Domestic Animal Endocrinology 47 (2014) 47–54

Contents lists available at ScienceDirect

Domestic Animal Endocrinology

journal homepage: www.domesticanimalendo.com

Effect of premilking stimulation and milking frequency on milking-induced prolactin release in lactating dairy cows

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ARTICLE INFO

Article history:
Received 22 October 2013
Received in revised form 19 November 2013
Accepted 24 November 2013

Keywords:
Lactation
Milking frequency
β-Endorphin
Cortisol

ABSTRACT

Four experiments were conducted to investigate the factors controlling prolactin (PRL) release at milking. Each experiment used 9 dairy cows in mid-lactation in a 3 × 3 Latin square design. Experiment 1 evaluated the effect of premilking stimulation. The milking unit was attached after 0, 20, or 120 s of manual stimulation. Blood samples were collected from 20 min before to 60 min after milking-unit attachment. The peak value and total PRL release (area under the curve) were not affected by the treatments, but the 120-s stimulation hastened PRL release. Stimulation (20 or 120 s) increased the β-endorphin peak value ($P = 0.02$), but the magnitudes of PRL and β-endorphin releases were not correlated. Experiment 2 evaluated the effect of milking frequency. Cows were milked twice, at 7 AM and 7 PM; 3 times, at 7 AM, 1 PM, and 7 PM; or 7 times, at 7 AM, 9 AM, 11 AM, 1 PM, 3 PM, 5 PM, and 7 PM. The amount of PRL released at the 7 PM milking decreased as the number of milkings increased ($P < 0.01$), and peak values were smaller with 7 milkings than with 2 and 3 milkings ($P < 0.05$). Beta-endorphin release was not affected by milking frequency and not correlated with the magnitude of PRL release. Experiment 3 evaluated the effect of manual stimulation between milkings on milking-induced PRL release. Cows received no stimulation; 5 stimulations (5 min each), at 9 AM, 11 AM, 1 PM, 3 PM, and 5 PM; or 1 stimulation at 5 PM. Manual stimulation reduced ($P < 0.5$) the amount of PRL released and the maximum PRL concentration at the 7 PM milking, but no difference were found between 1 and 5 stimulations. Manual stimulation did not affect the amount of cortisol released but did impair milk ejection. Experiment 4 evaluated the effect of milking frequency on the PRL release induced by manual stimulation. Cows were milked at 7 AM only; at 7 AM, 9 AM, 11 AM, 1 PM, 3 PM, and 5 PM; or at 7 AM and 5 PM. All cows then received manual stimulation at 7 PM. Milking every 2 h or once 2 h before manual stimulation reduced the amount of PRL released and the maximum PRL concentration but did not affect cortisol release. In conclusion, the length of premilking stimulation has no significant effect on milking-induced PRL release, but increasing milking frequency reduces the amount of PRL released at milking. This effect is due not to the number of milkings or the amount of milk harvested during the milking but to the interval since the preceding milking.

1. Introduction

Biological mechanisms exist to adjust milk output to demand. Some evidence, including photoperiod, bovine somatotropin administration, gestation status, and stage of lactation, suggests that milk production is regulated primarily systemically, whereas other evidence, such as

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http://dx.doi.org/10.1016/j.domaniend.2013.11.007

milking frequency and milk stasis, suggests that milk production is also regulated locally at the level of the mammary gland. Milking and suckling induce an important release of hormones in the female circulation and, therefore, provide a link between local and systemic regulation of milk production.

Prolactin (PRL), as its name implies, is the most important hormone for the control of lactation. In most mammals, suppression of PRL with bromocriptine strongly inhibits lactation [1,2]. Although the role of PRL in the control of ruminant lactation has been controversial, studies that involve the present authors found that administration of a specific and potent PRL release inhibitor, quinagolide, decreased milk production in dairy cows [3,4] and that PRL injection attenuated the inhibitor effect [5]. Even though the basal level of PRL has not been clearly associated with the level of milk production, milking-induced PRL release has been correlated with the level of milk production in both PRL-inhibited and control cows. In addition, the effect of PRL inhibition on milk production was found to be modulated by milking frequency [3]. Therefore, factors that affect PRL concentration, especially milking-induced PRL release, are likely to play an important role in the control of milk production.

Milking- and suckling-induced PRL release is a poorly understood neuroendocrine reflex. The β-endorphin antagonist naloxone [6] and dopamine agonists suppress milking-induced PRL release [3,7], suggesting that β-endorphin and dopamine are important players for the control of this reflex. This reflex is not related to the milk harvest itself, because PRL release can be induced in non-lactating animals by nipple stimulation [8]. Therefore, the objective of the experiments in the present study was to determine the effects of premilking stimulation and milking frequency on milking-induced PRL release.

2. Materials and methods

All the experiments were conducted at Agriculture and Agri-Food Canada's Dairy and Swine Research and Development Centre (Sherbrooke, QC, Canada) in accordance with the guidelines of the Canadian Council on Animal Care. Cows were kept in a tie-stall barn and were normally milked twice a day with a 12-h milking interval.

2.1. Experimental design

2.1.1. Effect of premilking stimulation on milking-induced prolactin release

Experiment 1 used 9 cows in mid-lactation (171 ± 6 DIM) in a 3 × 3 Latin square design in which the milking unit was attached a) without prior manual stimulation of the mammary gland, b) after 20 s of manual stimulation, or c) after 120 s of manual stimulation. Cows were milked at approximately 8 AM at the stall under the close supervision of a milkman and followed by teat dipping. On each experimental day, blood samples were collected before, during, and after the morning milking (−20, −10, −2, 0, 3, 5, 7, 10, 15, 20, 25, 30, 40, and 60 min relative to the start of milking) in Vacutainer tubes without additives (BD, Mississauga, ON, Canada) from a Silastic catheter (i.d. 1.02 mm, o.d. 2.16 mm; Dow Corning Corp, Midland, MI, USA) inserted into the jugular vein. In addition, blood samples were collected 9 times (−2, −1, 0, 1, 2, 3, 5, 7, and 10 min relative to the start of milking) in EDTA-coated Vacutainer tubes that contained aprotinin. All blood tubes without additives were left for approximately 2 h at room temperature for clotting before centrifugation (1,900× g 4°C, 15 min). Then, the serum was stored at −20°C until determination of PRL concentration. All blood tubes that contained EDTA were placed on ice immediately after collection and centrifuged (1,900× g, 4°C, 15 min). Then, the plasma was stored at −80°C until β-endorphin determination. The 3 sampling days were separated from each other by 1 d of rest.

2.1.2. Effect of milking frequency on milking-induced prolactin release

Experiment 2 used 9 cows in mid-lactation (178 ± 6 DIM) in a 3 × 3 Latin square design in which they were milked on the sampling day at a) 7 AM and 7 PM (2×); b) 7 AM, 1 PM, and 7 PM (3×); or c) 7 AM, 9 AM, 11 AM, 1 PM, 3 PM, 5 PM, and 7 PM (7×). Cows were milked at the stall under the close supervision of a milkman. The mammary gland was washed for about 25 sec, and the milking units were attached immediately after. Blood samples were collected at the 7 PM milking as described for Exp. 1, with 2 exceptions: the samples at −2 and −1 min were omitted, and a sampled was collected in an EDTA-coated tube at −10 min. The 3 sampling days were separated from each other by 1 d of rest.

2.1.3. Effect of manual stimulation of the mammary gland between milkings on milking-induced prolactin release

Experiment 3 used 9 cows in mid-lactation (170 ± 5 DIM) in a 3 × 3 Latin square design in which all the cows were milked 2 times, 12 h apart (7 AM and 7 PM). The treatments were a) no manual stimulation between milkings; b) 5 manual stimulations at 2-h intervals, starting 2 h after the morning milking (at 9 AM, 11 AM, 1 PM, 3 PM, and 5 PM); and c) 1 manual stimulation at 5 PM. The milkings were performed as described in experiment 2, and the manual stimulations were performed by rubbing the udder and teats vigorously with bare hands for 5 min. At the 7 PM milking, blood samples were collected in uncoated Vacutainer tubes at −20, −10, 0, 3, 5, 7, 10, 15, 20, 25, 30, 40, and 60 min relative to the start of milking. Serum samples were prepared as described for Exp. 1 and stored at −20°C until determination of PRL and cortisol concentrations. The 3 sampling days were separated from each other by 1 d of rest.

2.1.4. Effect of milking frequency on prolactin release induced by manual stimulation

Experiment 4 used 9 cows in mid-lactation (177 ± 5 DIM) in a 3 × 3 Latin square design in which all the cows were milked at 7 AM and manually stimulated at 7 PM. The treatments were a) no additional milking; b) 5 additional milkings at 2-h intervals, starting 2 h after the morning milking (at 9 AM, 11 AM, 1 PM, 3 PM, and 5 PM); and c) 1 additional milking at 5 PM. Blood samples were collected at the 7 PM manual stimulation. Milking, manual stimulation,