Inhibition of milk ejection in cows by oxytocin receptor blockade, α-adrenergic receptor stimulation and in unfamiliar surroundings

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Summary. Inhibition of milk ejection in cows by oxytocin receptor blockade (Atosiban) and α-adrenergic receptor stimulation (phenylephrine) prior to prestimulation was compared with inhibition of milk ejection in unfamiliar surroundings. In addition, Atosiban and phenylephrine were administered after a 1 min prestimulation or 1 min after the start of milking. Oxytocin concentrations increased during milking in all treatments. The spontaneously removed milk fraction (before oxytocin was injected) was similar for Atosiban and phenylephrine treatments and in unfamiliar surroundings, but lower than in controls. Peak flow rates were similar in all treatments, but reduced as compared with controls when phenylephrine and Atosiban were administered before prestimulation. Peripheral (Atosiban, phenylephrine) and central (unfamiliar surroundings) inhibition of milk ejection reduced the amount of available milk similarly. Drug treatments resulted in similar peak flow rates; however, teats were contracted after phenylephrine administration but not after Atosiban. The inhibition induced by Atosiban could be abolished by oxytocin injection, but not that induced by phenylephrine, which was antagonized by α-adrenergic receptor blockade. These results indicate that inhibition of milk ejection through activation of α-adrenergic receptors is based on blockade of milk flow into the cistern, but not through the teats.

Milk ejection is essential to make the main milk fraction, the alveolar fraction, available for removal, whereas the cisternal fraction can be obtained by simply overcoming the teat sphincter barrier (Bruckmaier *et al.* 1994*a*). Milk ejection is elicited by tactile teat stimulation through oxytocin release beyond a threshold level (Schams *et al.* 1984). For complete milk removal oxytocin needs to be elevated and milk ejection maintained throughout the entire milking (Bruckmaier *et al.* 1994*b*). Milk ejection can be disturbed at both central and peripheral levels. Central inhibition of oxytocin release was found during milking in unfamiliar surroundings where milk ejection had already been normalized by small amounts of exogenous oxytocin (Bruckmaier *et al.* 1993). In contrast, peripheral inhibition of the oxytocin effect in the mammary gland was observed in response to catecholamine administration mediated by α-adrenergic receptor stimulation (Gorewit & Aromando, 1985; Blum *et al.* 1989; Bruckmaier *et al.* 1991) and could not be abolished by exogenous oxytocin. Furthermore, milk ejection was shown to be inhibited after administration of an oxytocin receptor blocking agent in the goat (Knight *et al.* 1994).

Inhibition of milk ejection can be employed to remove cisternal and alveolar

fractions separately. Thus, milking in unfamiliar surroundings proved to be practicable for the determination of the cisternal fraction (Bruckmaier *et al.* 1994*a*; Pfeilsticker *et al.* 1996).

The goal of this investigation was to test the hypothesis that the inhibition of oxytocin release in unfamiliar surroundings and of oxytocin effect by oxytocin receptor blockade and α-adrenergic receptor stimulation have similar effects on milk ejection and milk removal. In addition, the experiments were designed to show that milk ejection can still be interrupted by α-adrenergic receptor stimulation and oxytocin receptor blockade after prestimulation and even after the start of milking, i.e. after milk ejection has already occurred.

Materials and methods

Animals

The experimental cows belonged to the herd of the Swiss Federal Research Station for Animal Production, Posieux (Simmental × Red Holstein and Swiss Braunvieh × Brown Swiss breeds) and were in weeks 4–44 of their first to seventh lactations. For technical reasons different cows had to be used for the different experiments. However, lactational stages, lactation numbers and average milk yields were comparably distributed in all experiments. Cows were kept in tie stall barns and fed on maize silage, hay and concentrates according their individual production levels.

Materials and experimental procedures

Milking was performed during routine milking times from 06.00 to 08.00 or from 16.00 to 18.00 at a vacuum level of 45 kPa and a pulsation rate of 60 cycles/min at a ratio of 70:30, using Harmony clusters (Alfa Laval, S-147 21 Tumba, Sweden). Milking machine characteristics were similar in the barn and in the operating theatre (the 'unfamiliar surroundings'). To avoid influences of the previous treatment, experimental milkings were performed only once daily; the second milking was routine only. If the udder could not be completely emptied owing to drug treatments, milking was repeated 1 h after the experiment. Milk flow was continuously recorded by a strain gauge system and conveyed to a strip chart recorder as described by Schams *et al.* (1984). In all treatments the milking cluster was attached immediately after a 1 min teat stimulation. In each experiment, both milking order and treatment order were randomized.

Experiment 1

Phenylephrine (PE; L-phenylephrine-HCl) was purchased from Sigma Chemical Co. (St Louis, MO 63178, USA) and is an α-adrenergic agonist. Phentolamine (PA; Regitin) was donated by Ciba-Geigy AG (CH-4001 Basle, Switzerland) and is an α-adrenergic blocking agent. Six cows were milked at three subsequent morning milkings in their familiar barn without or with i.v. injection of 30 μg PE/kg body weight (BW) before prestimulation. After milk flow had ceased, 10 i.u. oxytocin (OT; Chassot, CH-3123 Belp, Switzerland; 10 i.u./ml) were injected i.v., or 100 μg PA/kg BW were injected i.v. after milk flow cessation and 10 i.u. OT after milk flow had ceased again.

Teat length, diameter at the barrel and at the apex, and thickness at the barrel and at the apex from one front and one rear quarter were measured with a ruler

(length) and a cutimeter (diameter and thickness) as described by Hamann *et al.* (1996), before and 1 min after PE injection. Teat thickness was defined as the distance (mm) between the spring-loaded caliper jaws at a pressure of 10–15 kPa.

Experiment 2

Atosiban (ATO; (1-deamino-2-D-Tyr(O-Et)-4-Thr-8-Orn)-OT) was donated by Ferring Research Institute AB, S-20061 Malmö, Sweden and is an OT receptor blocking agent. Six cows were milked in their familiar barn at three subsequent morning milkings without or with i.v. injection of 10 μg ATO/kg BW or 30 μg PE/kg BW 1 min before prestimulation. Milk flow was also recorded in the unfamiliar operating theatre where the cows were relocated immediately before milking. These unfamiliar surroundings have previously been shown to cause central inhibition of milk ejection (Bruckmaier *et al.* 1993). After milk flow had ceased 10 i.u. OT were injected i.v. in all treatments to induce maximal udder evacuation. To test the responsiveness to OT in PE treatment, 0·2 and 1 i.u. OT were injected i.v. at 1 min intervals prior to the administration of 10 i.u. OT.

An indwelling catheter was inserted in the external jugular vein $\sim$ 6 h before the first milking and blood samples (10 ml) were taken at 1 min intervals from 2 min before the start of milking until the injection of OT after milk flow had ceased. Blood samples were treated with anticoagulant (Na-EDTA), cooled on ice and centrifuged at 1500 ***g*** for 20 min immediately after the experiments. The plasma was stored at -18 °C until the OT concentration was determined by radioimmunoassay as described previously (Bruckmaier *et al.* 1996).

Experiment 3

Six cows were milked in their familiar barn at three subsequent evening milkings without or with i.v. injection of 10 μg ATO/kg BW or 30 μg PE/kg BW immediately after prestimulation and before milking. After milk flow had ceased 10 i.u. OT were injected i.v. to induce maximal udder evacuation.

Experiment 4

Six cows were milked in their familiar barn at three subsequent morning milkings without or with i.v. injection of 50 μg ATO/kg BW or 30 μg PE/kg BW 1 min after the start of milking. After milk flow had ceased 10 i.u. OT was injected i.v. to induce maximal udder evacuation.

Evaluation and statistical analyses

For statistical evaluation of oxytocin concentrations, the means for periods of 2 min were calculated before the start of milking (two blood samples), from the start of milking until 2 min after the start of milking (three blood samples) and from 2 to 4 min after the start of milking (three blood samples). Milk yields were divided into two fractions: before OT administration (spontaneous fraction) and in response to OT injection and stripping (OT fraction). Because in PE treatments the udders could not be completely emptied even after OT injection, total milk yield of the control was used to calculate the fractions in all experiments.

Values are presented as means $\pm$ SEM. For statistical evaluations the SAS program package (SAS, 1995) was used. Treatment effects within and between experiments were tested for significance ($P < 0{\cdot}05$) using the General Linear Model procedure (GLM). Within experiment the model Y_{ijk} = general mean + animal$_i$ + treatment$_j$ +

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residual error$_{ijk}$ was used. To test effects of drug administration at different timepoints in Expts 2–4 the model Y_{ij} = general mean + experiment$_i$ + residual error$_{ij}$ was used. Differences between treatments and experiments were localized using the Least Significant Difference test (LSD).

RESULTS

Experiment 1

Teat length was reduced ($P < 0{\cdot}05$) from 49 ± 2 to 37 ± 2 mm (23 ± 2 %) within 1 min after PE injection. However, the diameter of the barrel was 24 ± 1 mm both before and after PE administration, and changes in the apex from 18 ± 1 mm before to 16 ± 1 mm after PE administration were not significant. Teat barrel thickness increased ($P < 0{\cdot}05$) from 9 ± 0 to 12 ± 1 mm (25 ± 5 %) and thickness of teat apex increased from 9 ± 0 to 10 ± 0 mm (8 ± 3 %).

Total milk yield was 16 ± 1 kg in controls. The spontaneous milk fraction was 14 ± 1 kg (90 ± 4 %) in controls, but only 4 ± 0 kg (24 ± 2 %) after PE administration. When only 10 i.u. OT, a supraphysiological dosage, was injected after milk flow cessation in PE treatment, total milk yield remained reduced ($P < 0{\cdot}05$) at 10 ± 1 kg (62 ± 6 % of the total milk yield in controls). In response to PA injection milk flow commenced again within $2{\cdot}4 \pm 0{\cdot}3$ min. Mean milk yield after PA injection was 15 ± 2 kg (91 ± 6 % of the total milk yield in controls). When OT was injected in addition after PA, the disturbance of milk ejection was fully abolished, and total milk yield was similar to that in controls.

Experiments 2–4

In Expt 2 (Table 1, Fig. 1) premilking OT concentrations were similarly low in controls, ATO and PE treatments (6 ± 1, 7 ± 1 and 7 ± 1 ng/l respectively) and increased in all treatments during prestimulation and milking (pooled concentrations from 0 to 2 min of milking, 27 ± 7, 57 ± 19 and 59 ± 20 ng/l respectively) and remained increased during the milking procedure (pooled concentrations from 2 to 4 min of milking, 25 ± 5, 44 ± 11 and 65 ± 20 ng/l respectively).

Total milk yields were similar in controls, in unfamiliar surroundings and for ATO treatment, but were reduced ($P < 0{\cdot}05$) by PE treatment. The spontaneously removed milk fraction was much lower ($P < 0{\cdot}05$) than in controls and similar for ATO and PE treatments and in unfamiliar surroundings. Peak flow rates during removal of the spontaneous milk fraction were similar in unfamiliar surroundings and for ATO and PE treatments, and reduced ($P < 0{\cdot}05$) compared with controls. During PE treatment 0·2 and 1 i.u. OT did not induce any additional milk removal (results not shown), whereas milk flow commenced after injection of 10 i.u. OT. However, the udder could not be completely emptied even by 10 i.u. OT (total milk yield compared with total milk yield of controls). The peak flow rate of the OT fraction was low for PE treatment. Most of this fraction was removed during stripping.

In Expt 3 (Table 2, Fig. 2), when PE or ATO was administered immediately after prestimulation, total milk yield was reduced ($P < 0{\cdot}05$) in PE treatment whereas total milk yields for controls and ATO treatment were similar. The spontaneous fraction was reduced ($P < 0{\cdot}05$) by ATO and by PE treatment. Peak flow rate of this fraction was not reduced by PE or ATO.

Table 1. *Inhibition of milk ejection in cows by intravenous injection of oxytocin receptor blocking agent Atosiban or the α-adrenergic receptor stimulator phenylephrine before prestimulation or by milking in unfamiliar surroundings*

(Values are means ± SEM for $n = 6$)

Removed milk	Traits	Control	Unfamiliar surroundings	Atosiban	Phenylephrine†
Spontaneous‡	Yield, kg	$11{\cdot}7 \pm 1{\cdot}0^{a}$	$3{\cdot}0 \pm 0{\cdot}8^{b}$	$2{\cdot}6 \pm 0{\cdot}6^{b}$	$2{\cdot}2 \pm 0{\cdot}6^{b}$
	Fraction, %	80 ± 3^{a}	20 ± 4^{b}	17 ± 3^{b}	15 ± 4^{b} (22 ± 4)§
	Peak flow rate, kg/min	$3{\cdot}3 \pm 0{\cdot}2^{a}$	$2{\cdot}3 \pm 0{\cdot}4^{b}$	$2{\cdot}2 \pm 0{\cdot}2^{b}$	$2{\cdot}2 \pm 0{\cdot}2^{b}$
In response to oxytocin injection and stripping	Yield, kg	$3{\cdot}0 \pm 0{\cdot}4^{a}$	$11{\cdot}1 \pm 0{\cdot}7^{b}$	$12{\cdot}3 \pm 0{\cdot}7^{b}$	$7{\cdot}2 \pm 0{\cdot}7^{c}$
	Fraction, %	20 ± 3^{a}	80 ± 4^{b}	83 ± 3^{b}	50 ± 4^{c} (78 ± 4)
	Peak flow rate, kg/min	$1{\cdot}6 \pm 0{\cdot}2^{a}$	$3{\cdot}2 \pm 0{\cdot}2^{b}$	$3{\cdot}0 \pm 0{\cdot}2^{b}$	$1{\cdot}9 \pm 0{\cdot}2^{a}$
Total	Yield, kg	$14{\cdot}7 \pm 1{\cdot}1^{a}$	$14{\cdot}1 \pm 1{\cdot}1^{a}$	$14{\cdot}9 \pm 0{\cdot}2^{a}$	$14{\cdot}7 \pm 1{\cdot}1$ ($9{\cdot}4 \pm 1{\cdot}1$)[b]

† In phenylephrine treatment, total milk yield of control was used.
‡ Spontaneous milk yield: milk yield without exogenous oxytocin.
§ Actual values (based on incomplete total milk yield) and fractions affected are given in parentheses.
[a, b, c] Treatment means without common superscript letters were significantly different: $P < 0{\cdot}05$.

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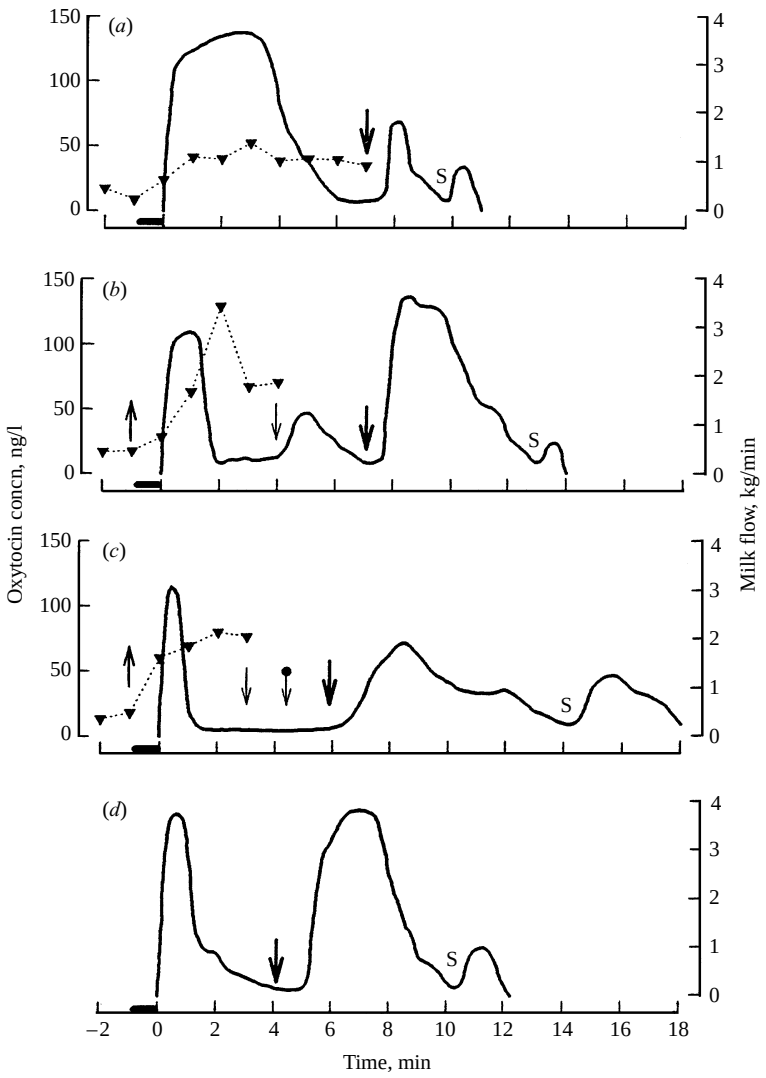

Fig. 1. ▬, Milk flow and ▼---▼, oxytocin concentrations in a single representative cow (*a*) without and with administration of (*b*) the oxytocin receptor blocking agent Atosiban or (*c*) the α-adrenergic receptor stimulator phenylephrine before prestimulation and (*d*) in unfamiliar surroundings. Injections of ↓, 0·2 i.u. oxytocin; ↕, 1 i.u. oxytocin; ↓, 10 i.u. oxytocin; ↑, Atosiban or phenylephrine; ▬, prestimulation; S, start of stripping.

In Expt 4 (Table 3, Fig. 3), when ATO or PE was administered 1 min after the start of milking, the spontaneous fraction was reduced ($P < 0{\cdot}05$) in response to ATO injection and numerically but not significantly by PE treatment. Peak flow rate of this fraction was similar for controls, ATO and PE treatment. Total milk yield was reduced ($P < 0{\cdot}05$) by PE treatment.

The spontaneous milk fraction increased ($P < 0{\cdot}05$) with ATO and PE treatment when the drugs were administered before stimulation from 17 ± 3 and 22 ± 4% respectively to 34 ± 3 and 42 ± 8% respectively after stimulation and to 66 ± 5 and 79 ± 7% respectively after the start of milking with respect to total milk fraction.

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Table 2. *Inhibition of milk ejection in cows by intravenous injection of oxytocin receptor blocking agent Atosiban or the α-adrenergic receptor stimulator phenylephrine after prestimulation*

(Values are means ± SEM for $n = 6$)

Removed milk	Traits	Control	Atosiban	Phenylephrine†
Spontaneous‡	Yield, kg	$9{\cdot}9 \pm 1{\cdot}4^{a}$	$3{\cdot}6 \pm 0{\cdot}3^{b}$	$4{\cdot}8 \pm 0{\cdot}6^{b}$
	Fraction, %	83 ± 4^{a}	34 ± 3^{b}	42 ± 8^{b} (68 ± 9)§
	Peak flow rate, kg/min	$3{\cdot}0 \pm 0{\cdot}4^{ab}$	$2{\cdot}8 \pm 0{\cdot}1^{a}$	$3{\cdot}4 \pm 0{\cdot}3^{b}$
In response to oxytocin injection and stripping	Yield, kg	$2{\cdot}1 \pm 0{\cdot}6^{a}$	$7{\cdot}3 \pm 1{\cdot}1^{b}$	$2{\cdot}8 \pm 1{\cdot}2^{a}$
	Fraction, %	17 ± 4^{a}	66 ± 3^{b}	22 ± 8^{a} (32 ± 9)
	Peak flow rate, kg/min	$1{\cdot}1 \pm 0{\cdot}3^{a}$	$2{\cdot}5 \pm 0{\cdot}3^{b}$	$0{\cdot}6 \pm 0{\cdot}2^{a}$
Total	Yield, kg	$12{\cdot}1 \pm 1{\cdot}8^{a}$	$10{\cdot}9 \pm 1{\cdot}2^{a}$	$12{\cdot}1 \pm 1{\cdot}8$ $(7{\cdot}6 \pm 1{\cdot}3)^{b}$

† In phenylephrine treatment, total milk yield of control was used.
‡ Spontaneous milk yield: milk yield without exogenous oxytocin.
§ Actual values (based on incomplete total milk yield) and fractions affected are given in parentheses.
[a,b] Treatment means without common superscript letters were significantly different: $P < 0{\cdot}05$.

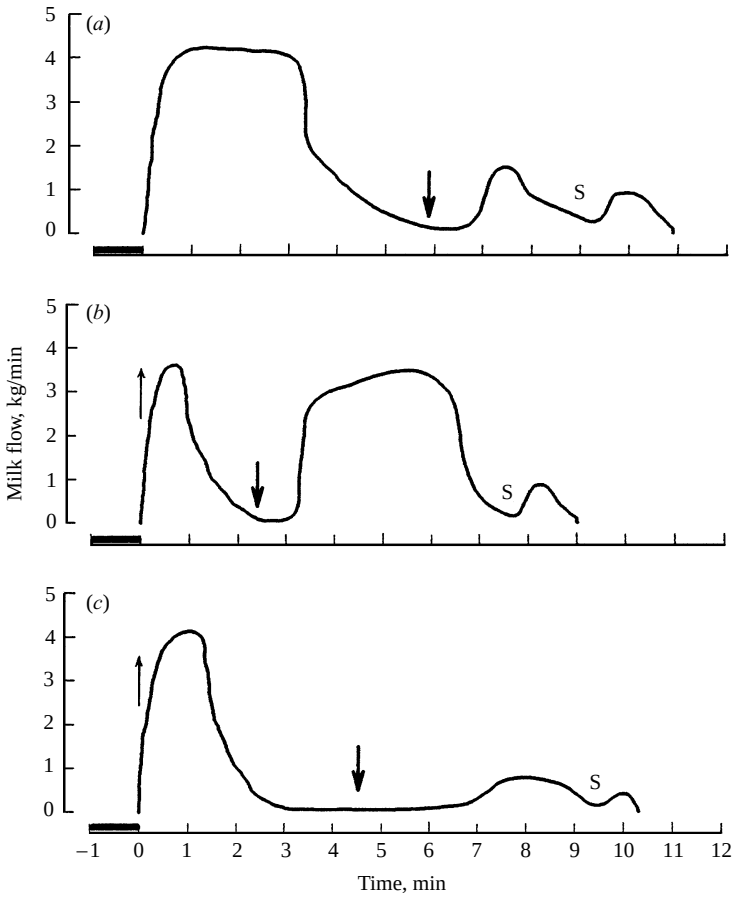

Fig. 2. Milk flow in a single representative cow (*a*) without and with injections of (*b*) the oxytocin receptor blocking agent Atosiban or (*c*) the α-adrenergic receptor stimulator phenylephrine after prestimulation. Injections of ↓, 10 i.u. oxytocin; ↑, Atosiban or phenylephrine; ▬, prestimulation; S, start of stripping.

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Table 3. *Inhibition of milk ejection in cows by intravenous injection of oxytocin receptor blocking agent Atosiban or the α-adrenergic receptor stimulator phenylephrine 1 min after the start of milking*

(Values are means ± SEM for $n = 6$)

Removed milk	Traits	Control	Atosiban	Phenylephrine†
Spontaneous‡	Yield, kg	$9{\cdot}9 \pm 0{\cdot}5^{a}$	$7{\cdot}7 \pm 0{\cdot}7^{b}$	$9{\cdot}0 \pm 0{\cdot}8^{ab}$
	Fraction, %	91 ± 2^{a}	66 ± 5^{b}	79 ± 7^{a} (94 ± 1)§
	Peak flow rate, kg/min	$3{\cdot}1 \pm 0{\cdot}4^{a}$	$3{\cdot}1 \pm 0{\cdot}4^{a}$	$3{\cdot}1 \pm 0{\cdot}3^{a}$
In response to oxytocin injection and stripping	Yield, kg	$1{\cdot}0 \pm 0{\cdot}2^{a}$	$4{\cdot}2 \pm 0{\cdot}7^{b}$	$0{\cdot}5 \pm 0{\cdot}1^{a}$
	Fraction, %	9 ± 2^{a}	35 ± 5^{b}	4 ± 1^{ab} (6 ± 1)
	Peak flow rate, kg/min	$0{\cdot}5 \pm 0{\cdot}1^{a}$	$2{\cdot}2 \pm 0{\cdot}3^{b}$	$0{\cdot}1 \pm 0{\cdot}03^{a}$
Total	Yield, kg	$10{\cdot}9 \pm 0{\cdot}5^{ab}$	$11{\cdot}8 \pm 0{\cdot}8^{a}$	$10{\cdot}9 \pm 0{\cdot}5$ $(9{\cdot}5 \pm 0{\cdot}8)^{b}$

† In phenylephrine treatment, total milk yield of control was used.
‡ Spontaneous milk yield: milk yield without exogenous oxytocin.
§ Actual values (based on incomplete total milk yield) and fractions affected are given in parentheses.
[a,b] Treatment means without common superscript letters were significantly different: $P < 0{\cdot}05$.

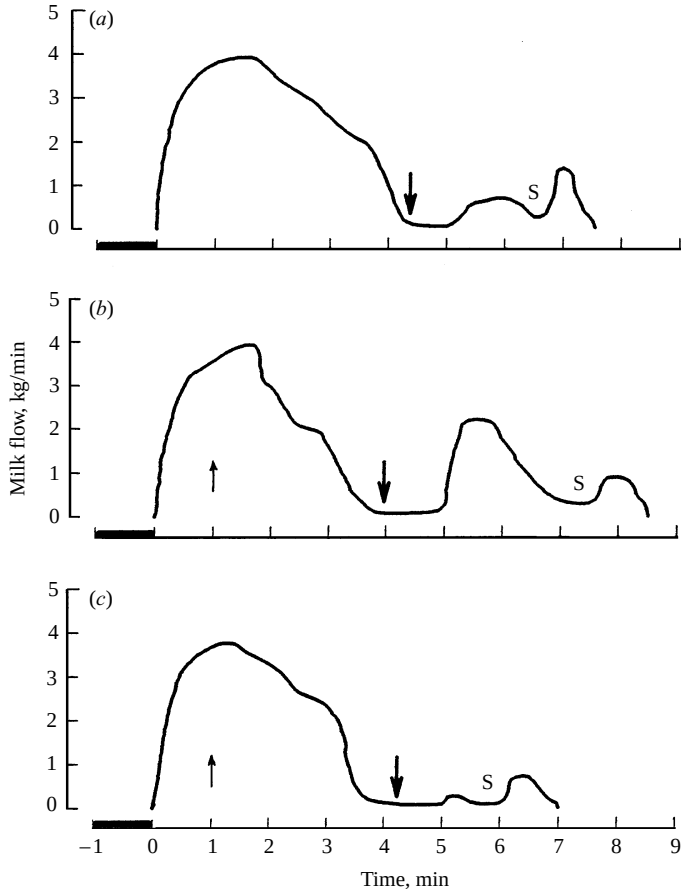

Fig. 3. Milk flow in a single representative cow (*a*) without and with injections of (*b*) the oxytocin receptor blocking agent Atosiban or (*c*) the α-adrenergic receptor stimulator phenylephrine after the start of milking. Injections of ↓, 10 i.u. oxytocin; ↑, Atosiban or phenylephrine; ▬, prestimulation; S, start of stripping.

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DISCUSSION

It was previously shown that in unfamiliar surroundings milking related OT release was absent and that the administration of exogenous OT in physiological amounts provoked normal milk ejection (Bruckmaier *et al.* 1993, 1994*b*). In contrast, release of OT was normal or even elevated during milking after OT receptor blockade or α-adrenergic agonist administration as compared with control milkings. Because milk ejection after PE or ATO administration was inhibited despite normal OT release, the inhibitory effect was located at the level of the mammary gland. This confirms our previous investigations (Blum *et al.* 1989; Bruckmaier *et al.* 1991), but contradicts the findings of others, that α-adrenergic receptor stimulation inhibits OT release in response to milking stimuli (Barowicz, 1979; Gorewit & Aromando, 1985).

The OT receptor blocking agent ATO inhibited milk ejection, although OT was normally released. Thus, ATO was shown to have the same inhibitory effect on milk ejection in cows as already demonstrated in goats (Knight *et al.* 1994). ATO binds to OT receptors, competes with OT and prevents the activation of the intracellular signalling pathways that mediate the effect of OT on myometrial contraction, although OT has a much higher affinity for the receptor than ATO and can replace the antagonist very easily (Melin, 1994).

The administration of PE caused mainly longitudinal teat contraction. Obviously the contraction caused increased thickness due to increased tissue density. It was previously reported that teat contraction, including contraction of the teat sphincter muscle, occurs in response to α-adrenergic agonist administration (Lefcourt, 1982).

Total milk yield after PE administration was reduced when PE was injected before or after prestimulation or after the start of milking. This confirms previous investigations in which specific α-adrenergic receptor stimulation reduced milk yield markedly (Blum *et al.* 1989; Bruckmaier *et al.* 1991). However, despite teat contraction and interrupted milk ejection milk flow rate was not reduced as long as milk was available in the cistern. Only when PE was administered before prestimulation was the amount of available milk small, and milk flow did not then reach a plateau and its potential maximum. Therefore, milk flow rate was reduced after PE treatment in Expt 2 as in unfamiliar surroundings and after ATO administration, when no teat contraction occurred. Inhibition of milk ejection through α-adrenergic receptor action is therefore most likely located in the pathway from alveolar tissue to the cistern, i.e. in the milk ducts. We have demonstrated the presence of α- and β-adrenergic receptors in the tissue around the cistern where the large milk ducts are located, but only in small numbers in the proximal mammary tissue (Hammon *et al.* 1994). Therefore, an α-adrenergic receptor action on milk ejection via blood vessels or myoepithelial cells within the mammary parenchyma is most unlikely and contradicts the hypothesis that OT cannot reach its receptors on the myoepithelial cells although total blood flow to the mammary gland is reduced in response to catecholamine administration (Gorewit & Aromando, 1985). Nevertheless, it is most unlikely that as a consequence of blood vessel contraction blood flow to the mammary gland would be totally stopped, because other organs with an even smaller blood supply may function properly (Blum *et al.* 1978), and even extremely high amounts of exogenous OT could not elicit normal milk ejection.

Relationships between adrenergic receptor density and milk flow were previously described by Roets *et al.* (1986). It was even demonstrated that measurement of the α_2-adrenergic receptors on blood platelets of bulls could be used to estimate the milking characteristics of the daughters (Roets *et al.* 1995). It is therefore possible

that the adrenergic receptor density in the teat is correlated with the density in gland tissue and reflects the receptor density in the tissues around the gland cistern. Surprisingly we observed no direct effect of the adrenergic tone in the teat, at least in response to short-lasting catecholamine treatment. The vacuum of the milking machine was obviously able to remove the milk stored in the cistern without being affected by the teat contraction induced by α-adrenergic receptor stimulation.

After PE administration, injection of OT in physiological amounts did not allow any milk removal, and only small amounts of milk could be removed in response to pharmacological amounts of OT. It was previously shown that pharmacological amounts of OT cause an intramammary ejection pressure beyond the pressure maximum that is reached in response to physiological dosages of OT (Bruckmaier *et al*. 1994*b*). Possibly the powerful contraction of myoepithelial cells induced by high amounts of OT forced milk even through contracted milk ducts.

The inhibitory effect of PE was antagonized by PA and the udder was completely emptied, at least if high amounts of OT were administered after PA injection. PA in the dosage used was obviously able to reduce mammary duct contraction and thus facilitated the flow of milk from the alveolar tissue into the cistern.

The amounts of available milk and milk flow rates were quite similar in peripheral (by ATO and PE) and central (in unfamiliar surroundings) inhibition of milk ejection. When ATO or PE was injected before prestimulation, milk ejection was completely inhibited and the spontaneously available milk represented the cisternal fraction, just as in milking in unfamiliar surroundings (Bruckmaier *et al*. 1994*a*; Pfeilsticker *et al*. 1996). When ATO or PE was injected after prestimulation or during milking, milk ejection was interrupted and milk flow ceased before the udder was emptied. However, in PE and in ATO treatments the spontaneously available milk fraction increased ($P < 0{\cdot}05$) the later the drug was injected (e.g. before or after stimulation or after the start of milking). These findings provide additional evidence that milk ejection during normal milking must continue throughout the milking procedure to induce continuous milk ejection and complete udder evacuation (Bruckmaier *et al*. 1994*b*).

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