Prolactin Release in Response to Milking Stimulus in the Cow and Goat Estimated by Radioimmunoassay

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Synopsis

The effect of milking stimulus on the plasma prolactin concentration of lactating cows and goats was investigated by a radioimmunologic procedure. Plasma prolactin level in seven cows was increased abruptly 1 to 2 mins. after the start of milking. On the other hand, the level of the hormone was increased shortly before the onset of milking in two cows at early lactation stage. In the goats at mid-lactation stage, milking was followed by a dramatic increase in plasma prolactin concentration within 2 mins. In all the cases, plasma prolactin level reached a peak near the end of milking. The stimuli associated with milking induced an unequivocal and rapid prolactin release from the adenohypophysis in the cow and in the goat.

Several studies showed that suckling stimulus caused a fall in the prolactin stores of the pituitary gland of lactating rat by bioassay (Reece and Turner, 1937; Grosvenor and Turner, 1958; Grosvenor et al., 1967) and by cytological inspection (Pasteels, 1963). However, measurement of prolactin level in blood has been impeded by a lack of a sensitive method.

With a radioimmunoassay by double antibody method which is capable of detecting immunoreactive prolactin in unextracted plasma, an acute change in the hormone level in blood of lactating cow and goat in relation to the stimuli associated with milking is demonstrated in this experiment.

Materials and Methods

Radioimmunoassay

a) Hormones and chemicals

Bovine prolactin and other pituitary hormones are generously supplied by the Endocrinology Study Section, National Institute of Health (Bethesda, Md., USA). ACTH and bovine serum alubumine (BSA) were obtained from the Armour Pharmaceutical Ltd. Bovine prolactin (NIH-P-B2, 19.9 I.U./mg) was further purified by DEAE-Sephadex A-50 column chromatography. The major fraction obtained by column chromatography with stepwise elution of sodium chloride in 0.01 M phosphate buffer, pH 6.8 was collected and lyophilized after dialysis. Other chemicals were those of highest purity available.

b) Preparation of $^{131}$I-prolactin

The purified bovine prolactin was iodinated according to the method developed for human growth hormone (Greenwood et al., 1963). To 2 mCi aliquots of carrier-free Na$^{131}$I (ISO/Serve Division of Nuclear Corp., Cambridge, Mass., U.S.A.), 0.02 ml of 0.5 M sodium phosphate buffer, pH 7.5 and 5 mg prolactin were added. Twenty mg of chloramine T in 0.02 ml of 0.05 M phosphate buffer, pH 7.5, was added and the reaction mixture was agitated for 30 seconds. The reaction was stopped with addition of 240 mg sodium metabisulfite, followed by 0.1 ml of 1 % KI. This reaction mixture was placed on the surface of a 1 x 15 cm Sephadex G-50 column equilibrated with 0.05 M barbital buffer, pH 8.6. Before use, 1 ml of 20 mg/ml BSA in the buffer was passed through the column. The elution of the sample was carried with 0.05 M barbital buffer, pH 8.6. One ml aliquot of

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protein fraction were collected in tubes containing 0.1 ml of 2 % BSA in the buffer. Specific activities between 200 and 300 mCi/mg were obtained by this procedure. The damage to the labeled prolactin during the preparation was less than 7 % as judged by Whatman DEAE paper electrophoresis (Johke, 1969). More than 90 % of 131I-prolactin was precipitable with excess antibody in the assay system.  

**c) Antisera to prolactin and guinea pig gamma globulin**

Antisera to bovine prolactin (NIH-P-B2) were prepared in guinea pigs. Water solution of 1 mg prolactin was emulsified with equal volume of complete Freund’s adjuvant. The mixture was injected into the foot pads of hind legs. The animals were given additional injections intraperitoneally and subcutaneously at intervals of 2 to 3 weeks, and bled 7 to 10 days after the animals had received their fourth injection. Each animal was given approximately 5 mg of prolactin. The sera were decomplemented by incubation for 30 mins. at 56°C. They were absorbed with small amount of diluted bovine serum (1:10). The antiserum of highest energy of reaction was used in all assays. Rabbit, anti-guinea pig gamma globulin sera (anti-GPGG) were obtained from adult rabbits by repeated injections of GPGG emulsified in complete Freund’s adjuvant. The sera were decomplemented by incubation for 30 mins. at 56°C.  

**d) Assay procedure**

Plasma prolactin was measured by a modified double antibody method (Johke, 1968) which was originally devised for the radioimmunoassay of insulin by Morgan et al., (1964), and for human growth hormone by Schalch and Parker (1964). The diluent used consisted of 0.5 % BSA in 0.05 M barbital buffer pH 8.6 containing 0.01% merthiolate. The reaction was carried out as follows. To 0.2 ml of diluent containing 0.035 M tetrasodium ethylene diamine tetraacetate (pH 8.6±0.2) were added 0.1 ml of standard hormone or unknown, 0.1 ml of 131I-prolactin (0.07~0.13 mCi), and 0.1 ml of guinea pig anti-bovine prolactin serum (1:100000~1:200000). The antiserum was diluted appropriately so that the diluted antiserum bound 50 to 60 % of the added 131I-prolactin when no competing unlabeled prolactin was present in the system. After incubation for four days at 4°C, 0.1 ml of anti-GPGG (1:5~1:10), which would assure complete precipitation of all GPGG in the system, was added. Finally, 0.1 ml of normal guinea pig serum (1:300) was added. After reincubation at 4°C for another 24 hrs. the mixtures were counted in a well type scintillation counter. Then, they were centrifuged at 3000 rpm for 15 mins. and the supernatants (free 131I-prolactin) were discarded. By counting the precipitate (antibody bound 131I-prolactin), the percent radioactivity of the precipitate was calculated. From the standard curve, the concentration of prolactin in each plasma sample was determined. Bovine prolactin (NIH-P-B2) was used as the standard hormone in the assay. The radioimmunoassay of bovine prolactin by double antibody method was not interfered by other pituitary hormones. Ovine prolactin reacted quantitatively in the system (Fig. 1). Goat pituitary extracts also showed strong cross-reaction with bovine prolactin, and purified prolactin added to plasma was recovered quantitatively (unpublished data). Plasma samples were assayed beyond a final dilution 1:25.  

**Experimental animals**

The plasma samples were obtained from lactating Holstein cows and Saanen goats in the Institute herds. In order to minimize emotional disturbance
and to get serial samples, the blood was taken from an indwelling polyethylene catheter previously inserted into one of the external jugular veins by venipuncture with a bleeding needle. In cows, the catheter was 40 cm in length with an outside diameter of 2 mm and in goats, 25 cm in length with an outside diameter of 1.5 mm. The catheter had a stopcock at the external end. Blood samples (4 ~ 6 ml) were collected into a glass centrifuge tube containing heparin at 1 to 20 mins. intervals. In cows, when the stopcock of the catheter was open, the blood flowed freely from the catheter. To shorten the sampling time in goats, the blood was withdrawn by suction using a 5 ml hypodermic syringe. The catheter was flushed with 2 to 3 ml of heparinized saline after each sampling. Blood samples were chilled by ice immediately. At the end of each experiment the blood was centrifuged and the plasma was stored at -20°C until assayed. The experiments were done either on a stall platform or in a pen. Seven cows were milked by a bucket type milking machine and two cows were milked by hand. Washing the udder with warm water and foremilking were carried out within 2 mins. before the start of milking. Concentrate was given to the cows immediately preceding milking except cows H5 and H8, which were not fed during milking. The goats were milked by hand with neither washing the udder nor feeding concentrate.

**Results and Discussion**

Plasma prolactin level in seven cows (H3 ~ H9) was increased abruptly 1 to 2 mins. after the start of milking (Fig. 2 and Table 1). On the other hand, levels of the hormone increased shortly before the onset of milking in the cow H1 and H2 (Fig. 3). In the goats milked neither washing the udder nor feeding,
Table 1. Prolactin level in the external jugular vein plasma of cows and goats

<table>
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<tr>
<th></th>
<th>Cow</th>
<th>Goat</th>
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<tr>
<td></td>
<td>H1</td>
<td>H2</td>
</tr>
<tr>
<td>Calving no.</td>
<td>3</td>
<td>2</td>
</tr>
<tr>
<td>Stage of lactation (day)</td>
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<td>15</td>
</tr>
<tr>
<td>Plasma prolactin before milking (mμg/ml)</td>
<td>13</td>
<td>19</td>
</tr>
<tr>
<td>Maximum plasma prolactin after the start of milking (mμg/ml)</td>
<td>87</td>
<td>87</td>
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<tr>
<td>Time required to reach maximum plasma prolactin after the start of milking (min.)</td>
<td>12</td>
<td>7</td>
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<tr>
<td>Duration of milking at the experiment (min.)</td>
<td>13</td>
<td>10</td>
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<tr>
<td>Milk yield at the experiment (kg)</td>
<td>15.4</td>
<td>10</td>
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<td>Milk yield per day (kg)</td>
<td>31.2</td>
<td>25</td>
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<td>Milking method</td>
<td>M*</td>
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* Machine milking
** Hand milking

milking was followed by a dramatic increase in plasma prolactin concentration within 2 mins. (Fig. 4). In all the cases, plasma prolactin level reached a peak near the end of milking (4 ~ 15 mins., see Table 1).

Although milking itself will be the most potent stimulus to elevate plasma prolactin level in the cow and goat, the results suggest that washing the udder and other stimuli associated with milking also can elicit a rapid increase in plasma prolactin concentration in the cows. Exteroceptive stimuli associated with nursing cause the release of prolactin from the adenohypophysis of the lactating rat to the same extent as the resulting from nursing (Grosvenor, 1965). Folley and Knaggs (1966) showed that the application of the teat cups of milking machine was the most potent stimulus to elevate plasma oxytocin level in the cows. It was also demonstrated that auditory and visual stimuli associated with milking process, washing the udder and foremilking stimulated the release of oxytocin in the cows (Cleverley, 1968).

It seems reasonable to assume that the increase of immunoreactive prolactin in jugular vein plasma gives an indication of release of the hormone from the adenohypophysis. In the present study, it has been clearly shown that the stimuli associated with milking induced an unequivocal and rapid prolactin release from the pituitary gland of cow and goat.

The elevation of plasma prolactin level in plasma was detected in the cows at the end of lactation period and goats at mid-lactation stage which had not been previously milked for 24 hrs. The data suggested that the prolactin release response of the cows at the end of lactation period was weaker than that of the cows at early lactation period. On the other hand, Grosvenor and Turner (1958) showed by bioassay that 30 mins. of suckling induced a considerable fall in pituitary prolactin concentration if applied during the first two-thirds of lactation, but failed to do so on the 21st day of lactation, the normal time of weaning in the rat. Grosvenor et al., (1967) also noted that suckling by pups following 8 hrs. of nonsuckling on the 7th day postpartum induced a rapid fall in pituitary prolactin concentration, while suckling was in-
effective in reducing the pituitary prolactin concentration, if the mothers had been not suckled for 16 hrs. The study is under way to clarify the effects of the stage of lactation and milk yield on the prolactin release response to milking stimulus in the same cows.

It has been demonstrated that prolactin is one of the essential hormones for the maintenance of milk secretion in hypophysectomized rats, guinea pigs, and goats (see Meites and Nicoll, 1966, for review). Prolactin was found to stimulate directly the synthesis of milk proteins in organ culture of mouse’s mammary gland (Turkington, 1968). Milking or suckling stimulus causes removal of milk from the mammary gland and at the same time it induces rapid prolactin release from the adenohypophysis. In the lactating rat, it has also been demonstrated that suckling stimulated release of growth hormone (Grosvenor et al., 1968), ACTH (Gregorie, 1947; Taleisnik and Orias, 1966), MSH (Taleisnik and Orias, 1966), antidiuretic hormone and oxytocin (see Cross, 1961, for review) from the pituitary gland. It is postulated from the above evidences that, although the duration of high prolactin concentration in plasma after milking is relatively short time, prolactin might stimulate directly the synthesis of milk constituents in the mammary gland after removal of

![Graph](image-url)
milk together with other hormones in cows as well as goats.

Plasma prolactin levels soon after cannulation by venipuncture with bleeding needle (outside diameter 3 mm) without anesthesia were usually higher than those immediately before the start of milking in the cows (Figs. 2 and 3). There was considerable individual variation in the magnitude of the response. In the preliminary experiments, the data suggested that stressful stimuli associated with venipuncture also could elicit nonspecifically an acute prolactin release from the pituitary gland in the cow. Grosvenor et al., (1965) found that laparotomy with bleeding in lactating rats anesthetized with ether and cervical stunning prior to decapitation, each resulted a fall in pituitary prolactin concentration similar to that which followed nursing.

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References


Grosvenor, C. E., L. Krulich and S. M. McCann (1968). Endocrinology 82, 617.