Studies of Prolactin and Growth Hormone Secretion in Dairy Cattle by Radioimmunoassay

By TETSU JOHKE

Department of Physiology, National Institute of Animal Industry

For the studies of endocrine control of lactation and growth of dairy farm animals, it is essential to examine the hormone secretion as well as hormonal action. Until the appearance of radioimmunoassay procedure around 1960, there had been no reliable and sensible assay method to determine hormone concentration in blood[1]. Bioassay techniques for hormone determination provided valuable information but were often lacking specificity, sensitivity and precision. Attempts of direct chemical assay have always failed because of the low concentration of hormones in the presence of plasma proteins at concentration of millions of times greater. The introduction of radioimmunoassay into the field of endocrinology has revolutionized the techniques of assay of hormones in blood[1,2,6]. Since the later years of 1960', radioassay techniques, mainly radioimmunoassay, for protein and steroid hormones in blood of farm animals, have been developed successfully[6,10]. With these techniques it has become possible to assay a series of pg to ng levels of immunoreactive hormones in a large number of samples at a time.

In recent years, development of radioimmunoassay techniques for bovine prolactin (PRL) and growth hormone (GH) have been studied successfully in our laboratory bringing in new information on PRL and GH secretion in cattle[2~4,8].

Radioimmunoassay for bovine prolactin and growth hormone

The principle of radioimmunoassay is based on the ability of unlabeled hormone to compete with radioisotope labeled hormone for specific antibody, and hence to inhibit the binding of labeled hormone[1]. Although the theoretical principle and methodological aspects of radioimmunoassay are generally applicable, technical details vary according to characteristics of the different hormones. It has not been easy to obtain antisera to anterior pituitary hormones which have specific reactions and high affinity. Accordingly, in our radioimmunoassay for bovine PRL and GH, special consideration has been given to the production and selection of antisera. Specific antisera to PRL were obtained from guinea pigs immunized with bovine PRL[2~4] On the other hand, antisera to bovine GH were prepared in monkeys[8]. After studying several experimental conditions, the specific radioimmunoassays for bovine PRL and GH using double antibody method have been developed.

By these methods, as little as 0.1 ng PRL and GH in bovine plasma or serum can be estimated without extraction. The method is more than ten thousand times sensitive as compared with bioassay for PRL or GH and allows accurate and specific estimation of the hormones in plasma and other biological fluids. Furthermore, relative simplicity of the assay method permits large numbers of estimations simultaneously. It is also possible to estimate the hormones in goats and sheep by these method[4,8].

Serum hormone level during lactogenesis

During the late stage of pregnancy, serum

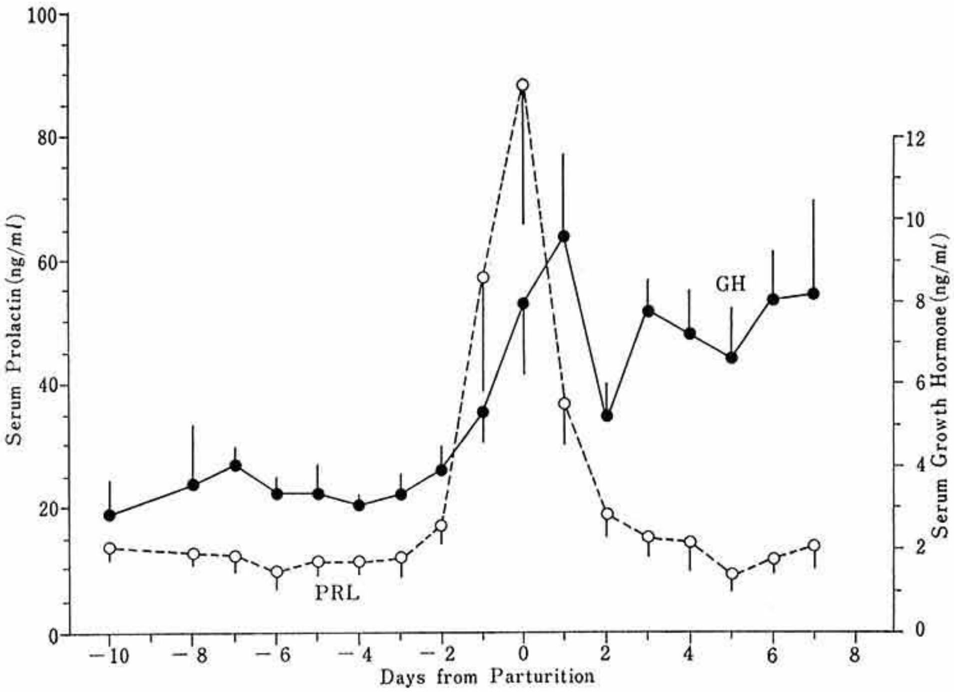

Fig. 1. Serum PRL (○) and GH (●) concentrations around parturition in the dairy cows. Each point and vertical bar indicate the mean and standard error (S. E.) of 5 determinations.

PRL in Holstein cows maintained a relatively low level. A conspicuous rise of circulating PRL levels occurs at the end of pregnancy[7]. The peak value was observed from one day before to the day of parturition (Fig. 1). The PRL surge around parturition continued for 2 to 3 days. Bovine serum GH level during the late stage of pregnancy was also low until the end of pregnancy. The highest level was observed on the day of parturition[7]. In postpartum, mean serum GH level was higher than that during late stage of pregnancy, though considerable day to day variation was observed. Subcutaneous administration of a potent PRL inhibitor, 2-Br-α-ergocryptine methane sulfonate (CB154, Sandoz) to multiparous Holstein cows during 2 weeks before parturition induced a decrease of basal serum PRL level followed by complete disappearance of PRL surge around parturition, but did not in serum GH and triiodothyronine[9]. In all the cases, lactogenesis or initiation of lactation was suppressed. The mean milk production during a week postpartum was 58.5% of that of the previous lactation[9]. It took more than 50 days to attain the milk yield of the previous lactation. Furthermore, the suppression of PRL secretion around parturition induced the reduction of syntheses of α-lactalbumin (B protein of lactose synthetase), lactose, and caseins[9,10].

Milking stimulus and release of hormone

Milking stimulus induces a remarkable but temporal rise of plasma PRL level in lactating cows and goats[3,5]. Stage of lactation as well as season affects the magnitude of PRL release to milking stimulus in the lactating cows[5,6] The response diminished markedly at late stage of lactation. PRL release to tactile stimulus to udder is not peculiar to lactating animals. Teat manipulation or sham-milking for 5 minutes can induce PRL release in non-lactating heifers and goats[5]. Circulating GH level in lactating goats increased by milking, but not in cows[10].

Seasonal variation of serum hormone

Bovine and caprine PRL secretion showed a considerable seasonal variation. Circulating basal and stimulated PRL level in the ruminant increased during spring and summer and decreased during autumn and winter[6,10]. The highest level was observed in summer and the lowest was in winter. A significant positive interrelation was obtained between plasma PRL concentration and ambient temperature (+0.835) or photoperiod (+0.764) in Holstein heifers[6,10]. Similar results were obtained in male and non-lactating goats[10]. On the other hand, no significant seasonal variation of of plasma GH in cattle was observed. Though several possibilities of seasonal variation of PRL secretion in ruminant exist, i.e. relation to season of nursing in wild and domesticated ruminants, breeding season, or salt retension, etc., the physiological significance remains to be elucidated.

Effects of TRH on hormone secretion

The administration of synthetic thyrotropin releasing hormone (TRH) not only elevates plasma thyrotropin, thyroxine, and triiodothyronine concentration but also causes release of PRL and GH in cattle[8]. Injection of TRH as small as 0.1 μg per kg body weight can increase bovine plasma PRL and GH concentrations (Fig. 2). In contrast to GH, the basal and post-injection plasma PRL levels of lactating

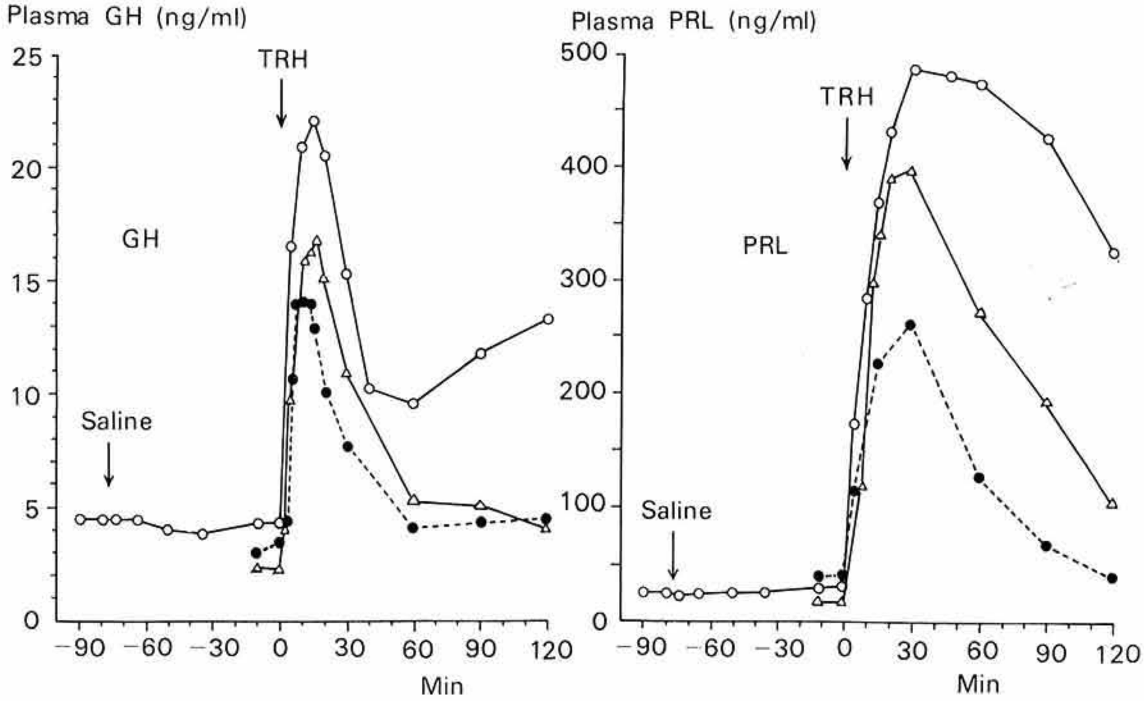

Fig. 2. Mean plasma GH and PRL responses in two lactating cows
Aadministered 0.1 (●), 1.0 (△), and 5 μg (○) of synthetic TRH per kg of bw at time 0. The peak values of GH and PRL increased proportionally to the dose of TRH. Saline injection did not change plasma GH and PRL concentration.

cows and heifers differed significantly in seasons, though the increase rate did not show a remarkable difference. TRH may be useful to evaluate the capacity of the bovine pituitary gland to release TSH, GH, and PRL at the same time.

Conclusion

Recent development and application of radioimmunoassay procedures for PRL and GH have revealed several factors affecting circulating levels of PRL and GH in cattle. Radioimmunoassay has been and will continue to be a most powerful tool for the study of the dynamics of hormone secretion in farm animals. Further investigations of endocrine control of growth and lactation by radioimmunoassay will not only contribute to the physiology of dairy animals but also to the improvement of the technology of dairying.

References

1) Berson, S. A. & Yalow, R. S.: Immunoassay of protein hormones. *In* The Hormones. eds. Pincus, G., Thimann, K. V. and Astwood, E. B. Academic Press. New York and London, 4, 557–630 (1964).
2) Johke, T.: Prolactin. *Igakunoayumi*, 67, 436–442 (1968) [In Japanese].
3) Johke, T.: Prolactin release in response to milking stimulus in the cow and goat estimated by radioimmunoassay. *Endocrinol. Japon.*, 16, 179–185 (1969).
4) Johke, T.: Radioimmunoassay for bovine prolactin in plasma. *Endocrinol. Japon.*, 16, 581–589 (1969).
5) Johke, T.: Factors affecting the plasma prolactin level in the cow and the goat as determined by radioimmunoassay. *Endocrinol. Japon*, 17, 393–401 (1970).
6) Johke, T.: Studies of prolactin secretion in dairy farm animals by radioimmunoassay. *Jap. J. Zootech. Sci.*, 47, 183–191 (1974) [In Japanese].
7) Johke, T. & Hodate, K.: Bovine serum prolactin, growth hormone and triiodothyronine levels during late pregnancy and early lactation. *Jap. J. Zootech. Sci.*, 48, 772–776 (1977).
8) Johke, T.: Effects of TRH on circulating growth hormone, prolactin and triiodothyronine levels in the bovine. *Endocrinol. Japon.*, 25, 19–26 (1978).
9) Johke, T. & Hodate, K.: Effects of CB154 on serum hormone level and lactogenesis in dairy cows. *Endocrinol. Japon.*, 25, 67–74 (1978).
10) Johke, T.: Hormone secretion during lactogenesis and maintenance in dairy farm animals. *In* The Physiology of Mammary Gland. eds. Yokoyama, A., Mizuno, H. and Nagasawa, H. University of Tokyo Press, Tokyo (1978).