Comparative study of the galactopoietic effect of oxytocin during and between milkings in cows and goats

V. Lollivier & P. G. Marnet

INRA/Agrocampus Research on Milk Production, 65 rue de Saint-Brieuc, 35042 Rennes Cedex, France
E-mail: vanessa.lollivier@rennes.inra.fr

Abstract

Oxytocin is released during milking and induces milk ejection i.e. the transfer of alveolar milk into the cistern by contraction of the myoepithelial cells. The galactopoietic action of oxytocin could result of this transfer, but also of a potential effect on the mammary epithelium. Our works aimed to study the galactopoietic effect of oxytocin in ruminants, by developing :

- a zootechnical approach. Increases of milking frequency with and without injections of oxytocin receptor blocking agent were compared to injections of physiological doses of oxytocin in lactating cows and goats, which differ in udder morphology. The galactopoietic effect of oxytocin was different according to the species. In cows, oxytocin doses induced a galactopoietic effect only when they are accompanied by milk removal, whereas they increased milk yield in goats, proportionally to their capacity of cisternal storage. This effect can mainly be explained by the transfer of alveolar milk but also by an additional, limited and unidentified, action of oxytocin on milk yield.
- a tissue/cellular approach to test the hypothesis that oxytocin has a direct effect on the lactating mammary epithelium. Immunohistochemistry studies showed the presence of the oxytocin receptor in rabbit and cow epithelial cells and specific and different effects of oxytocin on epithelial and on myoepithelial cells. Oxytocin provoked an acceleration of the intracellular transfer of caseins throughout epithelial cells into lumen, followed by the contraction of myoepithelial cells.

Our results suggest that oxytocin has an effect on the secretory processes in the mammary gland in addition of its effect of milk ejection.

Key words: *Oxytocin, milking, milk yield, mammary epithelial cell, secretory process, cow, goat, rabbit*

Introduction

In ruminants, milk yield can be modulated by milking frequency. Milking can stimulate milk production by a local effect of milk removal and by systemic effects. Stimulations of the udder during milking provoke the release of oxytocin into the general circulation from the neural lobe of the pituitary. Oxytocin binds to specific receptors located on mammary myoepithelial cells, which surround the alveoli and the small intralobular ductules and induces myoepithelium contraction and milk ejection into the cistern (Ely et al., 1941). It decreases the intra-alveolar pressure due to milk accumulation, which avoid deleterious effects such as crushing of mammary epithelium (Richardson et al., 1947, Stelwagen et al., 2001) and reduces the negative effects of the Feedback Inhibitor of Lactation (Wilde et al., 1987). Milking can also be beneficial to the udder since mammary stimulations cause the release of pituitary lactogenic hormones like prolactin (Kann et al., 1977, Kelly et al., 2002) or oxytocin, which could assume additional role to its effect of milk ejection. Indeed, *in vitro* studies suggested that oxytocin may have effects on cell proliferation (Bussolati et al., 2001) and that lactating mammary epithelial cells could be a target for oxytocin (Kimura et al., 1998, Lollivier et al., 2001, Wagner et al., 1997). Our aim was to elucidate the potential roles of oxytocin on lactating mammary gland and to respond to several questions :

- Did physiological doses of oxytocin have a galactopoietic effect in ruminants ?
- Injections of oxytocin have already been used to study their galactopoietic effect on ruminant. However, extraphysiologic doses were often used, that are described to be deleterious on milk ejection, milk quantity and quality in dairy cows (Allen et al., 1990, Bruckmaier et al., 2003).
- Is this galactopoietic effect different according to species (cow vs goat) ?
- Small ruminants and especially goats have proportionally larger cistern compartment compared to cows. Because of these udder morphology, most of the milk is stored in the cisternal cavities between milkings, which may facilitate the oxytocin effect.
- Is this galactopoietic effect different according to the milk repartition into the udder in goats ?
- Could this galactopoietic effect result from a direct effect on the mammary epithelium, more precisely from an effect on the intracellular process of milk secretion ?

A zootechnical approach (with increases of milking frequency, injections of physiological doses of oxytocin with and without milk removal and milking with injection of an oxytocin receptor blocking agent) and a tissue/cellular approach (with localisation of the oxytocin receptor in rabbit and cow epithelial cells and study of the oxytocin effect on milk secretory processes) were developed.

Materials and methods

Zootechnical approach : Oxytocin effect on milk yield and composition in ruminants.

8 Holstein cows and 20 Alpine goats from the INRA experimental farm of Le Rheu (France) were used. For one week before the beginning of the trials : 1) performance of each animal was recorded during milking at 6.30 and 18.30 as control, 2) cisternal and alveolar milk fractions were measured for each goat by administration of an oxytocin receptor-blocking agent (Atosiban) followed by injection of 2.5 IU of oxytocin. Goats were separated in 2 groups : one with a mean cisternal milk fraction inferior to 80 % of total milk yield and the other with a mean cisternal milk fraction superior to 80 %, 3) individual and physiological doses of oxytocin to inject in order to mimic natural events were determined by measuring for each animal the mean endogenous oxytocin discharge during milking and the oxytocin pharmacokinetic parameters. We decided to inject intravenous (iv) doses varying between 0.1 and 0.6 IU for goats and between 0.25 and 4 IU for cows.

The experiments were conducted according to a Latin Square design with 5x 14-d periods (10 d of treatment and 4 d without treatment i.e. twice daily milking at 6.30 and 18.30). Animals were assigned to 5 treatments :

- TD (twice daily milking at 6.30 and 18.30) as control,
- FD (4 daily milking at unequal intervals i.e. 6.30, 10.30, 14.30 and 18.30) to measure the additional milking effect,
- OT (twice daily milking at 6.30 and 18.30 and 2 iv injections of oxytocin at 10.30 and 14.30), to measure the oxytocin effect without milking,
- AT (twice daily milking at 6.30 and 18.30 and 2 milkings occuring 1 min after Atosiban injection at 10.30 and 14.30), to measure the milking effect without oxytocin,
- C+OT (twice daily milking at 6.30 and 18.30, and 2 udder drainages by canula followed by oxytocin injection at 10.30 and 14.30), to measure the oxytocin effect with udder emptying but with a limited systemic hormonal discharge due to stimulation of the udder.

Animals were fed to provide 110 % of requirements allowing a milk production increase without a negative energy balance.

Daily milk yield, milk composition parameters (protein, fat and lactose contents) and plasma concentrations of oxytocin and of prolactin (to assess normal milk ejection and hypothalamic-pituitary stimulation) were measured.

Analysis of variance were conducted with the general linear procedure of SAS (1990). Daily milk yields were averaged for statistical analysis. All data were analysed with models that included cow, treatment and period and goat, cisternal milk fraction, treatment, period, treatment´cisternal milk fraction and period´cisternal milk fraction.

Tissue/cellular approach : effect of oxytocin on the mammary epithelial cells

Lactating New Zealand female rabbits and Holstein cow were originating from our laboratory.

Rabbits were killed and their mammary glands were excised and cut into fragments, which were incubated for 1 and 7 minutes in the absence or presence of oxytocin (10^{-6} IU/mL).

Cows were injected with 5 IU of oxytocin and fragments of the mammary gland were obtained by biopsy (biopsy needle BSA 14/15, Biosphere Medical, France) before the injection and after 1 and 7 min.

Mammary fragments were treated for morphological (rabbit and cow) and immunohistochemical (rabbit) studies and sectioned. For immunofluorescence, sections were labelled with antibodies (anti-rabbit a_{s1} casein and anti-annexin II).

Some rabbit mammary fragments were used to prepare enzymatically dissociated acini. Acini were stained with Fluo-Oxytocinä and with an antihuman oxytocin receptor monoclonal antibody (Ito et al, 1996).

Results and discussion

Zootechnical approach

Additional milkings increase milk production by 8% (24.19 vs 22.40 kg/d, P<0.05) in cows and by 9% (3.70 vs 3.39 kg/d, P<0.05) in goats, confirming their galactopoietic effect.

In cows, the limitation of systemic hormonal releases other than oxytocin during additional milkings provoke a non significant increase of milk production (+ 4.4%, 23.40 vs 22.40 kg/d, ns). Such a limitation of hormonal discharges other than oxytocin do not inhibit milk yield increase compared to additional milkings in goats (+ 8 %, 3.66 vs 3.39, P<0.05).

Table 1. Effect of the treatments on milk yield and composition in cows (least square means ± SEM).

	TD	FD	OT	AT	C+OT	SEM	n
Milk (kg/d)	22.40 ^{ab}	24.19 ^{c}	22.54 ^{ab}	21.91 ^{a}	23.40 ^{bc}	0.411	39
Fat (g/kg)	41.8	41.4	41.7	42.0	41.8	1.05	39
Protein (g/kg)	32.8	32.7	32.5	32.9	32.7	0.32	39
Lactose (g/kg)	48.2	48.3	47.5	48.4	48.0	0.36	39

TD : twice daily milking, FD : 4 daily milking, OT : TD + 2 injections of oxytocin, AT : TD + 2 milkings with Atosiban, C+OT : TD + 2 teat canulations and 2 injections of oxytocin.
a b c : values within a line differ at P<0.05, SEM : standard error of means, n : number of observations.

Table 2. Effect of the treatments milk yield and composition in goats (least square means ± SEM).

	TD	FD	OT	AT	C+OT	SEM	n
Milk (kg/d)	3.39 ^{a}	3.70 ^{bc}	3.75 ^{c}	3.54 ^{ab}	3.66 ^{bc}	0.072	94
Milk (kg/d) Cisternal capacity < 80%	3.49 A	3.59 A	3.50 A	3.50 A	3.75 A	0.107	45
Milk (kg/d) Cisternal capacity < 80%	3.30 ^{a} A	3.80 ^{bc} A	4.00 ^{c} B	3.57 ^{ab} A	3.57 ^{ab} A	0.099	49
Fat (g/kg)	33.2	34.7	34.3	33.8	33.7	0.95	94
Protein (g/kg)	28.9 ^{b}	27.5 ^{a}	27.6 ^{a}	27.5 ^{a}	28.1 ^{ab}	0.39	94
Lactose (g/kg)	44.9 ^{a}	45.9 ^{b}	45.3 ^{ab}	45.8 ^{b}	45.7 ^{ab}	0.34	94

$^{a\ bc}$: values within a line differ at P<0.05, ^{A,B} : values within a column differ at P<0.05

These results suggest a limited role of systemic hormones by comparison to oxytocin effect for the expression of the galactopoietic effect of milking in the medium term (10 d) in goats. On the other hand, a higher sensibility for systemic hormones is shown in cows, maybe due to the lower ability of this species for alveolar milk transfer.

The major effect of oxytocin for the expression of a galactopoietic effect of milking was proven by injections of Atosiban before the additional milkings, that inhibit milk yield increase in cows (21.91 vs 24.19 kg/d, P<0.05) and goats (3.54 vs 3.70 kg/d, P<0.05) compared to additional milkings.

In cows, injections of physiological doses of oxytocin induce an intermediate galactopoietic effect between twice and four daily milking only when they are accompanied by milk removal (22.54 vs 22.40 and 24.19 kg/d, ns), whereas in goats they induce a galactopoietic effect similar to additional milking (+ 10 %, 3.75 and 3.70 vs 3.39 kg/d, P<0.05). Moreover, no significant galactopoietic effect of additional milking or oxytocin injections is observed in the group of goats with the lowest cisternal storage capacity. At the opposite, increases of milk yield reach +15% (3.80 vs 3.30 kg/d, P<0.05) and + 21% (4.00 vs 3.30 kg/d, P<0.05) respectively in groups of goats with the largest cisternal storage capacity. All these results show that the prevention of alveolar milk stasis is primordial to measure a galactopoietic effect and that the transfer of milk from alveoli to cistern is the main effect of oxytocin, probably by reducing the negative effects of milk stasis. An additional role of oxytocin co expressed with alveoli emptying is suggested by the remanent galactopoietic effect observed on morning milking after oxytocin injections.

Tissue/cellular approach : effect of oxytocin on the mammary epithelial cells.

Immunolocalization of oxytocin receptors by immunofluorescence showed that oxytocin receptors are detectable in lactating rabbit mammary epithelial cells. Moreover, oxytocin bound specifically to epithelial cells, as previously shown (Lollivier et al, 2001).

Oxytocin added *in vitro* (to lactating rabbit mammary fragments) and *in vivo* (to lactating cows) provoke after 1 minute a modification of the morphology of the epithelial cells. Moreover, the localization of alpha S1 caseins and proteins associated with the secretory traffic is modified in rabbit mammary epithelial cells, which suggest a striking acceleration of the transport leading to exocytosis. The contraction of myoepithelial cells was only detectable after 7 minutes. These results strongly suggest that oxytocin has a dual effect on lactating mammary gland : an acceleration of the intracellular transfer of caseins in mammary epithelial cells and an emptying of these cells followed by the contraction of myoepithelial cells.

Conclusion

Our study confirmed that physiological doses of oxytocin have a galactopoietic effect in ruminants. This effect is different in cows and in goats, maybe due to the udder morphology. This effect is also different according to the milk repartition into the udder. Indeed, it is more pronounced in animals with larger cisterns. Thus, the galactopoietic effect of oxytocin can mainly be explained by the transfer of alveolar milk, thereby limiting the negative effects of milk stasis. Furthermore, an additional role of oxytocin was shown: it provokes an acceleration of the intracellular transfer of caseins in lactating mammary epithelial cells and an emptying of these cells followed by the contraction of myoepithelial cells.

Our results suggest that oxytocin has an effect on the secretory processes in addition of the effect of alveolar milk transfer, which may together contribute to an optimal milk secretion. This last hypothesis remains to be confirmed.

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