Effects of morphine and naloxone on the release of oxytocin and on milk ejection in dairy cows

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Summary. The aim of this study was to investigate the action of opioids (the μ receptor agonist morphine) and the antagonist naloxone on inhibition of oxytocin release and milk let-down in response to milking in dairy cows. In the first experiment, cows were injected with 0, 21, 70 and 210 mg morphine 10 min before milking on four successive days. Plasma oxytocin levels after 1 min manual stimulation of the udder were reduced by 70 and 210 mg morphine, and milk let-down was inhibited at the latter dose. In the second experiment, cows were injected after a control milking with 210 mg morphine (or 350 mg at 10 min before milking the following day if not effective) to inhibit milk flow. On the following day the inhibiting dose of morphine was given with 210 mg naloxone. Naloxone injection given before morphine had no effect on plasma oxytocin concentrations, but abolished the inhibition of oxytocin release by morphine and potentiated oxytocin release in response to milking. Naloxone alone injected the day after control milking increased oxytocin levels during milking, suggesting involvement of the opioid system in milking. A model has been developed for the control of opioid effects during milking. Morphine suppressed oxytocin release during milking in a dose-dependent manner and the effect was reversible by naloxone.

The release of oxytocin and milk ejection during manual stimulation of the teats and machine milking are important for rapid and complete milk removal in dairy cows (Schams *et al*. 1984; Gorewit *et al*. 1992; Tančin *et al*. 1995). Milk ejection occurs when oxytocin release results in concentrations above a threshold level of ~ 3–5 ng/l (Schams *et al*. 1984). More recently, it has been shown that oxytocin release is an absolute requirement for successful milk ejection and complete milk removal in dairy cows (Bruckmaier *et al*. 1994). Without milk ejection, only cisternal milk is available, and this represents ~ 5–25% of the total milk in the udder (Knight *et al*. 1994).

In practical dairying, cows are exposed to stressful situations. The acute stress from novelty immediately before milking could result in central suppression of the milk ejection reflex by inhibiting the release of oxytocin (Bruckmaier *et al*. 1993), but the mechanisms responsible for this inhibition are not clearly understood in dairy cows. There is general agreement that in rats endogenous and exogenous opioids

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inhibit oxytocin secretion in response to stimuli (Bicknell & Leng, 1982; Bicknell 1985; Russell *et al*. 1993). It has been confirmed that administration of the opioid antagonist naloxone can abolish the central inhibition of oxytocin release in rats (Pumford *et al*. 1991; Russell *et al*. 1993; Douglas *et al*. 1998) and sows (Lawrence *et al*. 1992). Surprisingly, in dairy cows naloxone fails to abolish inhibition of oxytocin secretion under stress conditions during milking or at parturition (Aurich *et al*. 1993; Wellnitz *et al*. 1997). Another opioid antagonist, naltrexone, is also without effect on oxytocin during suckling (Ehrenreich *et al*. 1985). There is no information currently available about relationships between opioids and secretion of oxytocin during milking in dairy cows that could explain the mechanisms of disturbed milk ejection.

The aim of the present study was to determine the role of opioids in the inhibition of oxytocin release and milk let-down factors in response to machine milking in dairy cows. Exogenous morphine (a μ receptor agonist) was used as a model to study the effects of opioids. The reversibility of morphine effects by the opioid antagonist naloxone was also investigated. In addition, the effect of naloxone alone on oxytocin release during milking was tested.

Materials and methods

Animals

A total of 16 Brown Swiss dairy cows in their first to fifth lactations with body weights (BW) 700 ± 14 kg (mean $\pm$ SEM) were used for the experiments. The cows were 1–10 months in lactation, not pregnant, and had daily milk productions from 17 to 40 kg just before the experiments. The cows had free access to a mixed ration providing energy and nutrients for the production of 22 kg milk and received additional concentrates depending on their milk production. At 2 d before the experiment, the cows were separated from the herd and brought to another part of the building, where they could be housed in individual tie stalls. The cows were milked using a bucket milking installation with an integrated Lactocorder (Werkzeug- und Maschinenbau Berneck AG, CH-9442 Berneck, Switzerland; Göft, 1996) for recording the actual nature of the milk flow during milking. The experiment started when the milk flow characteristics and the milk yields were at the same levels as during previous milkings in the dairy parlour.

At 1 d before the experiment, the cows were fitted with a cannula inserted into the jugular vein. After sedation with 20 mg xylazine (Bayer Vital Tiergesundheit, D-51368 Leverkusen, Germany), the neck was shaved and disinfected and a needle (o.d. 5·0 mm, i.d. 3·0 mm) was positioned in a jugular vein. Through this needle, a catheter of medical grade silicone (i.d. 1·5 mm, o.d. 2·5 mm; Dow Corning, Ulrich Comp., D-89030 Ulm, Germany) was pushed $\sim$ 400 mm to a position close to the heart. After withdrawal of the needle, a flexible plate of double layer polyester felt was glued to the catheter and sutured to the skin. The free end of the catheter was closed with a Luer lock steel tip and a plastic lock and packed into a leather pocket fixed just above the catheter plate. All catheters were rinsed at least twice daily and filled with heparin solution (500 i.u./ml in 9 g NaCl/l) and were patent until the end of the experiment.

Treatments and blood collections

Experiment 1. Six cows were given four i.v. doses of morphine (0, 21, 70 and 210 mg, equal to 0, 0·03, 0·1 and 0·3 mg/kg BW respectively dissolved in 10 ml sterile saline) on four consecutive days 10 min before the start of manual stimulation

(0 min) of the udder to prepare the cows for morning milking (at 07.00). During the intensive blood collections around the morning milkings, 10 ml samples were collected into tubes containing 200 μl of a solution containing EDTA (ethylene dinitrilotetra-acetic acid disodium salt, 300 μmol/l; Merck, D-64293 Darmstadt 1, Germany) and acetylsalicylate (10 g/l; Serva, D-69042 Heidelberg 1, Germany). These were cooled in an ice bath, centrifuged at 3000 ***g*** and stored in divided samples at -20 °C until assayed. Blood samples were taken at -20, -15, -10, -5 and -1 min before, at time 0, and at $+0{\cdot}5$, $+1{\cdot}0$, $+1{\cdot}5$, $+2{\cdot}0$, $+2{\cdot}5$, $+3{\cdot}0$ and $+4{\cdot}0$ min after the start of hand stimulation, and then at 1 min intervals until the end of milking. At $+1{\cdot}0$ min, milking and recording the milk flow began. If the morphine dose inhibited the milk flow, oxytocin (1 i.u.) was injected i.v. (Albrecht, D-88323 Aulendorf, Germany) ~ 2 min after cessation of milk flow. In one cow with incompletely suppressed milk flow after 210 mg morphine, an additional dose of 350 mg morphine was administered on the following day. All injections were carried out via the catheter, which was then flushed with saline (9 g/l). At the evening milking (18.00), the animals were not injected with morphine and no blood was collected, but the milk flow was recorded.

Experiment 2. This experiment was performed in two parts. In the first, we studied the reversibility of the effects of morphine by naloxone and in the second possible direct effects of naloxone alone on oxytocin secretion. On the basis of results from the first experiment, two cows were used to test the effective naloxone dose. After the control milking 1 d before, they were treated with 210 mg morphine and then if this was not effective with 350 mg morphine on the next day to inhibit milk flow (the 'inhibiting dose'). One day later 350 mg naloxone was administered at -15 min followed by the effective dose of morphine at -10 min (210 and 350 mg in the two cows). On the following day, the experiment with these two cows was repeated with 210 mg naloxone. Since 210 mg naloxone was effective, for the next two cows only 210 mg naloxone was used with the inhibiting dose of morphine. In the second part of the experiment six cows were used to study the effect of naloxone alone on oxytocin. After the control milking (without naloxone) 300 mg naloxone was injected at the next milking 10 min before the start of the 1 min manual stimulation and machine milking. Blood was collected in the same order as in the previous experiments.

Hormone determinations

Oxytocin was determined by radioimmunoassay as originally described for cattle (Schams *et al.* 1979) and with a method of improved sensitivity (Schams, 1983) after extraction with SEP-PAK C_{18} cartridges (Waters, Milford, MA 01757, USA). The antiserum did not cross react with related peptides such as lysine- or arginine-vasopressin or anterior pituitary hormones. The extraction recovery at 0·8, 1·6, 3·2 and 6·4 ng/l was 76 ± 10 % (mean $\pm$ SD for $n = 9$). The within-assay CV varied from 5·9 to 7·8 % and the between-assay CV from 11·2 to 16·9 % in samples with high ($17{\cdot}2 \pm 1{\cdot}9$ ng/l) and low ($1{\cdot}6 \pm 0{\cdot}3$ ng/l) oxytocin concentrations.

Statistical evaluation

Calculations were performed on an area under the curve/min basis for the period before and from 1·5 to 4·0 min after the start of machine milking. For statistical evaluation, a repeated measures analysis of variance was calculated using the MIXED procedure of SAS (1995). The animal was the repeated subject. Differences on a least square means basis were localized using Bonferoni's *t* test. For statistical

Table 1. *Oxytocin levels in blood plasma and milking characteristics of dairy cows in Experiment 1 before and after intravenous treatment with saline or three different doses of morphine 10 min before the start of morning milking*

(Values are means ± SEM for $n = 6$)

Day of treatment	1	2	3	4	4
Morphine dose, mg	0	21	70	210	210
Oxytocin injection, i.u.†	0	0	0	0	1
Oxytocin before stimulation, ng/l‡	2·7 ± 0·3	2·8 ± 0·5	2·7 ± 0·4	3·9 ± 0·8	—
Oxytocin during milking, ng/l§	25·5 ± 7·7^{b}	26·4 ± 10·9^{b}	9·2 ± 1·6^{c}	4·1 ± 0·4^{c}	44·4 ± 8·7^{a}
Morning milk yield, kg	13·8 ± 2·1^{ab}	13·0 ± 1·8^{ab}	11·0 ± 2·6^{b}	2·0 ± 0·4^{c}	15·2 ± 2·2^{a}¶
Highest milk flow in morning, kg/min	2·7 ± 0·3^{ab}	2·5 ± 0·3^{ab}	2·7 ± 0·4^{ab}	1·7 ± 0·3^{b}	3·0 ± 0·3^{a}

† Oxytocin was injected intravenously after cessation of alveolar milk flow at the highest morphine dose during morning milking.

‡ Two blood samples taken at −5 min and −1 min prior to hand stimulation of the udder.

§ Six blood samples taken during the first 3 min of milking after 1 min hand stimulation or after oxytocin injection.

¶ Calculated cumulatively (includes cisternal content of 2·0 kg).

$^{a, b, c}$ Values in the same line without a common superscript letter were significantly different: $P < 0·05$.

comparisons, $P < 0·05$ was considered to be significant and all results are presented as means ± SEM.

RESULTS

Experiment 1

Milk yields are shown in Table 1. Morphine had a dose-related inhibitory effect on milk yield, with complete inhibition after 0·5–1·5 min milking (except for the removal of the cisternal milk) in five of the six cows given a dose of 210 mg. In the remaining cow, there was complete inhibition after 350 mg morphine. In one cow, inhibition was already complete at a dose of 70 mg morphine. The inhibition of milk flow was reversed after an i.v. injection of 1 i.u. oxytocin and resulted in a normal (or slightly increased) milk yield compared with the control (no morphine, no oxytocin) treatment (Table 1).

Plasma oxytocin levels (Table 1) did not differ before the start of 1 min hand stimulation of the udder. However, during the first 3 min of milking, oxytocin release was significantly reduced at a dose of 70 mg, and further suppressed below the threshold level for milk ejection at a dose of 210 mg morphine.

Experiment 2

The first part of the experiment showed that the milk flow was fully inhibited by 210 (two cows) or 350 (two cows) mg morphine (Table 2). Pretreatment with 210 mg naloxone abolished the effect of morphine, but did not increase the milk yield above control levels.

Morphine inhibited the increase in plasma oxytocin concentration with milking (Table 2), an effect prevented by naloxone pretreatment. Naloxone followed by morphine increased plasma oxytocin concentrations during milking to values significantly higher than in controls (Table 2).

The second part of the experiment showed that basal values before milking were not influenced by naloxone administration (Table 3). Naloxone stimulated ($P < 0·05$) oxytocin release in response to machine milking compared with controls (Table 3). Maximal peak flow rate was not influenced by treatment (2·4 ± 0·2 kg/min for both groups).

Table 2. *Oxytocin levels in blood plasma and milking characteristics of dairy cows in Experiment 2 before and after intravenous treatment with saline, with an inhibitory dose of morphine alone or of naloxone plus morphine*

(Values are means ± SEM for $n = 4$)

Day of treatment	1	2	2	3
Naloxone dose, mg	0	0	0	210
Morphine dose, mg	0	Inhibition†	Inhibition†	Inhibition†
Oxytocin injection, i.u.‡	0	0	1	0
Oxytocin before stimulation, ng/l§	2·4 ± 0·4	2·9 ± 0·8	3·4 ± 0·9	2·7 ± 0·8
Oxytocin during milking, ng/l¶	16·7 ± 4·8[b]	3·6 ± 1·1[a]	51·6 ± 4·4[c]	47·4 ± 10·8[c]
Milk yield morning, kg	11·6 ± 0·4[a]	1·2 ± 0·4[b]	13·0 ± 0·7[a]‖	12·1 ± 0·6[a]
Highest milk flow in morning, kg/min	2·6 ± 0·3[a]	1·7 ± 0·6[b]	2·9 ± 0·4[a]	2·7 ± 0·4[a]

† In two cows, the alveolar milk flow was totally inhibited after a single intravenous injection of 210 mg morphine; in the other two cows, a higher dose of 350 mg was needed for complete inhibition of the alveolar milk flow.

‡ Oxytocin was injected intravenously after cessation of alveolar milk flow at the highest morphine dose during morning milking.

§ Two blood samples taken at −5 min and −1 min prior to manual stimulation of the udder.

¶ Seven blood samples taken during the first 3 min of milking after 1 min manual stimulation.

‖ Calculated cumulatively (includes cisternal content of 1·2 kg).

[a, b, c] Values in the same line without a common superscript letter were significantly different: $P < 0{\cdot}05$.

Table 3. *Oxytocin levels in blood plasma of individual cows after intravenous treatment with naloxone or saline before and during milking*

(Values are ng/l, oxytocin levels for individual cows measured as area under curve/min)

		Cow no.						
Treatment	Sampling period	1	2	3	4	5	6	Mean ± SEM
Naloxone, 300 mg intravenous	Before milking	2·3	2·2	2·2	1·9	1·6	2·8	2·1 ± 0·2[a]
	During milking	320·3	108·5	269·9	94·0	143·5	185·6	186·9 ± 37·1[b]
Saline (control)	Before milking	2·1	3·5	1·7	3·7	1·4	2·8	2·6 ± 0·4[a]
	During milking	78·7	27·4	32·6	52·0	78·4	46·1	52·5 ± 8·9[c]

[a, b, c] Values without a common superscript letter were significantly different: $P < 0{\cdot}05$.

DISCUSSION

The presence of opioid receptors and opioids in the bovine hypothalamus and pituitary (Pesce *et al.* 1987; Leshin *et al.* 1992; Zadina *et al.* 1997) supports the assumption that endogenous opioids can influence oxytocin secretion during milking in dairy cows. The effects of opioids on the release of cortisol, prolactin and luteinizing hormone under basal and stress conditions are well documented in cows (Peck *et al.* 1988; Nanda *et al.* 1989, 1992). It has been demonstrated that centrally inhibited oxytocin secretion during milking in unfamiliar surroundings is accompanied by increased levels of cortisol and the endogenous opioid β-endorphin (Bruckmaier *et al.* 1993). However, opioid–oxytocin relationships in dairy cows have not previously been clearly demonstrated.

To our knowledge, this paper is the first to describe the inhibitory effect of i.v. administration of morphine on oxytocin secretion in response to manual stimulation and machine milking of dairy cows. Morphine had suppressive effects on oxytocin release during milking in one animal at a dose of 21 mg and in all other cows with 70 mg, except for one cow in which milk ejection was not inhibited. At the highest dose, milk ejection was inhibited when oxytocin concentrations remained below the

threshold level (defined as the level needed to elicit milk ejection, ~ 5 ng/l). We have confirmed the same general inhibitory effect of morphine administration on oxytocin secretion in response to teat stimuli described in the rat (Clarke *et al.* 1979; Pumford *et al.* 1991) in a dose-dependent manner (Wright & Clarke, 1984), but using much lower doses than those generally used in rats. The inhibitory effect of morphine administration on milk ejection was abolished by exogenous oxytocin administration. It seems that morphine does not influence the sensitivity of the mammary gland to oxytocin, as observed in rats (Russell *et al.* 1993). From our results it is clear that morphine suppresses the milk ejection reflex in dairy cows during milking as a consequence of central inhibition of oxytocin release, not at the level of the udder.

In Expt 2, naloxone had no effect on basal oxytocin concentrations before milking, as has been reported for the goat (Seckl & Lightman, 1987) and mare (Aurich *et al.* 1996). However, naloxone administration overcame the inhibitory effect of morphine and potentiated oxytocin release in response to machine milking. Naloxone in combination with morphine can reverse the latter's inhibitory effect on oxytocin release (Pumford *et al.* 1991) and potentiate release in the rat (Sumner *et al.* 1989). It is clear from the second part of Expt 2 that naloxone alone can stimulate and potentiate oxytocin release in response to machine milking, suggesting that in dairy cows naloxone can reach the opioid receptors within the brain controlling oxytocin release. A similar effect of naloxone on oxytocin release has been reported during vaginal stimulation (Seckl & Lightman 1987; Aurich *et al.* 1996). Thus the endogenous opioid system seems to exert an inhibitory effect on oxytocin release during machine milking in dairy cows.

Our results suggest that endogenous opioids are involved in oxytocin release during milking by an inhibiting tonus. The question of why naloxone cannot overcome central inhibition of oxytocin release during milking under stress conditions (Wellnitz *et al.* 1997) or influence oxytocin levels during parturition (Aurich *et al.* 1993) as reported for the rat (Leng *et al.* 1988; Douglas *et al.* 1998) and sow (Lawrence *et al.* 1992) is still unresolved for dairy cows. One possible explanation is the dose of naloxone used. We gave a much lower naloxone dose (0·5 *v.* 2 mg/kg BW) than Wellnitz *et al.* (1997), who were unable to influence oxytocin levels during milking even under normal conditions. Naloxone doses of 1 mg/kg and below seem to be sufficiently effective to modulate a variety of pituitary hormones and corticoids in cattle (Nanda *et al.* 1992; Evans *et al.* 1992) in contrast to the situation in the rat. The possibility remains that stress-induced oxytocin inhibition is not actually opioid mediated.

Despite a significant increase in oxytocin release after naloxone administration, there were no differences in milk flow or yield compared with controls. This agrees with the threshold level hypothesis that oxytocin concentrations above this level have no additional effects on milk flow or milk yield. When milk ejection is induced, milk flow rate and milkability depend on the stimulation or non-stimulation of α- and β-adrenoreceptors in the smooth muscles of the udder tissue (Roets *et al.* 1986; Hammon *et al.* 1994). The potentiation effect of naloxone on oxytocin release suggests that endogenous opioid is released in response to udder stimulation and machine milking and could be considered a controlling factor in oxytocin inhibition in response to udder stimulation and machine milking.

In conclusion, a model has been developed for the control of opioid effects during milking. Morphine suppresses oxytocin release in response to machine milking in a dose-dependent manner and the effect can be reversed by naloxone. Naloxone alone potentiates oxytocin release during milking. However, we can not conclude from this

study that opioids are the only inhibitory factor in the regulation of oxytocin release in the cow with centrally blocked oxytocin secretion during milking. Further studies are needed to specify the mechanisms responsible for central inhibition of oxytocin release during machine milking under conditions of stress.

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