature (Staten et al., 1993). The estimates of K_d were somewhat variable and the d 3 estimates were considerably lower than d -12 or 17. The low estimate obtained on d 3 probably reflects our inability to accurately determine K_d in samples with low SB. If the low K_d on d 3 are indeed accurate then they suggest a unique GHR with different binding characteristics in early postpartum liver.

We conclude that the concentration of GHR in liver of dairy cattle is decreased on d 3 postpartum compared to 12 d before calving. The decrease in GHR concentration is associated with a specific decrease in GHR 1A mRNA. The total GHR mRNA remained unchanged on d 3. Thus, total GHR mRNA may be a poor indicator of GHR activity in the liver. The decrease in GH binding and the decrease in GHR 1A mRNA coincided with a decrease in liver IGF-I mRNA synthesis and a decrease in blood IGF-I concentrations. The decrease in blood IGF-I coincided with an increase in blood GH concentrations. The molecular control of GHR 1A mRNA expression may be the principal mechanism through which the GH axis is uncoupled in periparturient dairy cattle.

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