J. Dairy Sci. 98:1–13
http://dx.doi.org/10.3168/jds.2015-9853

In vivo inhibition followed by exogenous supplementation demonstrates galactopoietic effects of prolactin on mammary tissue and milk production in dairy cows

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ABSTRACT

It has been previously shown that the long-term inhibition of milking-induced prolactin (PRL) release by quinagolide (QN), a dopamine agonist, reduces milk yield in dairy cows. To further demonstrate that PRL is galactopoietic in cows, we performed a short-term experiment that used PRL injections to restore the release of PRL at milking in QN-treated cows. Nine Holstein cows were assigned to treatments during three 5-d periods in a 3 × 3 Latin square design: 1) QN: twice-daily i.m. injections of 1 mg of QN; 2) QN-PRL: twice-daily i.m. injections of 1 mg of QN and twice-daily (at milking time) i.v. injections of PRL (2 μg/kg body weight); and 3) control: twice-daily injections of the vehicles. Mammary epithelial cells (MEC) were purified from milk so that their viability could be assessed, and mammary biopsies were harvested for immunohistological analyses of cell proliferation using PCNA and STAT5 staining. In both milk-purified MEC and mammary tissue, the mRNA levels of milk proteins and *BAX* were determined using real-time reverse-transcription PCR. Daily QN injections reduced milking-induced PRL release. The area under the PRL curve was similar in the control and PRL injection treatments, but the shape was different. The QN treatment decreased milk, lactose, protein, and casein production. Injections of PRL did not restore milk yield but tended to increase milk protein yield. In mammary tissue, the percentage of STAT5-positive cells was reduced during QN but not during QN-PRL in comparison with the control treatment. The percentage of PCNA-positive cells was greater during QN-PRL injections than during the control or QN treatment and tended to be lower during QN than during the control treatment. In milk-purified MEC, κ-casein and α-lactalbumin mRNA levels were lower during QN than during the control treatment, but during QN-PRL, they were not different from the control treatment. In mammary tissue, the *BAX* mRNA level was lower during QN-PRL than during QN. The number of MEC exfoliated into milk was increased by QN injections but tended to be decreased by PRL injections. Injections of PRL also increased the viability of MEC harvested from milk. Although PRL injections at milking could not reverse the effect of QN treatment on milk production, their effects on cell survival and exfoliation and on gene expression suggest that the effect of QN treatment on the mammary gland is due to QN's inhibition of PRL secretion.

Key words: mammary gland, cell survival, cell differentiation, milk mammary epithelial cells, cell exfoliation

INTRODUCTION

Despite its etymology, where "pro" stands for before and "lact" stands for milk, prolactin (**PRL**) has not always been considered a galactopoietic hormone in ruminants. Although it has been well established in rodents and lagomorphs that PRL depletion reduces milk production, in ruminants the effects of PRL inhibition are less obvious, and the galactopoietic role of PRL has been a matter of debate for several decades (Knight, 2001). Several of the present authors recently showed that the long-term inhibition of PRL with a potent dopamine agonist, namely quinagolide (**QN**), reduced milk yield in dairy cows (Lacasse et al., 2011). However, a complete demonstration of the galactopoietic role of

Received May 22, 2015.
Accepted June 29, 2015.
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PRL in ruminants requires showing that the effect of QN on the mammary gland is due to the inhibition of PRL secretion.

The actions of PRL on mammary epithelial cells (**MEC**) have been described in rodent cell lines and in primary bovine MEC cultures. The signaling of PRL begins with the binding to 1 of 2 forms of PRL receptors (**PRLR**): long and short isoform. After inducing the dimerization of the long isoform of its receptor, PRL signaling is followed by the activation by phosphorylation of both its receptor and the protein tyrosine kinase JAK2, which in turn phosphorylates the signal transducer and activator of transcription STAT5. Once activated, STAT5 translocates to the nucleus and binds to specific regulatory sites in the promoters of target genes such as milk protein genes (Groner and Gouilleux, 1995). Also in primary bovine MEC cultures, PRL has been shown to upregulate the mRNA levels of several caseins (Choi et al., 1988). A high number of other genes are under the control of PRL stimulation, as has been shown in bovine MEC using microarray analyses (Stiening et al., 2008; Riley et al., 2010). In association with its role in MEC differentiation, PRL is also known to promote DNA synthesis and cell proliferation, as has been demonstrated in rabbit and bovine MEC (Suard et al., 1983; Olazabal et al., 2000). Thus, one way to demonstrate that the effect of QN on the mammary gland is related to its inhibition of PRL is to analyze the effects of QN treatment at the level of MEC differentiation and proliferation and the activation of STAT5, which are known to be modulated by PRL in vitro.

Some previous studies have attempted to analyze the effect of PRL in dairy ruminants via PRL injections. The short-term administration of exogenous PRL did not significantly affect the milk yield of dairy cows during the first 3 wk of lactation (Wall et al., 2006) or around peak of lactation (Plaut et al., 1987). Even though the galactopoietic role of PRL in lactating sows is well established, PRL failed to stimulate milk yield in that species (Farmer et al., 1999). Because milking-induced PRL release decreases as lactation advances (Koprowski and Tucker, 1973), early in lactation, endogenous PRL is secreted at relatively high levels in response to milking, and thus the mammary gland may already be saturated. The effect of endogenous PRL on mammary tissue could prevent the action of exogenous PRL on milk production. Therefore, the injection of exogenous PRL in a later lactation stage may be a more efficient way to affect milk production. Moreover, so that interaction with endogenous PRL can be avoided, a replacement therapy study may be a more efficient way to demonstrate the role of PRL in mammary tissue. Thus, to assess the galactopoietic role of PRL and the specific action of PRL in the mammary tissue of dairy cows, we inhibited its secretion using QN treatment and tried to restore milking-induced PRL release using a recombinant bovine PRL injection.

MATERIALS AND METHODS

Animals and Experimental Design

All the procedures applied to animals were approved by the Animal Care Committee of the French Ministry of Agriculture, in accordance with French regulations (decree no. 2001–464, May 26, 2001). The cows were housed at the INRA Méjusseaume experimental dairy farm, (Le Rheu, France). Cows were all managed in individual tie stalls.

Nine multiparous Holstein cows (620 ± 79 kg of BW) at 90 ± 12 DIM were randomly assigned to treatments during three 5-d periods according to a 3 × 3 Latin square design balanced for residual effects (Cochran and Cox, 1957). Each 5-d experimental period was separated from the next by 9 d of rest without treatment. The 3 treatments were as follows: 1) QN: twice-daily i.m. injections of 1 mg of QN (Ferring, Wallisellen, Switzerland); 2) QN-PRL: twice-daily i.m. injections of 1 mg of QN and twice-daily (at milking time) i.v. injections of bovine PRL (HMC, Torrance, CA) at 2 μg/kg of BW; or 3) control: twice-daily injections of the vehicles. Quinagolide was diluted at 1 mg/mL in sterile water, mixed with a magnetic stir bar for 5 min, and then sonicated for 45 min at room temperature. Prolactin was first diluted in $NaCO_3$, 0.01 *M*, and then diluted 50:50 in physiological saline. Quinagolide or water injections were given 30 min before each milking (2 milkings per day) for 5 d in each experimental period, whereas exogenous PRL at 0.67 mg/mL or saline buffer was given through a catheter at each milking. One cow was withdrawn for health reasons from the experiment during the last period, but her data from the other periods were included in the analysis.

The cows were milked twice a day at 0715 and 1715 h. They were fed ad libitum according to INRA guidelines. The cows were fed ad libitum a diet containing (on a DM basis) 59.4% corn silage, 5.7% dry hay, 20.9% corn grain, 13.2% formaldehyde-treated soybean meal, 12.9% nonmineral supplement, 8.6% dehydrated alfalfa, 3.9% soybean meal, and 1.9% mineral supplement. The total mixed ration was formulated to meet the energy requirement (NE_L = 1.6 Mcal/kg of DM) and to be above the MP requirement (i.e., PDI for protein digestible in the intestine in the French system with PDI = 110 g/kg of DM) in the control treatment according to INRA (2007). Feed intake and milk production were recorded daily during the treatment period. The cows