Continuously elevated concentrations of oxytocin during milking are necessary for complete milk removal in dairy cows

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Summary. The importance of elevated concentrations of oxytocin (OT) during the entire milking period was investigated in seven primiparous dairy cows with inherent disturbed milk ejection and in sixteen healthy cows with disturbed milk ejection induced by placing them in an operating theatre. Disturbance of milk removal in both groups has previously been demonstrated to be exclusively due to central blockage of the expected OT release in response to teat stimulation and milking. However, milk ejection can be induced by exogenous OT. OT (0·2 i.u.) was injected i.v. before milking and 49±6% of the total milk was removed. When plasma OT decreased, milk flow stopped. In response to a second and third injection of 0·2 i.u. OT, 30±4 and 7±2% of the milk were removed respectively. The remaining milk was removed with 10 i.u. OT. The lag time from injection of OT to the start of milk flow was inversely correlated with the amount of milk actually removed in response to the OT injection. If 0·2 i.u. OT was injected during intramammary pressure (IMP) recording, IMP immediately increased to its maximum value. After 2·5±0·3 min, IMP decreased to an intermediate IMP (between preinjection and maximum IMP). After two additional injections of 0·2 i.u. OT and after injection of 0·5 i.u. OT, IMP increased to a similar maximum. However, after injection of 0·5 i.u. OT, maximum IMP lasted longer (2·9±0·3 min; $P < 0·05$) than after injections of 0·2 i.u. If OT was continuously infused (0·15 i.u./min) during milking, milk flow lasted until the udder was completely emptied. IMP increased during OT infusion to a maximum which remained stable until infusion was stopped after 10 min. The same IMP maximum was reached after the first individual OT injection (0·2 i.u.), but when plasma OT decreased towards basal concentrations, milk flow ceased and IMP decreased to an intermediate level. Thus continuously elevated OT concentrations such as those during infusion or during normal milking are necessary for complete milk removal.

Elevated concentrations of oxytocin (OT) during milking are needed for ejection of alveolar milk, i.e. shifting of the main milk fraction (which is fixed by adhesive and capillary forces) into the cisternal cavities for milk removal (Sagi *et al.* 1980; Mayer *et al.* 1984*b*; Schams *et al.* 1984; Gorewit & Gassman, 1985). OT is released in response to manual or mechanical teat stimulation, i.e. during premilking stimulation and milking (Sagi *et al.* 1980; Mayer *et al.* 1984*a*, *b*; Schams *et al.* 1984; Gorewit & Gassman, 1985; Mayer *et al.* 1985; Lefcourt & Akers, 1991; Mayer *et al.* 1991; Gorewit *et al.* 1992). Both endogenous and exogenously administered OT cause

alveolar milk ejection, an increase of intramammary pressure (IMP) within the cistern (Bruckmaier *et al.* 1991; Mayer *et al.* 1991) and an enlargement of the cisternal volume (Bruckmaier & Blum, 1992). Premilking teat stimulation causes the release of OT and evokes milk ejection before milking begins; this is important for fast and complete milk removal (Sagi *et al.* 1980; Mayer *et al.* 1984*b*; Gorewit & Gassman, 1985). Although OT is released during the whole milking procedure (Schams *et al.* 1984; Mayer *et al.* 1991), it has not been shown whether a continuously elevated OT concentration is necessary for continuous and complete milk removal. Thus, it was not known until now whether the alveolar milk is completely ejected in response to premilking stimulation or whether milk is ejected during the whole milking procedure in response to continuously elevated concentrations of OT.

In recent investigations, central inhibition of milk ejection, i.e. lack of OT release, was demonstrated in dairy cows. An absence of OT release in response to teat stimulation and machine milking was found in primiparous parturient cows with disturbed milk removal (Bruckmaier *et al.* 1992) and in healthy cows if disturbed by placing them in unfamiliar surroundings (Bruckmaier *et al.* 1993). Because exogenous OT in physiological amounts completely normalized milk removal in these animals, a reduced effect of OT at the level of the mammary gland (peripheral disturbance) could be excluded. Because manual teat stimulation and teatcup liners were found not to induce OT release in these animals, milk ejection can be manipulated by exogenous OT. Based on these findings, the hypothesis could be tested that release of OT at the start of milking, which causes a maximal increase of IMP (Mayer *et al.* 1991), is not sufficient to shift the whole amount of alveolar milk into the cistern. Previous investigators have considered that on the contrary the OT release during prestimulation and early milking is the most important factor for complete milk ejection (Mayer *et al.* 1984*b*, 1985; Schams *et al.* 1984). The goal of this work was to show the importance of continuously elevated OT concentrations during the whole milking procedure for continuous milk ejection when the cistern is continuously emptied by the milking machine. Experiments were designed to compare the effects on milk flow and IMP of repeated injections of OT, when blood OT is elevated only transiently after each injection, and of continuously infused OT, resulting in continuously elevated blood OT.

It was shown previously that the time from the start of premilking teat stimulation until milk ejection occurs increases during the course of lactation with decreasing amounts of milk being stored in the udder, although OT release was not delayed (Bruckmaier, 1988). The response time from OT release to milk ejection obviously depends on milk yield. Therefore, the lag time from injection of OT to the onset of milk flow and its dependence on the amount of ejected milk was investigated.

MATERIALS AND METHODS

Animals and experimental procedures

In Expt 1 (Table 1), seven primiparous parturient cows (Simmental × Red Holstein) with disturbed OT release located on different farms as described previously (Bruckmaier *et al.* 1992) were milked within 10 d after parturition and milk flow curves were recorded (Treatment 1.1) while OT (340 i.u./mg) was injected repeatedly before milking and during milking when milk flow had ceased. The IMP was recorded 1 d later during repeated OT injections, but for only four cows because of local hygienic conditions (Treatment 1.2). During IMP recordings, no milk was removed from the udder. In Expt 2 (Table 1), 16 cows (Simmental × Red Holstein)

Table 1. *Experimental design of oxytocin treatments*

Expt	Animals	Treatment	*n*	Measurements	Oxytocin administration: sequence of events
1	Primparous parturient cows	1.1	7	Milk flow, oxytocin concn	0·2 i.u. injected 3 min before milking; 0·2, 0·2 and 10 i.u. during milking when milk flow ceased
		1.2	4	Intramammary pressure, oxytocin concn	0·2 i.u. injected 2 min after teat cannulation; then 0·2, 0·2, 0·5 and 10 i.u. at 6 min intervals
2	Cows in unfamiliar surroundings	2.1	4	Milk flow, oxytocin concn	0·2 i.u. injected 3 min before milking; 0·2, 0·2, 1·0 and 10 i.u. during milking when milk flow ceased
		2.2	4	Milk flow, oxytocin concn	Milking until milk flow ceased; 0·15 i.u./min infused until milk flow ceased; then 1·0 i.u. injected; then 10 i.u. injected when milk flow ceased
		2.3	4	Intramammary pressure, oxytocin concn	0·2 i.u. injected 2 min after teat cannulation; then 0·2, 0·2, 0·5, 1·0 and 10 i.u. at 6 min intervals
		2.4	4	Intramammary pressure, oxytocin concn	0·15 i.u./min infused for 10 min from 2 min after teat cannulation; 0·2 i.u. injected 7 min after and 10 i.u. 14 min after the end of infusion

in months 2–7 of their first to ninth lactations were studied during disturbed OT release by exposing them to the unfamiliar operating theatre of the research station (Bruckmaier *et al.* 1993). To avoid conditioning them to the unfamiliar surroundings, each animal was used once only in one of four treatments (Treatments 2.1–2.4). In Expt 2, OT was either repeatedly injected or continuously infused while milk flow or IMP was recorded. All experiments were performed between 15.30 and 18.00, i.e. at the approximate time of routine evening milking.

During milking, the milk removed was weighed continuously by a strain gauge system and milk flow curves were recorded as described previously (Schams *et al.* 1984; Bruckmaier, 1988; Bruckmaier *et al.* 1992). During IMP measurements, the pressure within the teat cistern was continuously recorded by inserting a steel cannula through the teat canal into the left front quarter. Pressure was transmitted through silastic tubing to a physiological strain gauge pressure transducer (Model P 23; Gould Inc., Oxnard, CA, USA) and the signal conveyed to a strip chart recorder. The pressure at the level of the teat base was calibrated to zero to eliminate influences of different teat lengths on the recorded pressure.

At ~ 1 h before the experiments, indwelling catheters were inserted into both jugular veins to administer OT and to collect blood samples for determination of OT concentrations. During the entire experiments, 10 ml blood was taken every 1 min. In Expt 2, an additional blood sample was taken 30 s after each injection of OT to measure the peak concentration of OT after injection more exactly.

Laboratory methods

Concentrations of OT were determined by radioimmunoassay as described previously (Schams, 1983). For technical reasons, OT concentrations after the injection of 10 i.u. OT were not determined in this investigation. These concentra-

tions have been found previously to be > 400 ng/l 1 min after injection, which is above the physiological range (our unpublished results).

Statistical analyses

Values are presented as means ± SEM. Changes during the course of experiment were tested for significance ($P < 0.05$) by means of a paired t test employing the UNIVARIATE procedure of the SAS program package, release 6.04 (SAS, 1990).

RESULTS

Experiment 1. Primiparous parturient cows

Treatment 1.1. Oxytocin injections during recording of milk flow. The concentration of OT (Table 2) increased ($P < 0.05$) within 1 min after the first OT injection (0·2 i.u.), then decreased immediately and reached almost basal concentrations before the succeeding OT injection. At the start of milking, OT concentration was 6·5 ± 0·7 ng/l. Because OT concentrations did not completely return to basal values before the next injection, both preinjection and postinjection OT concentrations increased moderately, but significantly, during the course of the experiment. Within 3·5–4·0 min after all injections of 0·2 i.u. OT, the OT concentration had fallen to half the value found after 1 min.

Absolute and relative amounts of milk (Table 2) were highest after the first injection of OT and the start of machine milking, and decreased ($P < 0.05$) in response to the second and third injections of 0·2 i.u. OT. In response to the injection of 10 i.u. OT, the amount of milk tended to increase again, but this was not significant.

The lag time from injection of OT to commencement of milk flow (Table 2) was not measured for technical reasons after the first injection of 0·2 i.u. OT. However, lag time increased ($P < 0.05$) from the second to the third injection of 0·2 i.u. OT, and there was a strong correlation between the actual amount of milk removed and the lag time (Fig. 1). Because the correlation and regression coefficients for the second and third injections of 0·2 i.u. OT were similar, the values from both were pooled to calculate the regression equation. Lag time decreased in a linear manner with increasing amounts of milk removed in response to injection. In response to the final injection of 10 i.u. OT, lag time was shorter again (and the amount of milk higher) than after the third injection of 0·2 i.u.

Treatment 1.2. Oxytocin injections during recording of IMP. The concentration of OT increased ($P < 0.05$) in response to OT injection (Table 3). Preinjection and postinjection concentrations of OT (Table 2) increased slightly but significantly with increasing amounts of OT during the course of the experiment.

The IMP (Table 3) increased to a plateau in response to the first injection of 0·2 i.u. OT, before decreasing again to another intermediate plateau between preinjection and postinjection pressure. In response to further injections of 0·2 and 0·5 i.u. OT, IMP increased again to a level similar to that after the first injection and decreased again to similar intermediate plateaux. In response to the injection of 10 i.u. OT, however, the pressure increased to higher values than after the preceding injections.

Lag time from OT injection until IMP started to increase (Table 3) was similarly short in response to all injections. The duration of increase (Table 3) was longer after the first injection of 0·2 i.u. and after the injection of 10 i.u. OT as compared with the other injections ($P < 0.05$). The duration of the IMP maximum (Table 3) was similar

Table 2. *Oxytocin concentrations, lag times from oxytocin injection to start of milk flow and milk fractions in response to oxytocin injections in primiparous parturient cows with disturbed oxytocin release (Treatment 1.1)*

(Values are means ± SEM for $n = 7$)

Oxytocin injections, i.u....	First 0·2	Second 0·2	Third 0·2	10
Oxytocin concn, ng/l				
Before injection	$1·6 \pm 0·2^{a}$	$3·1 \pm 0·4^{b}$	$3·7 \pm 0·3^{c}$	$4·0 \pm 0·2^{c}$
1 min after injection	$9·7 \pm 0·6^{a}$*	$12·0 \pm 0·3^{b}$*	$13·4 \pm 0·3^{c}$*	$> 400^{d}$
Lag time, min	NM	$0·49 \pm 0·06^{a}$	$0·81 \pm 0·04^{b}$	$0·59 \pm 0·05^{a}$
Amount of milk				
kg	$4·6 \pm 0·7^{a}$	$2·8 \pm 0·4^{b}$	$0·7 \pm 0·2^{c}$	$1·3 \pm 0·3^{c}$
Percentage	49 ± 6^{a}	30 ± 4^{b}	7 ± 2^{c}	14 ± 3^{c}

NM, not measured for technical reasons.

[a,b,c,d] Values within line without common subscript letters were significantly different ($P < 0·05$).

* Oxytocin concentrations before and after injection were significantly different ($P < 0·05$).

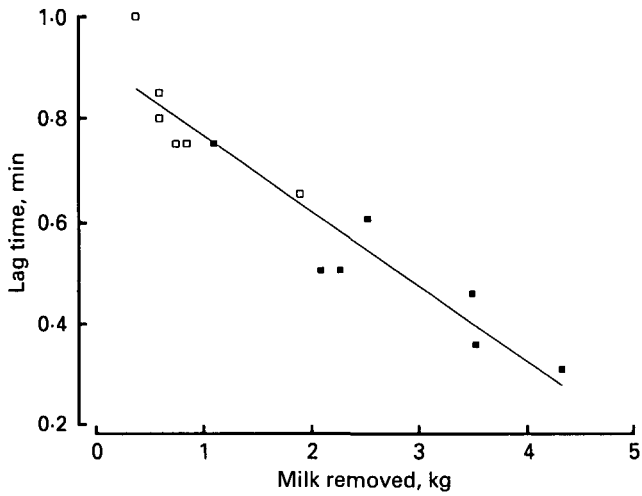

Fig. 1. Relationship between amount of milk (x) removed in response to the second (■) and third (□) injection of 0·2 i.u. oxytocin and the lag time from injection until beginning of milk flow (y) in primiparous parturient cows (Treatment 1.1; for details, see text.) The regression equation was $y = 0·92 - 0·15x$, $r = 0·95$, $P < 0·05$.

after all injections of 0·2 i.u., longer after injection of 0·5 i.u. ($P < 0·05$) and stable for > 10 min after injection of 10 i.u. OT.

Experiment 2. Cows in unfamiliar surroundings

Treatment 2.1. Oxytocin injections during recording of milk flow. Concentrations of OT (Table 4) were highest within 0·5–1 min of the first injection of 0·2 i.u. OT. Within 2 min after injection, the OT concentration started to decrease again. When the milking cluster was attached, OT concentration was $4·4 \pm 0·7$ ng/l. Preinjection concentrations of OT increased slightly, and postinjection concentrations increased in a dose-dependent manner, until the end of the experiment. Fig. 2(*a*) shows typical results for an individual cow.

Absolute and relative amounts of milk (Table 4, Fig. 2*a*) were highest after the first injection of OT and the onset of machine milking, and decreased (similarly to Treatment 1.1) in response to the succeeding injections.

The lag time from injection of OT to commencement of milk flow (Table 3)

Table 3. *Oxytocin concentration, intramammary pressure, lag time from oxytocin injection to beginning of pressure increase, duration of pressure increase and of pressure maximum in response to oxytocin injections in primiparous parturient cows with disturbed oxytocin release (Treatment 1.2)*

(Values are means ± SEM for $n = 4$)

Oxytocin injections, i.u....	First 0·2	Second 0·2	Third 0·2	0·5	10
Oxytocin concn, ng/l					
Before injection	$1·8 \pm 0·3^{a}$	$2·9 \pm 0·3^{ab}$	$2·9 \pm 0·2^{ab}$	$3·8 \pm 0·1^{b}$	$3·6 \pm 0·2^{b}$
1 min after injection	$11·6 \pm 0·8^{a}$*	$12·2 \pm 0·4^{a}$*	$12·4 \pm 0·3^{a}$*	$32·1 \pm 2·4^{b}$*	$> 400^{c}$*
Intramammary pressure, kPa					
Before injection	$3·4 \pm 0·7^{a}$	$5·3 \pm 0·6^{b}$	$5·4 \pm 0·6^{b}$	$5·4 \pm 0·6^{b}$	$5·3 \pm 0·6^{b}$
Maximum after injection	$6·4 \pm 0·5^{a}$*	$6·4 \pm 0·6^{a}$*	$6·4 \pm 0·5^{a}$*	$6·5 \pm 0·6^{a}$*	$7·2 \pm 0·5^{b}$*
Lag time, min	$0·30 \pm 0·02^{a}$	$0·36 \pm 0·03^{a}$	$0·32 \pm 0·05^{a}$	$0·30 \pm 0·02^{a}$	$0·33 \pm 0·02^{a}$
Duration of increase, min	$0·33 \pm 0·05^{a}$	$0·26 \pm 0·05^{b}$	$0·19 \pm 0·02^{b}$	$0·24 \pm 0·04^{b}$	$0·33 \pm 0·06^{a}$
Duration of maximum, min	$2·49 \pm 0·35^{a}$	$2·40 \pm 0·37^{a}$	$2·35 \pm 0·47^{a}$	$2·92 \pm 0·32^{b}$	> 10

[a,b,c] Values within line without common subscript letters were significantly different ($P < 0·05$).
* Intramammary pressure before and after injection was significantly different ($P < 0·05$).

Table 4. *Oxytocin concentration, lag time from oxytocin injection to beginning of milk flow and milk fractions in response to oxytocin injections in cows with oxytocin release disturbed by unfamiliar surroundings (Treatment 2.1)*

(Values are means ± SEM for $n = 4$)

Oxytocin injections, i.u....	First 0·2	Second 0·2	Third 0·2	1	10
Oxytocin concn, ng/l					
Before injection	$1·6 \pm 0·3^{a}$	$2·3 \pm 0·5^{ab}$	$2·8 \pm 0·6^{ab}$	$3·2 \pm 0·3^{b}$	$3·8 \pm 0·3^{c}$
1 min after injection	$9·9 \pm 1·9^{a}$*	$9·1 \pm 0·9^{a}$*	$10·3 \pm 1·3^{a}$*	$32·3 \pm 3·7^{b}$*	$> 400^{c}$*
Lag time, min	NM	$0·55 \pm 0·06^{a}$	$0·83 \pm 0·09^{b}$	$0·65 \pm 0·06^{ab}$	$0·70 \pm 0·04^{b}$
Amount of milk					
kg	$4·5 \pm 0·2^{a}$	$2·6 \pm 0·3^{b}$	$1·2 \pm 0·5^{bc}$	$0·5 \pm 0·05^{c}$	$0·4 \pm 0·08^{c}$
Percentage	49 ± 2^{a}	29 ± 4^{b}	13 ± 4^{bc}	5 ± 1^{c}	4 ± 1^{c}

NM, not measured for technical reasons.
[a,b,c] Values within line without common subscript letters were significantly different ($P < 0·05$).
* Oxytocin concentrations before and after injection were significantly different ($P < 0·05$).

increased ($P < 0·05$) from the second to the third injection of 0·2 i.u. OT (to levels similar to those in Treatment 1.1). After injection of 1 and 10 i.u. OT, lag time tended to be even shorter, but this change was not significant.

Treatment 2.2. Oxytocin infusion during recording of milk flow. Premilking concentrations of OT were $1·8 \pm 0·5$ ng/l; they remained basal during early milking, and were $2·0 \pm 0·5$ ng/l at the start of OT infusion. During infusion, plasma concentrations of OT increased ($P < 0·05$) continuously and linearly to $23·3 \pm 3·1$ ng/l at the end of the infusion. At 1 min after the single injection of 1 i.u., OT concentrations had increased to $55·8 \pm 11·3$ ng/l. Fig. 2(*b*) shows typical results for an individual cow.

Because no OT was administered before the start of milking, only a small amount of milk was removed during early milking ($1·4 \pm 0·5$ kg, 11 ± 3%, Fig. 2*b*). Milk flow commenced again $1·2 \pm 0·1$ min after infusion of OT had started. Plasma OT concentrations at commencement of milk flow were $5·7 \pm 0·5$ ng/l. During the infusion of OT, most of the milk stored in the udder was removed ($8·3 \pm 0·6$ kg, i.e. 78 ± 7%). The remaining small fractions of milk were removed in response to injections of 1 and 10 i.u. OT.

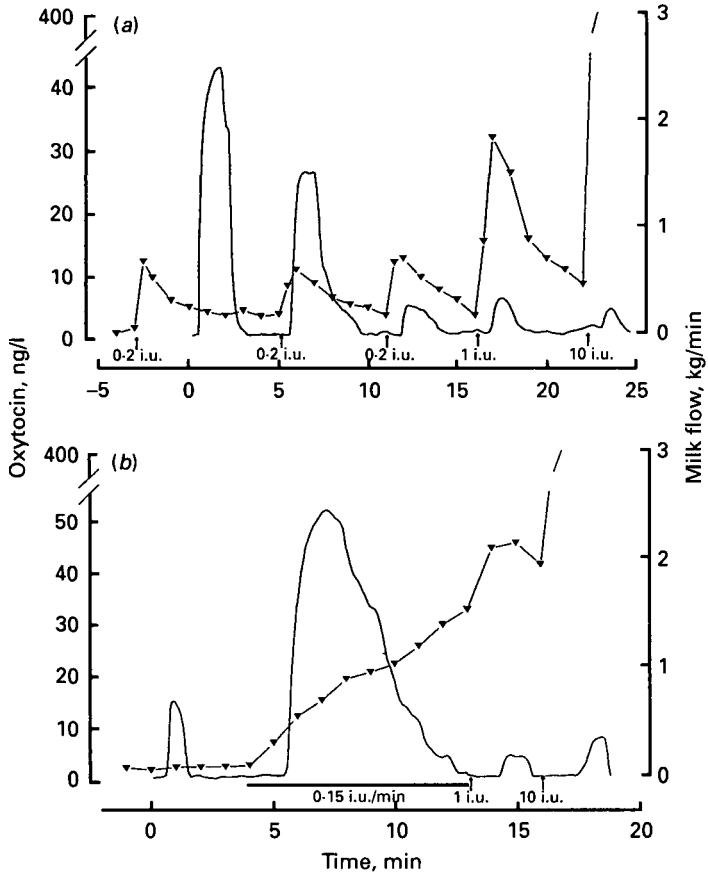

Fig. 2. Oxytocin concentrations (▼) and milk flow rate (——) of one cow in unfamiliar surroundings in response (*a*) to repeated oxytocin injections (Treatment 2.1) and (*b*) to oxytocin infusion (Treatment 2.2). For details of treatments, see text. ↑, Individual oxytocin injections; ——, continuous oxytocin infusion.

Treatment 2.3. Oxytocin injections during recording of IMP. Plasma concentrations of OT (Fig. 3*a*) increased in a dose-dependent manner after each injection. Preinjection concentrations increased slightly but significantly during the course of the experiment.

The IMP (Fig. 3*a*) increased after the first injection of 0·2 i.u. OT to a plateau. As in Treatment 1.2, IMP then decreased to another intermediate plateau. In response to the succeeding injections (0·2, 0·2 and 0·5 i.u. OT), similar maximum and intermediate IMP plateaux were obtained. After injection of 1 i.u. OT the pressure increase was not significantly higher than after the 0·2 i.u. injections, but after injection of 10 i.u. OT, a higher peak pressure was achieved than after the previous injections of 0·2 i.u. OT ($P < 0·05$).

The lag time from injection of OT to commencement of pressure increase (Fig. 3*a*) was similar in response to all OT injections ($0·25 \pm 0·03$ min). The duration of the increase was $0·70 \pm 0·05$ min in response to the first injection and was similar ($0·40 \pm 0·04$ min) after the other injections. The duration of the maximum in response to injections of 0·2, 0·5 and 1 i.u. OT was $0·90 \pm 0·07$, $1·10 \pm 0·10$ and $1·62 \pm 0·14$ min respectively, the last being greater ($P < 0·05$). In response to 10 i.u. OT, IMP remained at the maximum for > 10 min.

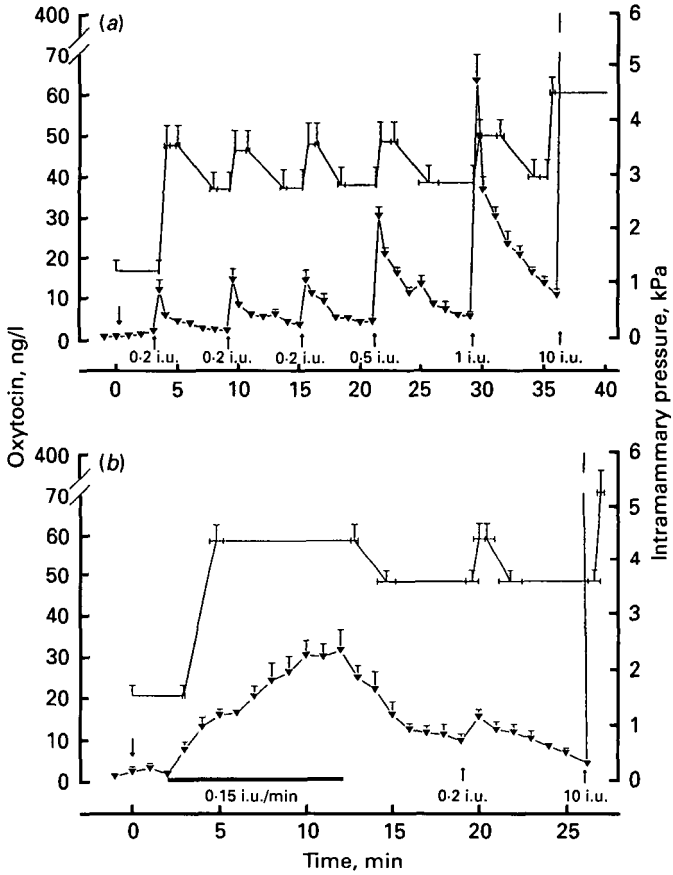

Fig. 3. Mean oxytocin concentrations (▼) and mean pattern of intramammary pressure (——) in cows in unfamiliar surroundings in response (*a*) to repeated oxytocin injections (Treatment 2.3) and (*b*) to oxytocin infusion (Treatment 2.4). For details of treatments, see text. ↓, Teat cannulation; ↑, individual oxytocin injections; ——, continuous oxytocin infusion.

Treatment 2.4. Oxytocin infusion during recording of IMP. The concentration of OT (Fig. 3*b*) remained basal until infusion of OT was started. During the infusion period (10 min), OT plasma concentration increased continuously and linearly, and started to decrease immediately after infusion was stopped. Succeeding injections of 0·2 and 10 i.u. OT caused the expected increases in OT plasma concentration.

The IMP (Fig. 3*b*) remained stable before OT was infused and began to increase at $0{\cdot}92 \pm 0{\cdot}08$ min after the start of infusion. At the commencement of pressure increase, OT concentration was $7{\cdot}4 \pm 1{\cdot}9$ ng/l. The duration of IMP increase was $1{\cdot}88 \pm 0{\cdot}29$ min, i.e. more than double that in response to OT injection (Treatment 2.3). The IMP maximum remained stable until the infusion was stopped, and started to decrease at $0{\cdot}80 \pm 0{\cdot}21$ min after the end of infusion. At this time, the OT concentration (calculated by interpolation) was 21 ± 4 ng/l. After the injection of 0·2 i.u. OT, IMP increased again to a maximum similar to that during infusion. In response to injection of 10 i.u. OT a higher IMP peak was obtained ($P < 0{\cdot}05$).

DISCUSSION

Concentrations of OT after injections or infusions were comparable to those found in other investigations (Wachs *et al.* 1984; Bruckmaier *et al.* 1992). Injections of 0·2 i.u. OT were found to cause plasma OT concentrations of 9–15 ng/l 1 min after

the injection, which is in the range of physiological OT release during milking (Schams *et al.* 1984, Mayer *et al.* 1984*a*, *b*, 1991; Bruckmaier *et al.* 1992). After injection of 0·5 and 1 i.u. OT, plasma OT concentrations were in the upper physiological range, whereas after injection of 10 i.u. OT concentrations increased to supraphysiological levels. In both experiments concentrations of endogenous OT remained basal during milking and IMP recording, and this is important, because elevated OT concentrations occurred only after administration of exogenous OT and the disappearance time of OT was similar after injections before and during milking. Disappearance time of OT was comparable to that found by Wachs *et al.* (1984).

Only ~ 10% of milk, obviously the cisternal milk fraction, was removed if the milking cluster was attached without previous OT administration (Treatment 2.2). Cisternal milk fractions of cows in unfamiliar surroundings have previously been found to be 5–10% of the total milk (Bruckmaier *et al.* 1993, 1994). Because of this small amount of cisternal milk, which is removed immediately by the milking machine, prestimulation is essential in milking practice to avoid blind milking. Only if OT release is stimulated before milk removal begins does opportune alveolar milk ejection occur (Mayer *et al.* 1984*b*; Gorewit & Gassman, 1985).

If OT was injected 3 min before milking, OT concentrations (Treatments 1.1 and 2.1) increased transiently and were already close to basal when milking started. About 50% of the milk stored in the udder could be removed during subsequent milking, owing to partial ejection of alveolar milk, which is made possible by enlargement of the cisternal cavities (Bruckmaier & Blum, 1992). In corresponding treatments with recording of IMP (Treatments 1.2 and 2.3), the IMP increased to a (physiological) maximum after the first injection of OT and did not increase beyond this maximum in response to succeeding 'physiological' amounts of OT. Therefore, if no milk was removed, all the alveolar milk could not be ejected because of limited space in the cistern. Only if milk was removed, and OT level was again elevated, were further portions of alveolar milk ejected.

Only a supraphysiological amount of OT (10 i.u.) was able to elicit an IMP increase to a higher level than the 'physiological' injections. This high dose of OT can obviously induce an additional contraction of the myoepithelial cells and therefore alveolar milk is ejected more completely than by concentrations in the physiological range. During removal of residual milk by supraphysiological amounts of OT after normal milking, up to 30% additional milk was available (Rajamannan *et al.* 1966; Andreae & Pfleiderer, 1972; Bruckmaier, 1988).

An OT level able to induce maximum milk ejection was reached after the first injection. Although no milk was removed during IMP recording, IMP decreased from its maximum plateau to another intermediate plateau when OT concentration decreased towards basal concentration. The duration of the IMP maximum was dependent on the OT dose. Because ejected milk is not likely to move back into the alveolar tissue, we assume that maximum IMP consists of two components, the pressure induced by the amount of milk present in the cistern, and the pressure contributed by the myoepithelial contraction. The IMP component, caused by myoepithelial contraction, disappears when plasma OT decreases towards basal concentrations. This would explain why in experiments with OT injections (Treatments 1.1 and 2.1) milk flow stopped after ejected milk was removed. In these experiments, no more milk was ejected when OT concentrations were too low to induce contraction of the myoepithelium. Thus, in experiments with a delayed start of milking after prestimulation (Mayer *et al.* 1984*a*; Rasmussen *et al.* 1992) the negative effects on milk flow and milk yield were possibly in part caused by

transiently interrupted milk ejection due to decreasing OT concentrations after prestimulation. If concentrations of OT have already decreased to basal levels after prestimulation, OT release and myoepithelial contraction must be newly evoked by the teatcup liner to refill the cisternal cavities during milk removal and to keep the IMP at a high level until the end of milking. High IMP is important to keep the teat cistern completely filled within each pulsation cycle.

Incomplete alveolar milk ejection in response to different stimuli, as observed in catheterized teats (Mielke, 1963; Wehowsky *et al.* 1986), is also most probably due to OT concentration decreasing towards basal values after the end of stimulation.

If OT was infused (instead of injected) after the cisternal milk fraction was removed, plasma concentration of OT increased continuously. Milk ejection and milk flow started. Most of the milk was removed during the infusion, because only small amounts of milk were removed in response to subsequent injections of 1 and 10 i.u. OT, which elevated the plasma OT concentration into the high physiological and supraphysiological ranges respectively (Treatment 2.2). In corresponding trials with OT infusion and IMP recording (Treatment 2.4), IMP started to increase at an OT concentration similar to that at which milk flow started during OT infusion, and remained at the maximum level until the end of infusion. In other words, if OT concentration stays continuously elevated, as is found in normal milking practice, the contraction of the myoepithelial cells is maintained until the alveolar milk is completely ejected.

As soon as the IMP maximum was reached, IMP remained unchanged although OT concentrations increased during the whole period of infusion. This supports the notion that a threshold level of OT must be reached for maximal alveolar milk ejection, as postulated by Schams *et al.* (1984). However, the duration of IMP increase was much longer during infusion (Treatment 2.4) than after injection of OT (Treatment 2.3). Obviously, partial milk ejection (and partial IMP increase) during OT infusion began when concentrations of OT were slightly elevated and continued with increasing OT concentration until the threshold level for maximal milk ejection was reached. During further increase of OT beyond this threshold level IMP remained stable.

During infusion of OT, the IMP started to increase at OT concentrations < 10 ng/l. This demonstrated high sensitivity to OT. In contrast, IMP started to decrease within 2 min after infusion of OT was stopped, although plasma concentrations of OT were still at ~ 20 ng/l at this time. This indicated development of desensitization of the myoepithelium to OT during the course of infusion. On the other hand, IMP increased again to its maximum in response to an injection of OT (0·2 i.u.) only 7 min after infusion was stopped, suggesting a resensitization if plasma OT decreased towards basal levels.

The lag time from injection of OT until the beginning of milk flow was closely correlated with the amount of milk actually removed in response to the injection, i.e. lag time increased with decreasing amount of milk. This correlation existed between animals and also within an animal when the amount of milk became smaller in response to a subsequent OT injection of the same dosage. If milk alveoli are less full, they probably need to contract further to shift milk into the cistern. Therefore it takes more time before milk ejection can be recorded in the cistern. This finding is in accordance with previous experiments (Mayer *et al.* 1991) in which milk ejection induced by teat stimulation was retarded at the end of lactation, although OT release in response to teat stimulation was enhanced. In contrast, lag time from OT injection until milk ejection was not retarded in response to succeeding injections in IMP

experiments, because no milk was removed and milk remained in the alveoli after the first milk ejection.

In conclusion, during normal milking only ~ 50% of the total amount of milk can be ejected by premilking milk ejection in early and mid lactation. The remaining alveolar milk fraction is ejected during the course of milking. An essential condition for continuous milk ejection during milking is continuous contraction of the myoepithelium induced by continuously elevated OT concentrations during the whole milking procedure. The original biological purpose of lactation is the nutrition of the suckling calf. Therefore, it is advantageous for the cow that milk ejection stops after the calf has suckled. As a consequence, cisternal cavities and teat sphincter are only transiently exposed to high IMP.

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