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Effect of stimulation intensity on oxytocin release before, during and after machine milking

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Release of oxytocin (OT) is essential for milk ejection in dairy cows (Lefcourt & Akers, 1983; Bruckmaier & Blum, 1998). During milk ejection, alveolar milk is shifted into the cistern, which causes an increase of intracisternal pressure (Bruckmaier et al. 1994). To initiate maximum milk ejection at the start of milking, increasing OT concentration beyond a threshold level is sufficient (Schams et al. 1983). Increasing OT concentration beyond this threshold has no additional effect on intracisternal pressure, i.e., milk ejection (Bruckmaier et al. 1994). Stimulatory effects of milking by hand or by machine or by suckling are well documented (Gorewit et al. 1992; Bar-Peled et al. 1995; Tancin et al. 1995; Bruckmaier & Blum, 1996). At the start of milking, stimulatory effects of machine milking without pre-stimulation or with a manual pre-stimulation and subsequent machine milking cause the release of comparable amounts of OT (Gorewit & Gassman, 1985; Mayer et al. 1985; Bruckmaier & Blum, 1996), whereas the timing of the applied pre-stimulation is important for the shape of the milk flow curve. Should the pre-stimulation period be too short, or absent altogether, the start of the main milk flow is delayed resulting in a bimodal milk flow profile (Bruckmaier & Blum, 1996). Furthermore, the stimulation of only one teat causes an OT release similar to that caused by stimulation of all four teats (Bruckmaier et al. 2001). However, milk production is greater for hand milking or suckling than for machine milking, possibly owing to higher OT concentrations (Gorewit et al. 1992; Bar-Peled et al. 1995). In addition, exogenous OT in supraphysiological doses improves udder evacuation and enhances milk production (Nostrand et al. 1991; Ballou et al. 1993; Bruckmaier et al. 1994; Knight, 1994). It has been shown that OT concentrations must be elevated throughout the entire milking process to allow complete milk removal (Bruckmaier et al. 1994). It is possible that the importance of OT concentrations beyond the pre-milking threshold might change towards the end of milk removal. The quarter distribution and the quarter milking time are often very unequal (Wellnitz et al. 1999). Therefore, in most cows individual quarters are over-milked while milk is still removed from others. The goal of this study was to test the hypothesis that OT release depends on the intensity of the stimulus and that a stimulus of low intensity is more effective at the start of milking than at the end of milking. Therefore the stimulatory effect of attached teat cups without pulsation before and after milking was tested. In addition, we tested whether a vibration stimulation of one single teat, without prolongation of the total milking time, towards the end of milking was able to induce additional OT release to improve udder evacuation.

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Materials and Methods

Animals and milking

The six experimental cows (Brown Swiss × German Braunvieh) were in months 2–12 of their first-to-fourth lactation. The cows were kept in loose housing and were milked in a 2 × 2 tandem milking parlour. The diet was maize and grass silage, hay and concentrate given according to individual milk production. Milking was performed with a vacuum of 42 kPa, a pulsation rate of 60 cycles/min and a 60 : 40 pulsation ratio using separate pulsators (Stimopuls CP, Westfalia Landtechnik GmbH, D-59299 Oelde) for each quarter. Machine stripping was performed after total milk flow dropped below 0·3 kg/min. ''Bio-Milker'' teat cups (Westfalia Landtechnik GmbH, D-59299 Oelde) with separate long milk tubes for each quarter were used.

Experimental design

Experiments were performed at the usual milking times starting at 05.00 and 16.00 for 4 consecutive days in a completely balanced crossover design. Three different treatments were tested during two milkings (a.m. and p.m.) in each cow. The treatments were randomly assigned to the individual cow at each experimental milking. Control milking (C) corresponded to the daily milking routine and included udder preparation by forestripping, short

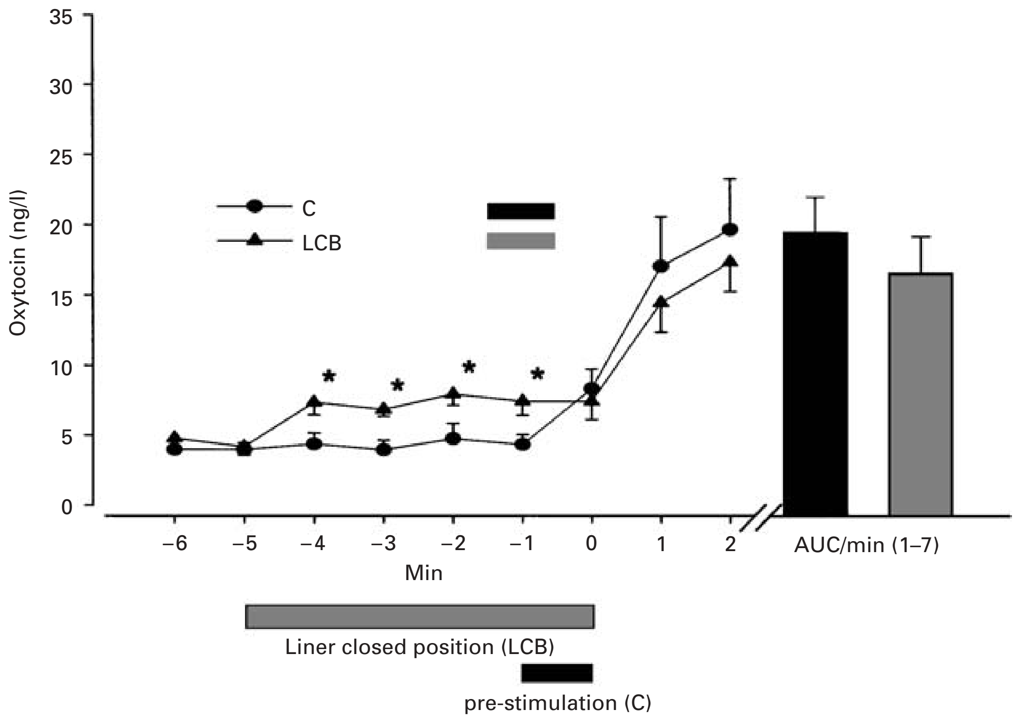

Fig. 1. Oxytocin concentrations (means ± SEM for $n=12$ milkings) for liner closed before milking (LCB) and control milking (C), 0 min = start of normal pulsation and milking. * signifies respective means are significantly different between treatments C and LCB ($P<0{\cdot}05$). Oxytocin values are also shown as areas under the curve (AUC) for the first 7 min of the milkings.

dry-paper cleaning for 15 s and additional vibration pulsation (Stimopuls, 300 min^{-1}, 25 kPa pulsation vacuum) for 60 s. In the treatment in which the liner was closed before milking (LCB) the teat cups were attached without pulsation and without touching the udder before attachment. After 5 min in the liner-closed position, milking pulsation was started without any additional pre-stimulation. In the treatment in which the liner was closed after milking (LCA), the milking process started with the usual routine (C), while pulsation was stopped after stripping, and teat cups remained attached in the liner-closed position for 5 min. In the treatment for which stimulation was applied during milking (STI), milking was started as for control milking. The quarter with the shortest milking time had been determined previously for each cow. One minute before the expected end of milk flow in this quarter, a 1-min vibration stimulation (Stimopuls) was applied to this quarter to provide additional stimulation during milking without prolongation of the total machine on-time.

Milk flow was recorded for individual quarters using four mobile recording units (Lactocorder, Werkzeug- und Maschinenbau Balgach, 9436 Balgach, Switzerland) as previously described (Wellnitz et al. 1999). Various milking characteristics were evaluated according to Bruckmaier et al. (1995). Total milk yield, stripping yield, peak flow rate, average flow rate and total milking time were analysed.

Cows were catheterized for blood sampling from the jugular vein the day before the start of the first experiment. Blood samples for determination of OT concentrations were taken at 1-min intervals. For C and LCB, blood sampling started 6 min before the start of milking. In C and LCA, blood sampling ended 5 min after the end of milking. For STI and LCA, blood sampling started 1 min before the start of teat stimulation. For STI and LCB, blood sampling was stopped after the end of milking. Blood samples were treated with EDTA to prevent coagulation and centrifuged at 1500 ***g*** for 15 min immediately after each milking. Plasma was stored at −20 °C until used for radioimmunological determination of oxytocin concentration according to Schams (1983).

Statistical analysis

Results are presented as means ± SEM. For statistical analysis, the SAS program package release 8.01 (SAS, 1999) was used. Treatment effects were tested for significance ($P<0{\cdot}05$) using the General Linear Model (GLM) procedure. The model included the individual animal and the treatment; the animal was defined as repeated subject. The date and the milking time entered the model as random factors. Differences between treatments were identified by using the Least Significant Difference (LSD) test.

Results

Oxytocin concentrations

Pre-milking OT concentrations (Figs 1, 2 and 4) were low and similar in all treatments. In LCB (Fig. 1) OT values

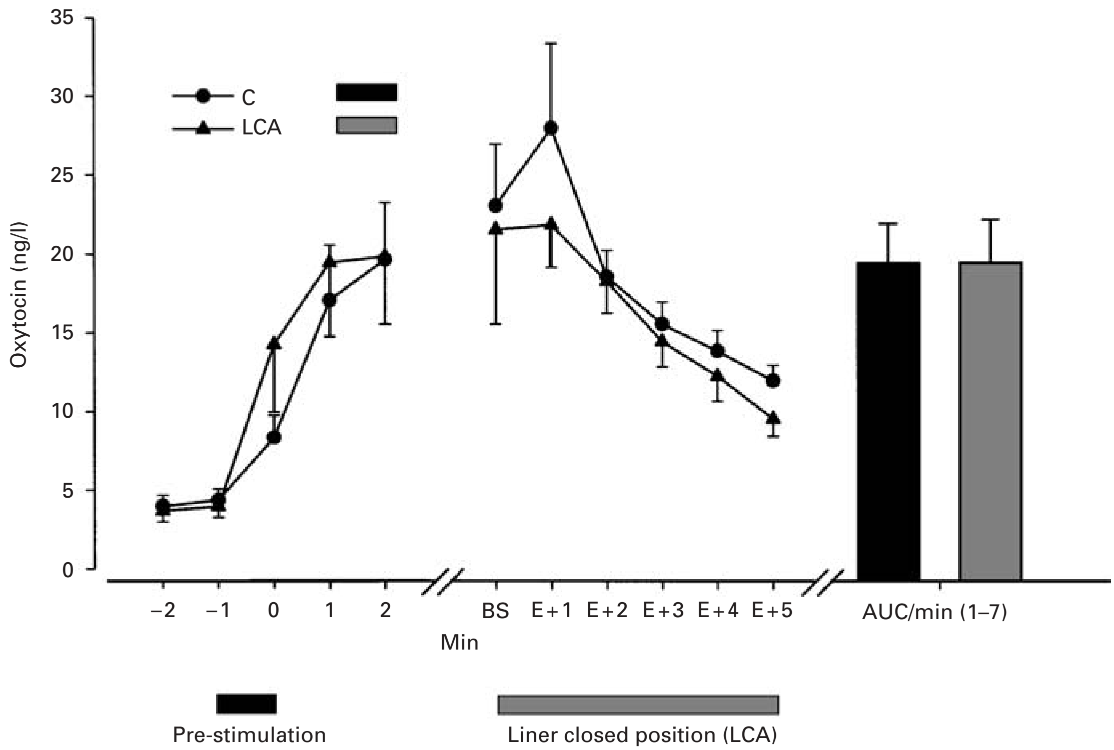

Fig. 2. Oxytocin concentrations (means±SEM for $n=12$ milkings) for liner closed after milking (LCA) and control milking (C), 0 min=start of milking after 1 min vibration stimulation, BS=before stripping, E=end of milking+1–5 min. Oxytocin values are also shown as areas under the curve (AUC) for the first 7 min of the milkings.

after cluster attachment were elevated and significantly higher than the basal levels in C. During subsequent milking, however, OT values increased further and were significantly higher than those during the liner-closed phase. Within 1 min after attachment of the teat cups (i.e., the start of pre-stimulation treatments in C, LCA and STI and start of milking in treatment LCB), OT values increased similarly for all treatments. OT values remained elevated over baseline levels for the entire milking and did not differ significantly from the control. Likewise, the area under the curve (AUC) of OT from 1–7 min of milking did not differ between LCB and C.

For LCA (Fig. 2) the OT pattern during milking was similar to that of the control. After cluster removal in C and the liner-closed phase in LCA, no differences in the steady decline of OT concentrations were observed between the two treatments.

For STI (Figs 3 and 4) OT release during milking was comparable to that of C, with no significant increase in response to the additional stimulation. Before and after the application of the additional 1-min vibration stimulation, OT values did not differ between STI and C.

Milking characteristics

As shown in Table 1, total milk yield, milking time, peak flow rate, average flow rate and stripping yield did not differ significantly between treatments. During the liner-closed phase in LCB and LCA, no milk flow was seen. In treatment STI a decrease in milk flow during the additional stimulation phase in the specific quarter could be observed (Fig. 3), without any significant change for the mentioned parameters. Bimodal milk flow curves, which indicate separate removal of cisternal and alveolar milk fractions due to delayed milk ejection, were not seen for any treatment.

Discussion

Release of OT in response to different tactile teat stimuli in cows has been described before (Lefcourt & Akers, 1983; Bruckmaier & Blum, 1998). Stimulatory effects are due to the milker's hands, movement of the liner or the sucking of the calf. Our results show that the amount of OT released varies with the intensity of teat stimulation. Application of the milking vacuum without pulsation (liner-closed position) caused OT release, but on a lower level than that caused by pulsation during normal milking. This OT release was sufficient to induce milk ejection, as indicated by the absence of bimodal milk flow curves in LCB, i.e., the presence of alveolar milk in the cistern already at the start of milking (Bruckmaier & Blum, 1996). For LCB, however, OT concentrations similar to these of C were only reached after the start of liner pulsation. The continuously elevated OT in LCB during the 5 min before the start of milking indicates that not only the attachment

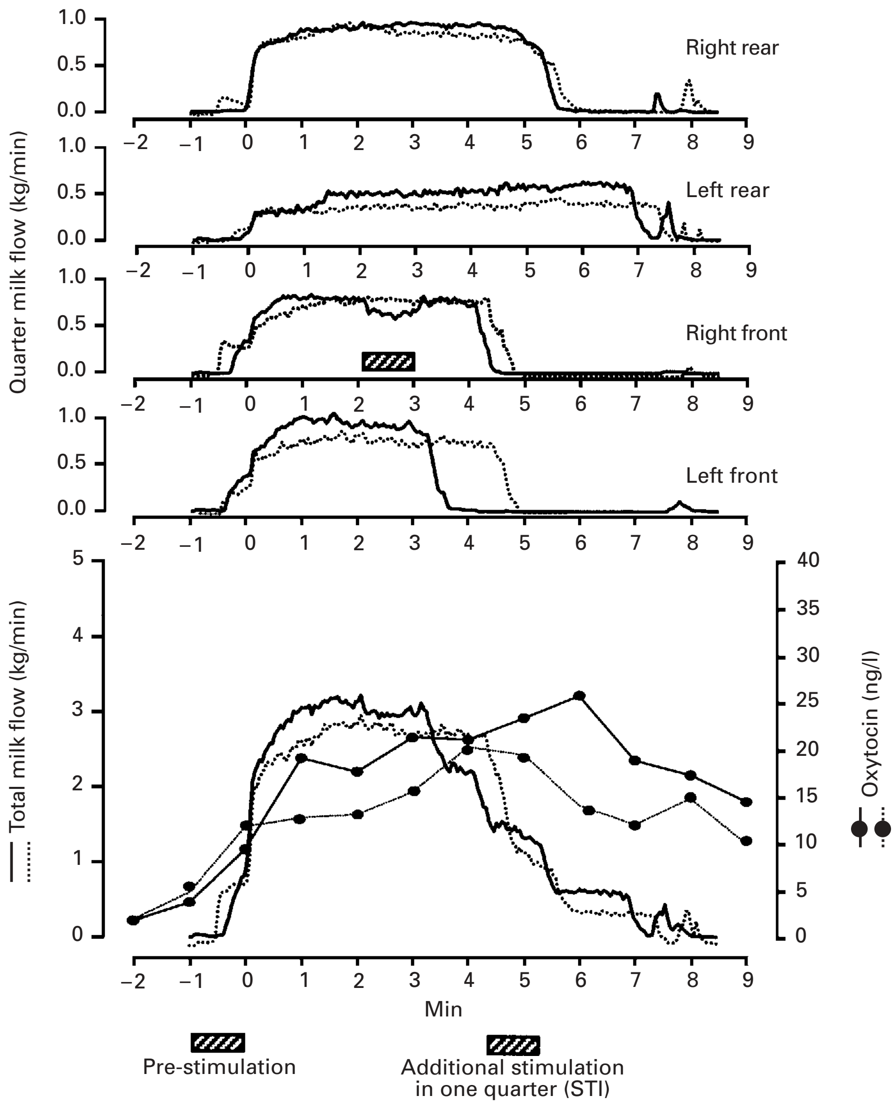

Fig. 3. Oxytocin concentrations (●) and quarter and udder milk flow with (—— STI) and without (········ C) an additional 1-min stimulation of the right front teat of one individual cow during milking.

procedure of the teat cups but also the applied milking vacuum, or the attached teat cup itself, was a stimulus for OT release. If attachment alone had been responsible for the OT release, an immediate decrease of OT concentrations would have occurred, owing to the rapid clearance of circulating OT (Wachs et al. 1984; Bruckmaier et al. 1994). After the start of the normal milking pulsation in LCB, the OT concentrations increased further, indicating that the amount of OT released is modulated by the intensity of the tactile stimulation of the teats.

Application of milking vacuum in the liner-closed position (LCA) after the end of normal milking, i.e., the same stimulus as applied in LCB, did not cause higher OT concentrations than C. Obviously, for both treatments, OT release ceased after the end of milking. The observed clearance of OT was similar to that seen previously (Wachs et al. 1984; Bruckmaier et al. 1994). Notably, the stimulus sufficient to induce a release of OT and to initiate the milk ejection before the start of milking had no effect at all on OT release after the end of milking. It should be remembered, however, that OT concentrations in this phase might have been too high to allow an effect of the stimulus during the liner-closed position in LCA. Nevertheless, OT values in LCA were numerically even lower than those in C during this period.

As for LCA, an additional vibration stimulation of one teat during milking (STI) had no effect on the OT pattern, i.e., it could not induce additional OT release. Obviously, the effects of specific stimuli in the late phases of the milking process were not similar to their effects during the early phase. The continuous pulsation during milking seemed to reduce the responsiveness to teat stimulation. Therefore the applied stimulus (vibration stimulation) in treatment STI, which was very effective before the milking,

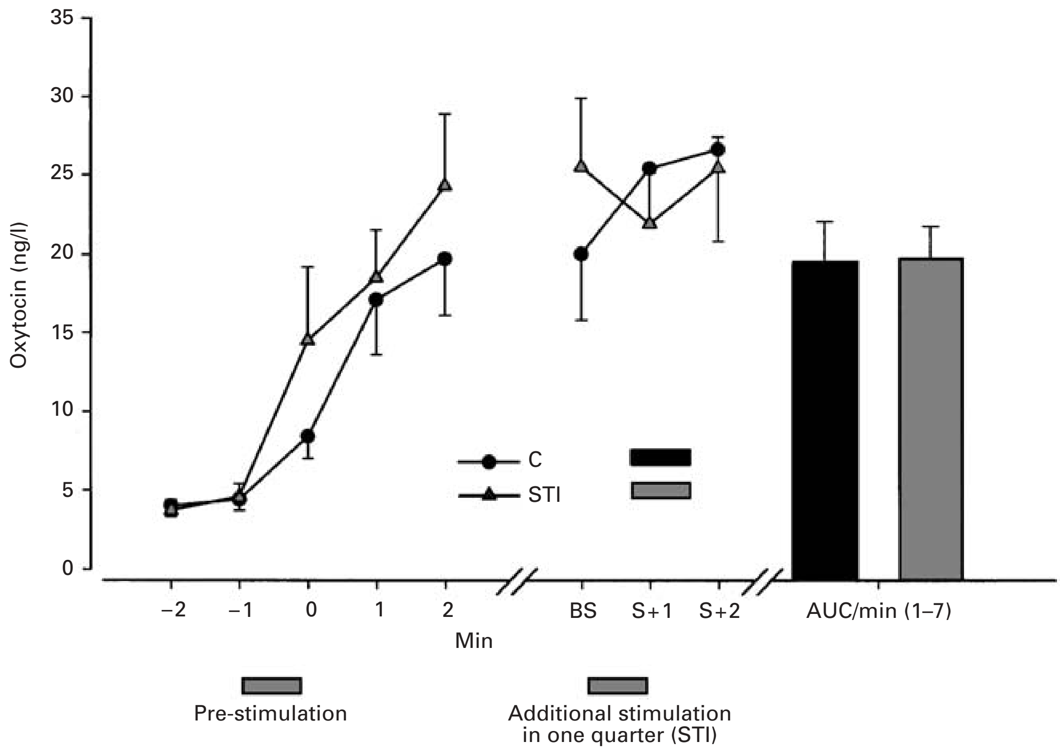

Fig. 4. Oxytocin concentrations (means ± SEM for $n=12$ milkings) with additional stimulation during milking (STI) and control milking (C), 0 min = start of milking after 1 min vibration stimulation, BS = before additional stimulation, S = stimulation + 1–2 min. Oxytocin values are also shown as areas under the curve (AUC) for the first 7 min of the milkings.

Table 1. Milking characteristics during control milking (C), liner closed before milking (LCB), liner closed after milking (LCA) and additional stimulation during milking (STI)

Values are means ± SEM for $n=6$

Parameters	C	LCB	LCA	STI
Total milk yield, kg	13·1 ± 1·3	13·0 ± 1·0	13·7 ± 1·3	13·7 ± 1·1
Stripping yield, kg	0·2 ± 0·1	0·6 ± 0·3	0·2 ± 0·1	0·2 ± 0·1
Peak flow rate, kg/min	3·0 ± 2·3	3·0 ± 2·2	3·0 ± 2·1	3·0 ± 2·2
Average flow rate, kg/min	1·6 ± 0·2	1·6 ± 0·1	1·7 ± 0·1	1·6 ± 0·1
Milking time, min	8·4 ± 0·3	8·0 ± 0·3	8·1 ± 0·5	8·2 ± 0·5

had no effect on the OT pattern during the late phases of milking.

The importance of enhanced OT concentrations throughout the whole milking has been shown previously (Bruckmaier et al. 1994). Therefore it is not surprising that milking with non-pulsating teat cups leads to lower lactation yields than milking with pulsating liners (Whittlestone, 1980). At the start of milking, when the udder is full, a relatively small increase in OT concentration is enough to induce milk ejection, as shown for treatment LCB. The degree of udder filling, however, is crucial for the start of milk ejection (Bruckmaier & Hilger, 2001). Therefore it is possible that the threshold at the start and at the end of milking is different. In this study the applied stimuli during and after milking were of too low an intensity to influence the OT pattern. However, the forces that are applied to the teat by a sucking calf are much greater than those applied by the milking machine (Rasmussen & Mayntz, 1998). It is possible that the stronger stimulus during sucking results in enhanced OT concentrations, which may improve udder evacuation.

In conclusion, OT release depends on the intensity of the tactile stimulation of the teat. The effect of tactile stimuli on the release of OT is different between early and late phases of milking, i.e., an OT response to low-intensity stimulation is observed only before the start of milking.

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