PROCEEDINGS OF THE SOCIETY FOR EXPERIMENTAL BIOLOGY AND MEDICINE 164, 115–119 (1980)

Clearance and Secretion Rates of Prolactin in Dairy Cattle in Various Physiological States (40833)[1]

R. MICHAEL AKERS, GORDON T. GOODMAN, AND H. ALLEN TUCKER

Animal Reproduction Laboratory, Department of Dairy Science, Michigan State University, East Lansing, Michigan 48824

Prolactin (PRL) is generally assumed to be necessary for initiation and maintenance of milk secretion (1). In addition, serum concentrations of PRL are positively associated with changes in milk yield during lactation in cows (2). However, it is also recognized that a variety of stimuli including milking, ambient temperature, and photoperiod influence the concentrations of PRL in serum of cattle (3–5). The concentration of PRL in serum at a given time is a function of secretion rate (SR) from the pituitary and clearance rate (CR) from serum. Although many factors influence the concentration of PRL measured in serum, there is a paucity of information to indicate whether such concentration changes result from variation in SR and/or CR of PRL. The objective of these experiments was to determine if SR and/or CR of PRL changed among various stages of lactation in dairy cows.

Materials and methods. Basal CR and SR for PRL were determined using the infusion to steady-state method of Tait (6). Calculations of CR, SR and half-life (T 1/2) were as follows: CR (ml/min) = PRL infusion rate (ng/min ÷ serum PRL (ng/ml) at steady state minus preinfusion serum PRL concentration (ng/ml); SR (ng/min) = CR (ml/min) × pre-infusion serum PRL concentration (ng/ml); and $T\ 1/2 = 0.693/k$; where k = slope × 2.303. Slope was calculated from linear regression equations generated from a plot of log PRL concentration (ng/ml) vs time (30 min) postinfusion of PRL. Because of variable body weights among cows studied, SR, CR, and T 1/2 data were adjusted and expressed per kilogram body weight. Statistical analysis of SR, CR, and T 1/2 results were conducted using log-transformed data to minimize heterogeneous variance. Analysis of variance of these transformed data and orthogonal contrasts were used for statistical comparisons among means (7).

In a first experiment eight Holstein cows in advanced lactation (average 309 days) and pregnancy (average 216 days gestation) were randomly assigned to one of two groups of four each to be infused with either 4 or 8 mg PRL/cow/hr for 4 hr in December. Infusions were repeated 24 hr later with doses reversed between groups.

In a second experiment, four nonpregnant Holstein cows lactating an average of 31 days and four nonlactating, pregnant (average 260 days) Holstein cows were infused with 6 mg PRL/hr/cow for 3.5 hr in April.

Because the influence of physiological state and season on basal CR and SR estimates could not be distinguished in comparisons made between Experiments 1 and 2, a third experiment was designed. Six nonlactating, pregnant (average 212 days), six early lactating (average 53 days), nonpregnant, and six late lactating (average 262 days) pregnant (average 196 days) Holstein cows were infused with 6 mg PRL/cow/hr for 3.5 hr. Two cows from each stage of lactation were infused on 1 of 3 consecutive days in August.

On the afternoon preceding start of infusion, cattle were fitted with polyvinyl cannulas in both jugular veins; blood was obtained through one cannula and PRL infused through the contralateral cannula. Baseline concentrations of PRL were determined in sera collected at 10-min intervals for 30 min prior to the start of PRL infusion at 0900 hr. PRL solutions were infused at the rate of 4.6 ml/hr using a constant infusion pump (Harvard Apparatus Co., Cambridge, Mass.). Blood was col-

[1] Michigan Agricultural Experiment Station Journal Article No. 9183. This research was supported in part by USPHS Grants AM-15899 and HD-09883.

0037-9727/80/050115-05$01.00/0

TABLE I. CLEARANCE RATES (CR), SECRETION RATES (SR) AND HALF-LIFE (T 1/2) OF PROLACTIN (PRL) OF COWS INFUSED WITH 4 OR 8 mg PRL/hr

Parameter	Infusion dose		
	4 mg PRL/hr		8 mg PRL/hr
No. animals[a]	8		8
Body weight (kg)		656 ± 20	
Preinfusion PRL (ng/ml)	11.7 ± 0.7		10.5 ± 0.7
CR (ml/min)	1512 ± 47		1520 ± 47
SR (μg/min)	16.6 ± 1.3		18.7 ± 1.3
CR (ml/min)/kg body wt	2.31 ± 0.06		2.31 ± 0.06
SR (μg/h)/kg body wt	1.52 ± 0.12		1.69 ± 0.12
T 1/2 (min)	32.0 ± 1.6		27.9 ± 1.6

[a] Multiparous cows pregnant (184–226 days), lactating (292–324 days).

lected at 30-min intervals during the first 2 hr of infusion and at 15-min intervals until the end of infusion. PRL assayed in sera collected following the initial 2 hr of infusion was used to estimate steady-state concentrations. After infusions were stopped, sera were collected at 5-min intervals for 30 min, and were assayed for PRL to calculate T 1/2.

Solutions of bovine PRL (NIH-B4)[2] were prepared within 24 hr of infusion in sterile saline (0.9% NaCl) containing 0.1% bovine serum albumin (BSA). The pH of solutions was adjusted (10.5–11.0) with 1.0 N sodium hydroxide. Infusion tubes were flushed before each experiment with 0.1% BSA in saline to minimize absorption of PRL.

Blood was stored at 24°C for 2 to 4 hr and at 4°C for 18 hr before centrifugation at 2300 × g for 30 min. Sera were decanted and stored at −20°C until assayed for PRL by double antibody radioimmunoassay (3). Bovine PRL (NIH B-4)[2] was used as reference standard.

Results. Among cows in advanced lactation, preinfusion serum PRL averaged (±SE) 11.7 ± 0.7 and 10.5 ± 0.7 ng/ml in cows subsequently infused with either 4 or 8 mg PRL/hr, respectively (Table I). Total PRL in serum averaged 55.7 ± 1.9 and 107.2 ± 5.5 ng/ml in cows at steady state during infusion with 4 or 8 mg PRL/hr, respectively (Fig. 1). Estimates of basal CR (1512 and 1520 ml/min), basal SR (16.6 and 18.7 μg/min) and T 1/2 (32.0 and 27.9 min) were similar ($P > 0.05$) in cows infused with either 4 or 8 mg PRL/hr.

In nonlactating, pregnant cows and in nonpregnant cows at the beginning of lactation (Experiment 2) serum PRL averaged 18.0 ± 8.0 and 36.5 ± 8.0 ng/ml respectively before infusion (Table II). At steady state total PRL averaged 92.5 ± 7.8 and 104.1 ± 8.5 ng/ml. When adjusted for body weight both basal CR and SR tended to be greater among lactating cows in comparison with nonlactating cows. Estimates of T 1/2 were greater ($P < 0.01$) among lactating cows.

In Experiment 3, PRL in serum of nonlactating, early lactating, and late lactating cows averaged 26.1 ± 2.2, 36.9 ± 2.2, and 30.9 ± 2.2 ng/ml, respectively, before infusion of PRL (Table III). At steady state during infusion of PRL, total serum concentrations of PRL averaged 75.3 ± 2.2, 69.1 ± 2.9, and 72.2 ± 3.1 ng/ml, respectively. Basal CR estimates of PRL were 75 and 25% greater and basal SR estimates 140 and 40% greater ($P < 0.05$) in early lactating and late lactating cows, respectively, than in nonlactating cows. However, T 1/2 estimates of PRL did not differ ($P > 0.05$) between nonlactating and lactating cows.

Differences in basal CR and SR of PRL among all lactating cows were correlated ($r = 0.36$; $P < 0.05$ and $r = 0.52$; $P < 0.005$, respectively) with mean daily milk production during the week prior to each experiment (Fig. 2).

Discussion. The constant infusion

[2] Kindly supplied by National Institutes of Health, Bethesda, Maryland.

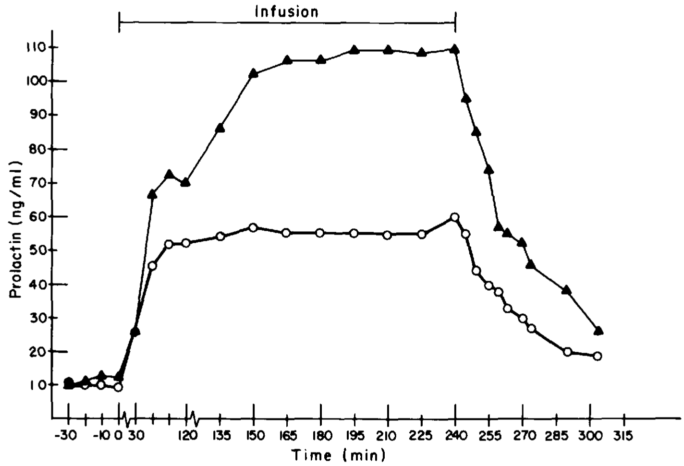

FIG. 1. Concentration of PRL in sera of lactating cows infused with 4 (○) or 8 mg/hr (▲) of NIH B4 PRL. Standard error before infusion was 0.7 ng/ml. At steady state (150 to 240 min) standard errors were 1.9 and 5.5 ng/ml for cows infused with 4 or 8 mg/hr, respectively.

method of estimating clearance rate depends upon the assumption that the infusate is in equilibrium with all compartments in the body. The pattern (Fig. 1) of temporal changes in serum PRL concentration, in response to infusion were similar among all groups of cattle in three experiments. Steady state concentrations of serum PRL were achieved 120 to 150 min after the start of infusion. Furthermore, doubling the infusion dose of PRL had no effect on estimates of basal CR or SR (Table I). In contrast, Grosvenor *et al.* (8) reported that CR estimates for PRL increased as the dose of PRL was increased in lactating or nonlactating rats. Reasons for this difference are unknown but may be related to the concentration of PRL achieved in response to infusion. For example, steady state serum PRL concentrations achieved in our studies (55–107 ng/ml) were considerably lower than the concentrations of 127 to 470 ng/ml reported by Grosvenor *et al.* (8). Also, the steady-state PRL concentrations in our studies, were similar to serum PRL concentrations observed in cattle under a variety of normal physiological conditions.

Estimates of basal CR and SR per unit body weight among nonlactating cows (2.0 to 3.2 and 2.2 to 5.2, respectively) were similar to values which may be calculated from data reported for Hereford steers (2.0 to 2.7 and 1.4 to 4.2, respectively) (9).

Our results (Table III) indicated that

TABLE II. CLEARANCE RATES (CR), SECRETION RATES (SR) AND HALF-LIFE (T 1/2) OF PROLACTIN (PRL) IN PREGNANT AND LACTATING COWS

Parameter	Nonlactating, pregnant[a]	Lactating, nonpregnant[b]
No. animals	4	4
Body weight (kg)	678 ± 42	603 ± 42
Preinfusion PRL (ng/ml)	18.0 ± 8.0	36.5 ± 8.0
CR (ml/min)	1367 ± 95	1259 ± 95
SR (μg/min)	24.3 ± 2.7	28.6 ± 2.7
CR (ml/min)/kg body wt	2.02 ± 0.09	2.10 ± 0.09
SR (μg/hr)/kg body wt	2.16 ± 0.3	2.91 ± 0.3[c]
T 1/2 (min)	27.0 ± 1.0	32.9 ± 1.0[d]

[a] Multiparous cows pregnant (182–227) days.
[b] Multiparous cows lactating (6–46) days.
[c] Greater ($P \simeq 0.10$) than nonlactating cows.
[d] Greater ($P \leq 0.01$) than nonlactating cows.

TABLE III. CLEARANCE RATES (CR), SECRETION RATES (SR) AND HALF-LIFE (T 1/2) OF PROLACTIN (PRL) OF COWS IN VARIOUS STAGES OF LACTATION

Parameter	Nonlactating, pregnant[a]	Early lactating, nonpregnant[b]	Late lactating, pregnant[c]
No. animals	6	6	6
Body weight (kg)	673 ± 30	567 ± 30	636 ± 30
Preinfusion PRL (ng/ml)	26.1 ± 2.2[d]	36.9 ± 2.2[e]	30.9 ± 2.2[e]
CR (ml/min)	2119 ± 79	3197 ± 302	2475 ± 130
SR (μg/min)	58.9 ± 4.3	118.5 ± 12.8	77.0 ± 8.8
CR (ml/min)/kg body wt	3.2 ± 0.14[d]	5.6 ± 0.50[e]	4.0 ± 0.26[d]
SR (μg/hr)/kg body wt	5.2 ± 0.26[d]	12.5 ± 1.3[e]	7.3 ± 0.68[f]
T 1/2 (min)	32.1 ± 2.7	28.5 ± 2.7	36.1 ± 2.7

[a] Multiparous cows pregnant (180–212) days.
[b] Multiparous cows lactating (30–72) days.
[c] Multiparous cows lactating (242–290) and pregnant (184–236) days.
[d,e,f] Entries with different superscript within a row differ ($P \leq 0.05$).

basal CR and SR estimates adjusted for body weight were greater in lactating than in nonlactating dairy cows. Similarly, Davis and Borger (10) reported greater CR and SR of PRL in lactating ewes than in nonlactating ewes. Yet, this conclusion must be reconciled with the results of Experiment 1 (Table I) in which basal CR and SR estimates of PRL were very low among cows during advanced lactation relative to nonlactating cows in Experiments 2 and 3 (Tables 2, 3). However, these basal CR and SR estimates among all cows tested within the same physiological state were determined at different seasons of the year (winter–spring, Experiments 1 and 2; vs summer, Experiment 3). Perhaps clearance and secretion mechanisms vary with season. Indeed, concentrations of PRL in sera are highest during summer and lowest during winter in cattle (11).

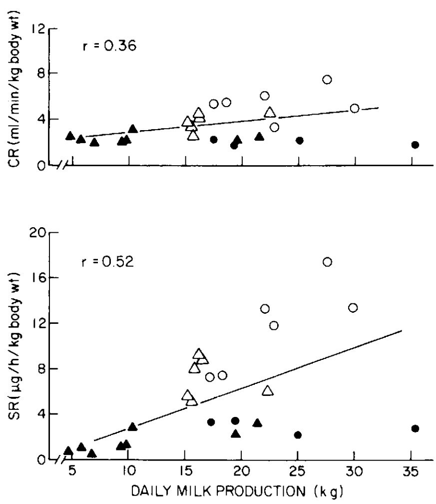

FIG. 2. Upper panel depicts relationship between PRL clearance rate (ml/min/kg body weight) and mean daily milk yield during the week prior to prolactin infusions. Lower panel is the relationship between PRL secretion and milk yield. ▲ = data from Experiment 1; ● = data from Experiment 2; and ○ △ = early and late lactating cows, respectively, data from Experiment 3.

Nonetheless, the data suggest that secretion of PRL is greatest during early lactation which coincides with the period of maximal milk yield. Furthermore, basal SR of PRL was correlated ($r = 0.52$) with milk production among cows at different stages of lactation (Fig. 2) irrespective of season of the year. It is speculated that the positive correlation between basal clearance rates of PRL and milk yield may result from greater tissue uptake and metabolism of PRL as milk production increases. In support of this possibility, Beck *et al.* (12) observed greatest uptakes of PRL by the mammary glands of cows during early lactation when milk yields were greatest.

Summary. Basal clearance rates (CR; ml/min/kg body wt) and secretion rates (SR; μg/hr/kg body wt) of prolactin (PRL) were calculated for Holstein cows. Among late lactating, pregnant cows (n = 8) tested in December, estimates of basal CR and SR were not different ($P > 0.05$) during infusion of either 4 or 8 mg PRL/cow/hr for 4 hr. In a second experiment, basal CR estimates

were similar ($P > 0.05$) in four nonlactating and four early lactating cows but basal SR was 35% greater ($P \sim 0.1$) in early lactating than in nonlactating cows tested in April. In a third experiment designed to avoid possible seasonal effects, basal CR estimates of PRL were 75 and 25% greater and basal SR estimates 140 and 40% greater ($P < 0.05$) in early lactating and late lactating cows than in nonlactating cows, respectively. Differences in basal CR and SR of PRL among all lactating cows were correlated with mean daily milk production ($r = 0.36$; $P < 0.05$ and $r = 0.52$; $P < 0.005$, respectively) during the week prior to each experiment. We conclude that increased basal CR and SR of PRL are associated with increased milk yields in dairy cows.

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Received October 15, 1979. P.S.E.B.M. 1980, Vol. 164.