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Mammary epithelium disruption and mammary epithelial cell exfoliation during milking in dairy cows

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ABSTRACT

The presence of mammary epithelial cells (MEC) in the milk of ruminants indicates that some MEC are shed from the mammary epithelium; however, the mechanisms that regulate the MEC exfoliation process are not known. Through the release of oxytocin, prolactin, and cortisol and through oxytocin-induced mechanical forces on the mammary epithelium, milking could participate in regulating the MEC exfoliation process. The aims of the present study were to determine the rate of MEC exfoliation throughout milking and to investigate its relationship to mammary epithelium integrity and milking-induced hormone release. Milk samples from 9 Holstein dairy cows producing 40.6 ± 1.36 kg of milk/d were collected at the beginning (after 1 and 2 min), in the middle, and at the end of milking. Milk MEC were purified using an immunomagnetic method. Blood samples were collected before, during, and after milking, and the oxytocin, prolactin, and cortisol concentrations in the samples were measured. Tight junction opening was assessed by plasma lactose concentration and the Na^+:K^+ ratio in milk. The somatic cell count in milk varied during the course of milking; it decreased at the beginning of milking and then increased, reaching the highest values at the end of milking. Exfoliated MEC were present in all milk samples collected. The presence of MEC in the milk sample collected during min 1 of milking, likely corresponding to the cisternal milk fraction, suggests that MEC were exfoliated between milkings. The observed increase in the Na^+:K^+ ratio in milk and in the plasma concentration of lactose indicated that disruption of mammary epithelium integrity occurred during milking. The MEC exfoliation rate at milking was not correlated with the variables describing milking-induced prolactin release but was negatively correlated with cortisol release, suggesting that cortisol may play a role in limiting exfoliation. In conclusion, milking induced a disruption of the mammary epithelial barrier. Mammary epithelial cells may be continuously exfoliated between milkings or exfoliated during milking as a consequence of the oxytocin-induced mechanical forces and the disruption of mammary epithelium integrity.

Key words: dairy cow, mammary epithelial cell exfoliation, milking, tight junction

INTRODUCTION

Milk is synthesized by mammary epithelial cells (**MEC**), and milk yield is determined by the metabolic activity and the number of these cells in the mammary gland (Capuco et al., 2003; Boutinaud et al., 2004). The number of MEC in the mammary gland depends primarily on the balance between cell proliferation and apoptosis (Capuco et al., 2003). The presence of MEC in the milk of ruminants (Kitchen, 1981) indicates that MEC are shed from the mammary epithelium into milk. It was recently shown that the MEC exfoliation process participates in the regulation of MEC number in the udder and thus in milk yield variations (Herve et al., 2016). The mechanisms that regulate the MEC exfoliation process, however, are currently unknown. The MEC exfoliation process could be linked to a disruption of mammary epithelium integrity. Indeed, the MEC exfoliation rate increases concomitantly with an increase in the mammary epithelial permeability in response to various physiological, breeding, and environmental factors (Herve et al., 2016). It is not yet clear, however, whether the exfoliation of MEC is a consequence or a cause of tight junction opening.

Hormones released at milking in response to teat stimulation—namely oxytocin (**OT**), prolactin (**PRL**), and cortisol (**Cort**)—are likely to be involved in the regulation of mammary epithelium integrity (Nguyen and Neville, 1998). However, it is not yet known whether milking induces a disruption of the mammary epithelium integrity. Exogenous OT administered at

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supraphysiological doses was shown to induce tight junction opening in the bovine mammary gland (Allen, 1990; Wall et al., 2016), and it was speculated that tight junction integrity is compromised as a result of the mechanical forces caused by myoepithelial cell contraction (Stelwagen and Singh, 2014). The physiological OT release induced by milking also induces the contraction of myoepithelial cells and thus mechanical pressures on the mammary epithelium, allowing the complete removal of the alveolar milk. Prolactin also participates in the regulation of tight junctions because PRL stimulates tight junction formation in vitro (Stelwagen et al., 1999) and reduces tight junction permeability in rabbit and rat mammary glands (Linzell et al., 1975; Flint and Gardner, 1994). Finally, Cort is known to be a key regulator of mammary epithelium integrity; it stimulates tight junction formation and maintenance in vitro (Zettl et al., 1992; Singer et al., 1994), is necessary for tight junction closure in vivo during the transition from pregnancy to lactation in mice (Nguyen et al., 2001), and reduces tight junction permeability in vivo in the bovine mammary gland (Stelwagen et al., 1998).

The milking-induced release of OT could disrupt mammary epithelium integrity during milking, whereas PRL and Cort, which are also released upon milking, could reduce mammary epithelial permeability. We therefore hypothesized that milking, through the release of OT, PRL, and Cort and the OT-induced mechanical forces on the mammary epithelium, could play a role in regulating the MEC exfoliation process by modulating the integrity of the mammary epithelium. Thus, the aim of the present study was to determine the rate of MEC exfoliation during the time course of milking and to investigate the relationships between milking-induced hormone release, mammary epithelium integrity, and the MEC exfoliation process in dairy cows.

MATERIALS AND METHODS

All procedures used on animals were approved by the local Ethics Committee in Animal Experiment of Rennes (France) in compliance with French regulations (decree no. 2001-464; May 29, 2001; https://www.legifrance.gouv.fr/eli/decret/2001/5/29/AGRG0001697D/jo/texte).

Animals and Experimental Procedures

Nine multiparous Holstein dairy cows (lactation 2 to 5), at peak lactation (57 ± 5 DIM) and producing 40.6 ± 1.36 kg of milk/d at the beginning of the experiment, were used in this study. The cows were selected to have a low SCC; thus, they presented on average 36 ± 11 × 10^3 cells/mL of milk before the beginning of the experiment. The experiment was conducted at the INRA experimental farm (UMR PEGASE, Le Rheu, France). Two weeks before the beginning of the experiment, the cows were housed in individual tiestalls to allow them to adapt to their new environment. The cows were fed according to the INRA recommendations and milked twice a day at 0700 and 1700 h. The milking routine included teat cleaning for approximately 30 s and foremilk hand milking to confirm the absence of mastitis, indicated by the absence of clotted milk. During one morning milking (after a routine 14-h milking interval), milk samples were collected separately each minute using 2 milking devices alternately. Milk yield was recorded each minute, and a milk sample (50 mL) was taken for SCC and milk composition (fat, protein, and lactose content) determination using an infrared method (Lillab, Châteaugiron, France).

Blood Sampling and Hormonal Assays

The cows were surgically equipped with permanent catheters (Silclear medical-grade silicone tubing, 1.02 mm i.d., 2.16 mm o.d.; Degania Silicone, Degania Bet, Israel) inserted into the jugular vein. The catheters were inserted 4 d before the beginning of the experiment. On the day of the experiment, blood samples were collected before, during, and after the morning milking at −5 −2, 1, 2, 3, 4, 6, 8, 10, 15, 25, 35, and 45 min relative to milking unit attachment. Monovette syringes coated with sodium heparin (Sarstedt, Nümbrecht, Germany) were used to collect samples for the measurement of plasma OT and lactose concentrations, and Monovette syringes coated with EDTA (Sarstedt) were used to collect samples for the measurement of plasma PRL and Cort concentrations. The plasma was separated by centrifugation at 3,000 × *g* for 15 min at 4°C and stored at −20°C until analysis. Plasma OT concentration was measured using the ELISA method as described by Marnet et al. (1994). The plasma concentration of Cort was assessed using the ELISA method developed by Komara and Marnet (2009). The ELISA plates were coated with 200 μL/well of mouse monoclonal antirabbit immunoglobulin antibody (Bertin Pharma, Montigny-le-Bretonneux, France) dissolved at 10 mg/L in 0.05 *M* phosphate buffer and incubated at 4°C overnight. The plates were then washed, and 300 μL of ELISA buffer (0.1 *M* phosphate buffer containing 0.15 *M* sodium chloride and 0.1% BSA, pH 7.4) was added to each well. Cortisol was extracted from the plasma samples as follows: 100 μL of plasma was mixed with 1 mL of absolute ethanol and incubated for 10 min on ice. After centrifugation at 8,000 × *g* for 10 min