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Inhibition of oxytocin release and milk let-down in postpartum primiparous cows is not abolished by naloxone

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Summary. About 10% of primiparous cows have no milk ejection during the first milkings after delivery. Therefore, 17 Brown Swiss dairy cows in their first lactation were used to evaluate the extent of disturbed milk let-down and the corresponding oxytocin (OT) plasma values in the 1st 5 days after delivery. The first milking was 9–22 h after parturition and served for classification of the cows to groups with inhibited (INH), bimodal (BIMO) or normal (NOR) milk let-down. The OT plasma levels before the start of manual teat stimulation and machine milking were comparably high during the first milking especially in NOR and BIMO cows. Ten minutes before the second milking (M2), 300 mg of the opioid antagonist naloxone was injected to test whether the disturbance was affected by the action of endogenous opioids on the neurohypophysis. The milk yield was not influenced by the naloxone treatment, and the INH cows had milk ejection only after a vaginal stimulation. Afterwards, the cows were milked twice every day, until the milk let-down and the OT release were unaffected (equal to control milking). Then, at the next milking, the cows were injected with 300 mg morphine 10 min before milking. The central OT release in response to manual teat stimulation and machine milking was completely blocked in all cows, but a vaginal stimulation was able to abolish this block, at least partially, in 16 cows. Thus, morphine produced a milk let-down characteristic as in the INH cows during the first three milkings. For the following milking, the cows were pre-treated with 300 mg naloxone (−15 min) plus 300 mg morphine (−10 min) before milking. The OT release and the milk yields were unaffected when compared with the control milking. This experiment demonstrates that exogenous opioids can affect the central release of OT in a naloxone-reversible manner even very soon after parturition. However, endogenous opioids are probably not the main mediators of disturbed central OT release and alveolar milk ejection in post-partum primiparous cows.

Keywords: Cow, primiparous, post-partum, oxytocin, morphine, naloxone.

Complete milk removal is necessary for optimum milk quality, high performance and good health of dairy cows. Disturbed milk ejection is observed in situations like

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oestrus, discomfort, stress, novel surroundings, and about 10% of the parturient primiparous cows are estimated to have disturbed milk ejection. In all these situations, the central release of oxytocin (OT) from the neurohypophysis is reduced or abolished (Bruckmaier *et al.* 1992, 1993; Bruckmaier & Blum, 1994). In the rat, the central release of endogenous opioids in stress situations is the probable mechanism for the inhibition of OT release (Zhao *et al.* 1988; Pumford *et al.* 1991; Russell *et al.* 1993). However, in the cow the mechanism blocking the OT release remains unclear. As in the rat, exogenous opioids can effectively suppress the central release of OT in the cow in a naloxone-reversible manner (Kraetzl *et al.* 1999; Tancin *et al.* 2000). However, naloxone may fail to restore OT release in stress situations (Wellnitz *et al.* 1997; Kraetzl *et al.* 2001) and the opioid system may lack sensitivity around parturition (Aurich *et al.* 1993). The situation therefore was quite unclear, and an experiment was carried out to evaluate the role of opioids in the release of OT during the first days of milking after parturition in primiparous cows by the use of an exogenously applied opioid agonist and antagonist.

Materials and methods

Seventeen primiparous Brown Swiss dairy cows were used for this experiment during the 1st 5 days of their first lactation. Two days before the expected calving, the cows were relocated from a loose housing environment to a separate calving box within the same stable. Immediately after delivery, the calf was separated from the cow to prevent suckling. After delivery (3–12 h), the cows were cannulated for blood collection from the jugular vein and relocated to a stand next to the common loose housing environment, where they remained for the next 5 days. Milking was twice daily at 07.00 and 18.00.

During all milkings, the milk flow was recorded with a Lactocorder® (Bruckmaier *et al.* 1996). All treatments and samplings were relative to the start of the milking record (0 min). From $\pm 0{\cdot}0$ until $+1{\cdot}0$ min, a manual teat stimulation was done, and at $+1{\cdot}0$ min, the teat cups of the milking machine were attached to the udder. The first milking (M1), was carried out 9–22 h after delivery and the cow received no further treatment during this milking. During all other milkings (M2–M8), a vaginal stimulation lasting 2 min, was done after the milk flow had ceased. The milk flow reaction was observed and after it had ceased again, 1 IU OT (Oxytocin®, Sanofi, D-40472 Düsseldorf, Germany) was injected intravenously (i.v.) to provide a physiological OT plasma level and to remove the remaining milk. During consecutive milkings (M2–M8), the effects of different pre-treatments before the start of stimulation were investigated (see Table 1). The morphine (morphine hydrochloride; Merck, D-64271 Darmstadt, Germany) and naloxone (naloxone hydrochloride; Tocris, Ballwin, MO 63011, USA) doses used for M2, M7 and M8, were determined according to the results of our recent studies on opioid effects (Kraetzl *et al.* 1999; Tancin *et al.* 2000). M3–M6 were used to make the animals familiar with the milking procedure and to normalise the milking characteristics of all participating cows.

Blood samples were taken at $-5{\cdot}0$, $-1{\cdot}0$, $\pm 0{\cdot}0$, $+0{\cdot}5$, and at 0·5 min intervals until $+4{\cdot}0$ min, and then every minute, relative to the start of milk recording until the injection of OT. The harvested blood was filled into tubes containing EDTA, cooled on ice, centrifuged at 3000 ***g***, aliquoted and frozen at -20 °C. The plasma samples were assayed for OT by radioimmunoassay as described (Schams *et al.* 1983), preceded by an extraction step with SEP-PAK cartridges (Waters, Milford, MA 01757, USA) to improve the sensitivity to 1·0 pmol/l. The antiserum did not cross

Table 1. *Treatments before the start of manual stimulation and during milk removal*

	Before manual teat stimulation		After 1st cessation of milk flow	After 2nd cessation of milk flow
	Time†	Treatment		
M1				
M3	−10	300 mg naloxone i.v.	2 min vaginal stimulation	1 IU oxytocin i.v.
M3–M6			2 min vaginal stimulation	1 IU oxytocin i.v.
M7	−10	300 mg morphine i.v.	2 min vaginal stimulation	1 IU oxytocin i.v.
M8	−15	300 mg naloxone i.v.	2 min vaginal stimulation	1 IU oxytocin i.v.
	−10	300 mg morphine i.v.		

† Time (min) relative to the start of 1 min manual stimulation.

react with related peptides such as lysine- or arginine-vasopressin or anterior pituitary hormones. The extraction recovery was on average 77 ± 9 % (SD). The intra-assay variation varied from 5·8 % to 7·4 % and the inter-assay variation from 10·5 % to 15·4 %.

For statistical calculations, the results from the blood samples were pooled and expressed as area under the curve/min (AUC/min) for −5·0 until −1·0 min (Phase I); +1·0 until +1·5 min (Phase II); +2·0 until +4·0 min (Phase III) and for 2 min after vaginal stimulation (Phase IV). A statistical evaluation was done by a two factorial ANOVA (SAS 1995), using a linear model for the effect of phase during milking, treatment, and animal. Differences were determined as significant when $P < 0{\cdot}05$. The data are presented as means ± SEM.

RESULTS

During the first three milkings after parturition, six cows had, at least once, a total inhibition of alveolar milk flow with an immediate release of only 0·1–0·9 kg milk followed by a complete cessation of the recorded milk flow during the next 3 min (Inhibition; INH). Seven cows had, at least once, a bimodal milk release (BIMO) with an immediate release of 1·0–2·3 kg milk followed by decreasing milk flow. A persistent release of the alveolar milk (additional 6·3–13·3 kg) started following a delay of 60–150 s after the start of milking in these cows. Only four cows had an immediate and complete milk ejection during the first three milkings, seen as continuous, undisrupted milk flow record (normal; NOR) and the corresponding OT plasma levels. Individual examples for the above mentioned milking characteristics are given in Fig. 1. The statistical analysis was done after submitting the cows to the above mentioned three types of milk release. For all further investigations, the cows were submitted to the mentioned three types of milk release. Table 2 gives the milking characteristics for all individual cows during the first three milkings after parturition. From the INH, BIMO or NOR groups, 0 (0 %), 2 (29 %), or 1 (25 %) cows had a hard delivery with manual expulsion, and 1 (17 %), 1 (14 %) or 2 (50 %) cows had a retention of the placenta for more than 12 h, respectively. The time between delivery and first milking was 17·8 ± 1·0, 15·5 ± 1·4 or 14·3 ± 2·3 h, respectively, for INH, BIMO or NOR cows (mean ± SEM).

Before the first milking (M1; Table 3) the cows with BIMO and especially the cows with NOR had significantly higher pre-stimulatory (Phase I) OT plasma levels than the cows with INH. After manual teat stimulation (Phase II), the OT plasma levels of INH cows increased only very moderately and did not reach the threshold for ejection of alveolar milk. Therefore, these cows had only a total amount of 0·9 kg

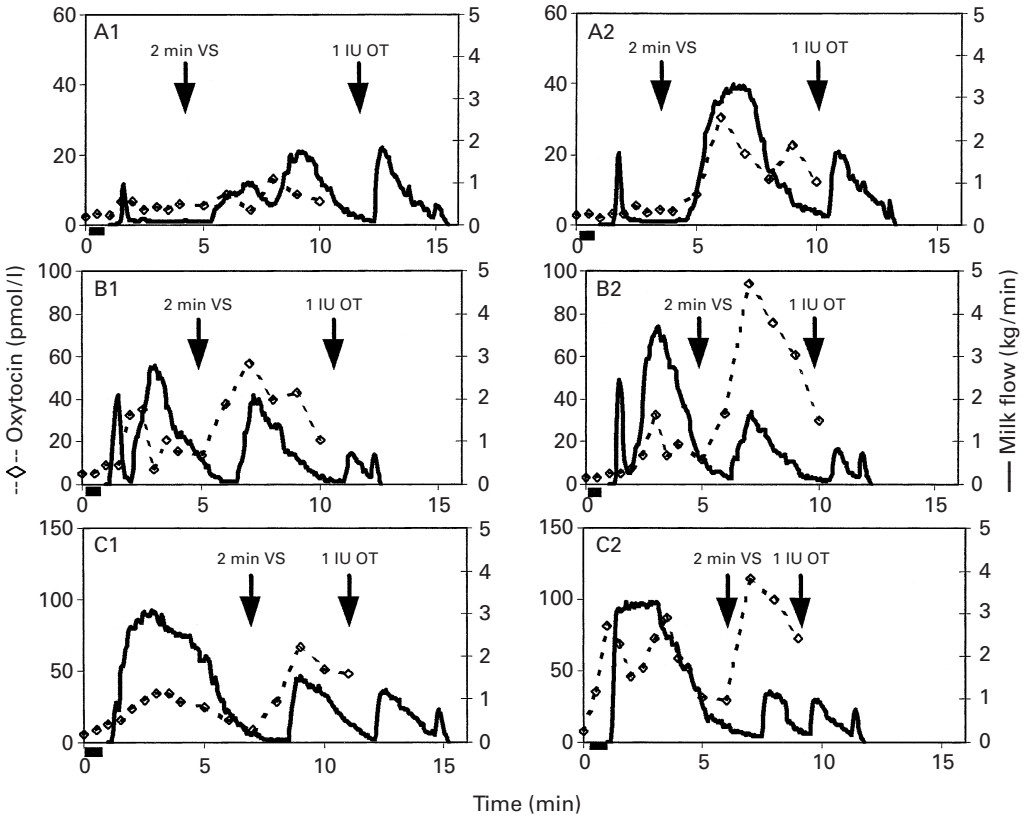

Fig.1. Examples for milk flow (kg/min; —) and oxytocin (OT) plasma levels (pmol/l; – – ◇ – –) during (*a*) inhibited (INH), (*b*) bimodal (BIMO) and (*c*) normal milk release (NOR) during the third (*a*1, *b*1, *c*1) and second (*a*2, *b*2, *c*2) milking after parturition (1 = M3 without or 2 = M2 with i.v. injection of 300 mg naloxone 10 min before start of 1 min teat stimulation, —, respectively). The applications of 2 min vaginal stimulation (VS) and i.v. injection of 1 international unit (1 IU) OT after cessation of milk flow are marked with an arrow.

Table 2. *Milking characteristics of individual cows (1–17) during the first three milkings (M1, M2, M3) after parturition*

INH-group				BIMO-group				NOR-group			
	M1	M2	M3		M1	M2	M3		M1	M2	M3
Cow				Cow				Cow			
1	INH	BIMO	INH	7	BIMO	NOR	NOR	14	NOR	NOR	NOR
2	INH	INH	INH	8	BIMO	BIMO	BIMO	15	NOR	NOR	NOR
3	INH	INH	INH	9	BIMO	BIMO	NOR	16	NOR	NOR	NOR
4	INH	INH	BIMO	10	BIMO	BIMO	NOR	17	NOR	NOR	NOR
5	INH	INH	INH	11	NOR	BIMO	NOR				
6	INH	INH	BIMO	12	NOR	NOR	BIMO				
				13	BIMO	BIMO	NOR				

M1 and M3 without pretreatment before manual teat stimulation.
M2 after 300 mg naloxone i.v. 10 min before manual teat stimulation.
INH inhibition of milk let-down after cisternal content.
BIMO bimodal milk let-down.
NOR normal (undisturbed) milk let-down.

Table 3. *Milk yield and OT AUC/min from the first (M1; without any treatment), second (M2; 300 mg naloxone 10 min before start of manual teat stimulation), and third (M3; without pretreatment) milking after parturition*

(Values are mean ± SEM)

	Milk yield (kg)				OT AUC/min (pmol/l)			
	Immediate†	Delayed†	After vag. stim.‡	After 1 IU OT‡	Phase I§	Phase II§	Phase III§	Phase IV§
M1								
INH	$0{\cdot}78 \pm 0{\cdot}46^{a}$	$0{\cdot}12 \pm 0{\cdot}02^{a}$			$4{\cdot}3 \pm 0{\cdot}7^{a,x}$	$6{\cdot}3 \pm 11^{a,y}$	$8{\cdot}7 \pm 1{\cdot}7^{a,z}$	
BIMO	$3{\cdot}01 \pm 0{\cdot}94^{b}$	$8{\cdot}98 \pm 1{\cdot}03^{b}$			$7{\cdot}0 \pm 1{\cdot}0^{b,x}$	$12{\cdot}4 \pm 2{\cdot}5^{b,y}$	$35 \pm 6^{b,z}$	
NOR	$9{\cdot}83 \pm 1{\cdot}60^{c}$	$0{\cdot}00 \pm 0{\cdot}00^{a}$			$12{\cdot}8 \pm 3{\cdot}3^{c,x}$	$29{\cdot}9 \pm 4{\cdot}1^{c,y}$	$60 \pm 11^{c,z}$	
M2								
INH	$0{\cdot}20 \pm 0{\cdot}04^{a}$	$0{\cdot}15 \pm 0{\cdot}02^{a}$	$8{\cdot}48 \pm 1{\cdot}01^{a}$	$1{\cdot}27 \pm 0{\cdot}33^{a}$	$3{\cdot}6 \pm 0{\cdot}8^{x}$	$5{\cdot}4 \pm 1{\cdot}2^{a,x}$	$9{\cdot}2 \pm 2{\cdot}5^{a,y}$	$103 \pm 26^{a,z}$
BIMO	$2{\cdot}86 \pm 1{\cdot}19^{b}$	$5{\cdot}72 \pm 1{\cdot}22^{b}$	$2{\cdot}41 \pm 0{\cdot}80^{b}$	$0{\cdot}64 \pm 0{\cdot}21^{b}$	$4{\cdot}6 \pm 1{\cdot}0^{x}$	$23 \pm 12^{a,x}$	$71 \pm 22^{b,y}$	$196 \pm 56^{b,z}$
NOR	$7{\cdot}65 \pm 0{\cdot}54^{c}$	$0{\cdot}0 \pm 0{\cdot}0^{a}$	$1{\cdot}0 \pm 0{\cdot}15^{b}$	$0{\cdot}35 \pm 0{\cdot}03^{b}$	$5{\cdot}7 \pm 1{\cdot}6^{x}$	$85 \pm 19^{b,y}$	$124 \pm 37^{b,y}$	$281 \pm 72^{b,z}$
M3								
INH	$1{\cdot}15 \pm 0{\cdot}89^{a}$	$1{\cdot}75 \pm 1{\cdot}11^{b}$	$3{\cdot}73 \pm 0{\cdot}70^{b}$	$1{\cdot}22 \pm 0{\cdot}35^{b}$	$3{\cdot}0 \pm 0{\cdot}5^{x}$	$9{\cdot}0 \pm 2{\cdot}4^{a,y}$	$12 \pm 3^{a,y}$	$74 \pm 21^{a,z}$
BIMO	$4{\cdot}81 \pm 1{\cdot}27^{b}$	$1{\cdot}11 \pm 0{\cdot}72^{b}$	$1{\cdot}31 \pm 0{\cdot}16^{a}$	$0{\cdot}49 \pm 0{\cdot}09^{a}$	$3{\cdot}5 \pm 0{\cdot}4^{w}$	$20 \pm 6^{b,x}$	$38 \pm 6^{b,y}$	$91 \pm 17^{a,b,z}$
NOR	$8{\cdot}53 \pm 1{\cdot}12^{c}$	$0{\cdot}0 \pm 0{\cdot}0^{a}$	$1{\cdot}50 \pm 0{\cdot}53^{a}$	$0{\cdot}80 \pm 0{\cdot}43^{a,b}$	$4{\cdot}0 \pm 0{\cdot}9^{x}$	$46 \pm 12^{b,y}$	$63 \pm 12^{c,y}$	$142 \pm 25^{b,z}$

Experimental groups: INH inhibited alveolar milk flow; BIMO bimodal milk flow; NOR normal, undisturbed milk flow.

† Immediate = milk flow without delay after attaching the teat cups.

† Delayed = additional milk yield from bimodal milk release after the first decline of the milk flow (for example see Fig. 1, no. B1, B2).

‡ Additional milk release after 2 min of vaginal stimulation or after OT injection.

§ Phase I, before stimulation –5·0 to –1·0 min; Phase II, after stimulation +1·0 to +1·5 min; Phase III, during milking +2·0 to +4·0 min; Phase IV, +1·0 to +3·0 min after vaginal stimulation.

^{a,b,c} Different superscripts within the same treatment and column indicate significant differences ($P < 0{\cdot}05$).

^{w,x,y,z} Different superscripts within the same row indicate significant differences ($P < 0{\cdot}05$).

milk from the first milking. Individual OT plasma levels during the first milking of INH cows reached from 5·3–20·1 (10·6 ± 2·5) pmol/l without ejection of alveolar milk. In the cows with BIMO, the OT plasma levels without alveolar milk ejection reached 4·5–14·9 (9·6 ± 1·4) pmol/l.

During the second milking after parturition (M2; Table 3), the milking characteristics were not altered by the naloxone pretreatment. From six cows with inhibition of milk ejection during M1 or M3, five cows also had this inhibition after naloxone pretreatment (M2), whereas one cow had BIMO. From seven cows with BIMO during M1 or M3, five cows also had BIMO during the naloxone pretreated milking, and two cows had normal milk flow. From the NOR cows, all four had normal milk flow during M2 (Table 2). The respective milk yields and OT data for the second and third milkings are presented in Table 3. The cows with NOR and BIMO characteristics, but not the cows with INH, had a significantly higher ($P < 0·05$) OT release during phase III after naloxone pretreatment (M2; Table 3) compared with M1 or M3 (Table 3). During the M2 milking, the OT plasma level in the BIMO cows increased only after a delay of 88 s and in the INH cows did not reach the threshold for alveolar milk ejection again. Only after 2 min of vaginal stimulation, the OT plasma levels in the INH cows increased sufficiently for alveolar milk ejection (Table 3).

The third milking (M3; Table 3) was without pretreatment and served therefore as a control for M2. From the INH cows, four had inhibition and two BIMO during M3. From the BIMO cows, two had BIMO and five had normal milk ejection during M3 (Table 2). The OT plasma levels of the INH cows were still significantly lower and those of the BIMO cows tended to be lower than in the NOR cows early after teat stimulation (Phase II; Table 3).

During subsequent milkings, the milking characteristics of disturbed milk ejection, as evaluated from the milk flow records, normalised after 4·2 milkings in INH cows and after 3·1 milkings in BIMO cows and were normal in every cow during M6. During M6, the OT plasma levels in Phase III of the INH cows were still lower than in the BIMO and NOR cows, but they were above the threshold for ejection of alveolar milk, and the BIMO cows reached their OT plateau after a delay. After vaginal stimulation (Phase IV), the NOR cows tended to the highest OT response (+173%). Of the total milk, 76, 80 and 78% were given without vaginal stimulation and OT injection by the INH, BIMO and NOR cows respectively, and the additional yield percentage after vaginal stimulation (17, 15, 15%) or OT injection (7, 5, 7%) was within a common range.

After the application of 300 mg morphine (M7), the release of OT after manual teat stimulation (Phase II) was inhibited in all groups. Therefore, the OT plasma levels remained basal and the milk flow ceased in all cows after the release of 0·33 ± 0·26 kg (range 0·1–1·0 kg). After vaginal stimulation (Phase IV), the OT plasma levels increased significantly in all groups, but were higher in the BIMO and NOR group. The milk flow was restored in 16 cows 84 ± 16 s (range 60–120 s) after the start of vaginal stimulation. However, after the yield of 5·87 ± 1·90 kg milk (3·0–8·6 kg), the milk flow ceased again. In one cow (BIMO group) no further milk was available by vaginal stimulation. 32 ± 1 s (range 25–40 s) after the injection of 1 IU OT i.v., the milk flow was restored in all 17 cows and an additional 3·13 ± 2·0 kg (range 0·6–8·0 kg) amount of milk was obtained.

After the pretreatment with 300 mg naloxone i.v. followed by 300 mg morphine iv, the milk flow characteristics were evaluated as normal in all cows and the milk amount spontaneously given (8·39 ± 2·01 kg; range 5·4–11·9 kg) was significantly

Table 4. *Milk yield and OT AUC/min from the opioid control milking (M6; first undisturbed milking after parturition, without pretreatment before the start of manual teat stimulation), after 300 mg morphine 10 min before manual teat stimulation (M7), and after 300 mg naloxone plus 300 mg morphine at 15 and 10 min before manual teat stimulation (M8), respectively*

(Values are mean ± SEM)

	Milk yield (kg)			OT AUC/min (pmol/l)			
	Immediate†	After vag. stim.‡	After 1 IU OT‡	Phase I§	Phase II§	Phase III§	Phase IV§
M6							
INH	$7{\cdot}72 \pm 0{\cdot}72^{a,b}$	$1{\cdot}77 \pm 0{\cdot}24^{b}$	$0{\cdot}65 \pm 0{\cdot}12^{b}$	$3{\cdot}1 \pm 0{\cdot}4^{x}$	$24 \pm 6^{a,y}$	$22 \pm 3^{a,y}$	$60 \pm 18^{a,z}$
BIMO	$6{\cdot}26 \pm 0{\cdot}49^{a}$	$1{\cdot}21 \pm 0{\cdot}15^{a}$	$0{\cdot}39 \pm 0{\cdot}05^{a}$	$3{\cdot}3 \pm 0{\cdot}4^{w}$	$28 \pm 7^{a,x}$	$56 \pm 13^{b,y}$	$118 \pm 24^{b,z}$
NOR	$8{\cdot}78 \pm 0{\cdot}97^{b}$	$1{\cdot}65 \pm 0{\cdot}47^{a,b}$	$0{\cdot}78 \pm 0{\cdot}44^{b}$	$3{\cdot}4 \pm 1{\cdot}2^{x}$	$60 \pm 13^{b,y}$	$50 \pm 9^{b,y}$	$134 \pm 28^{b,z}$
M7							
INH	$0{\cdot}23 \pm 0{\cdot}04^{(a)}$	$6{\cdot}30 \pm 0{\cdot}69$	$2{\cdot}98 \pm 0{\cdot}83$	$2{\cdot}7 \pm 0{\cdot}5^{x}$	$2{\cdot}7 \pm 0{\cdot}4^{(a),x}$	$3{\cdot}2 \pm 0{\cdot}5^{(a),x}$	$14{\cdot}8 \pm 2{\cdot}4^{a,y}$
BIMO	$0{\cdot}51 \pm 0{\cdot}18^{(b)}$	$4{\cdot}63 \pm 1{\cdot}05$	$3{\cdot}60 \pm 0{\cdot}83$	$2{\cdot}7 \pm 0{\cdot}5^{x}$	$2{\cdot}7 \pm 0{\cdot}4^{(a),x}$	$3{\cdot}4 \pm 0{\cdot}7^{(a),x}$	$30{\cdot}4 \pm 13{\cdot}5^{b,y}$
NOR	$1{\cdot}20 \pm 0{\cdot}83^{(b)}$	$5{\cdot}93 \pm 1{\cdot}23$	$2{\cdot}33 \pm 0{\cdot}94$	$3{\cdot}7 \pm 1{\cdot}2^{x}$	$4{\cdot}4 \pm 1{\cdot}2^{(b),x}$	$5{\cdot}0 \pm 0{\cdot}9^{(b),x}$	$29{\cdot}1 \pm 7{\cdot}2^{b,y}$
M8							
INH	$9{\cdot}98 \pm 0{\cdot}96^{a}$	$1{\cdot}34 \pm 0{\cdot}48^{(a)}$	$0{\cdot}54 \pm 0{\cdot}09^{a}$	$2{\cdot}4 \pm 0{\cdot}4^{x}$	$24{\cdot}4 \pm 5{\cdot}3^{a,y}$	$43 \pm 13^{a,y,z}$	145 ± 59^{z}
BIMO	$7{\cdot}38 \pm 0{\cdot}52^{b}$	$0{\cdot}73 \pm 0{\cdot}17^{(b)}$	$0{\cdot}27 \pm 0{\cdot}04^{b}$	$1{\cdot}9 \pm 0{\cdot}2^{x}$	$85 \pm 21^{b,y}$	$90 \pm 20^{b,y}$	211 ± 29^{z}
NOR	$7{\cdot}76 \pm 1{\cdot}01^{(b)}$	$0{\cdot}87 \pm 0{\cdot}17$	$0{\cdot}37 \pm 0{\cdot}12^{(b)}$	$1{\cdot}9 \pm 0{\cdot}3^{x}$	$114 \pm 24^{b,y}$	$125 \pm 26^{b,y}$	218 ± 10^{z}

Experimental groups: INH inhibited alveolar milk flow; BIMO bimodal milk flow; NOR normal, undisturbed milk flow.

† Immediate = milk flow without delay after attaching the teat cups.

† Delayed = additional milk yield from bimodal milk release after the first decline of the milk flow (for example see Fig. 1, no. B1, B2).

‡ Additional milk release after 2 min of vaginal stimulation or after OT injection.

§ Phase I, before stimulation −5·0 to −1·0 min; Phase II, after stimulation +1·0 to +1·5 min; Phase III, during milking +2·0 to +4·0 min; Phase IV, +1·0 to +3·0 min after vaginal stimulation.

[a,b,c] Different superscripts within the same treatment and column indicate significant differences ($P < 0{\cdot}05$).

[w,x,y,z] Different superscripts within the same row indicate significant differences ($P < 0{\cdot}05$).

higher than with morphine alone (0·54 ± 0·19 kg; Table 4). After vaginal stimulation, 0·98 ± 0·72 kg milk (range 0·4–3·1 kg) was given additionally, and after 1 IU OT i.v., 0·39 ± 0·20 kg (range 0·1–0·8 kg). The OT plasma levels increased significantly without delay after manual teat stimulation (Phase II) in all cows and were higher in the BIMO and NOR groups. After vaginal stimulation (Phase IV), the OT plasma levels were further increased.

discussion

Immediately after parturition, elevated OT plasma levels were found before teat stimulation, especially in cows with undisturbed milking characteristics during the first milking after parturition. The reason for this remains unclear, but an overhanging OT release after the utero-cervical stimulation during or after delivery or expulsion of the placenta (Ferguson reflex) or a changed sensitivity may be involved.

In the first milkings after parturition, we also observed relatively high OT plasma levels without milk ejection from the mammary alveoli, indicating a reduced sensitivity of the mammary myoepithelium for OT or a higher threshold level of OT for milk ejection, respectively. The sensitivity recurred after several milkings and then, the milk ejection occurred regularly at much lower OT plasma levels as during the first milkings. Similar findings are reported from the rat (Lau & Henning, 1987), where the sensitivity of the mammary gland to OT increased from 8 days post partum to 13 days post partum.

Thirteen cows (76%) had in their first milking after parturition a delay or a complete failure of OT release after teat stimulation as already reported by others (Bruckmaier *et al*. 1992; Schulz & Petzold, 1998), indicating an inhibition in the reflex arc from the udder via hypothalamus to the neurohypophysis. The reason for this inhibition can only be speculated, but the gradual normalisation of the OT release during the following milkings may suggest a learning effect.

Naloxone showed a differential effect on the release of OT: in cows with inhibited OT release and total failure of alveolar milk ejection, the injection of naloxone did not improve the release of OT and the ejection of alveolar milk. Recently we also could not overcome an inhibition of OT release in dairy cows during inexperienced suckling by the application of similar doses of naloxone (Kraetzl *et al*. 2001). Even much higher doses (2 mg/kg) were not effective to restore the OT release from the neurohypophysis and the milk ejection in emotional stress situations like milking in novel surroundings (Wellnitz *et al*. 1997). However, in the cows with initially high or delayed OT release and normal or bimodal milk ejection, the pretreatment with naloxone increased and potentiated the release of OT, as early as during the second milking after parturition. The reaction of these cows is therefore comparable with the response in advanced lactation (Kraetzl *et al*. 1999; Tancin *et al*. 2000).

All morphine-treated cows showed a strong and uniform reaction during a potentially undisturbed milking as recently described by our working group (Kraetzl *et al*. 1999; Tancin *et al*. 2000). The cows were injected with morphine (i.v.) very early postpartum, and all of them reacted with a total inhibition of OT release and in consequence a total failure of alveolar milk ejection, which contrasts with the conclusions of Aurich *et al*. (1993). However, the sensitivity of the mammary OT receptors was obviously not affected, because vaginal stimulation, providing sufficient OT plasma levels in all except one cow, resulted in a spontaneous ejection of alveolar milk. Therefore, vaginal stimulation must be much stronger than teat stimulation for the central release of OT or must act via different reflex arcs. Negoro

et al. (1987) postulated, for the rat, two different afferent pathways for mammary and vaginal stimulation, which can interact. Perhaps, in our case, the vaginal stimulation mimics the calving procedure and a learning of this reflex arc is not necessary, other than the suckling or teat reflex arc. Small bolus injections of 0·1 IU OT, given after the vaginal stimulation, provided physiological OT plasma levels for several minutes and led to a complete ejection of the remaining alveolar milk, indicating again no differences in the mammary sensitivity for oxytocin under opiates.

Naloxone is widely accepted as a general opioid antagonist, although there is some evidence, that its impact on *mu* receptors is stronger than on *kappa* receptors (Bicknell *et al*. 1985; Van de Heijning *et al*. 1991), and the stress-induced inhibition of OT release is mediated primarily via *kappa* receptors (Brown *et al*. 1998), but also via *mu* receptors (Carter & Lightman, 1987; Van de Heijning *et al*. 1991), at least in the rat. On the other hand, there is strong evidence from the rat, that naloxone can abolish a stress-induced inhibition of OT release, and other general opioid antagonists such as naltrexone have no advantage compared with naloxone (Ehrenreich *et al*. 1985). Combined treatment with naloxone and morphine in our experiment demonstrated that the dose of opiate given was effective to inhibit the OT release and alveolar milk ejection and that the antagonist, naloxone, could reverse this effect. As the OT plasma levels after morphine plus naloxone injection were even higher than during an untreated control milking, we suggest that naloxone moreover can effectively suppress a permanent endogenous opioid tone and therefore enhance the release of OT. From the OT plasma levels after vaginal stimulation, which were much higher during M2 than during M7 (103 vs. 15 pg/ml, respectively) in the INH cows, we can again conclude that the applied dose of 300 mg naloxone per cow, which corresponds quite well with the dose given in *in vitro* experiments (Bicknell *et al*. 1985), must have been sufficient to abolish the inhibition of OT release, if opioid dependent. Similar experiments were also done with much higher doses (1 mg/kg BW) and no effect of naloxone (Macuhova *et al*. 2001).

Additionally, there is still a possible role of other mediators like dopamine, noradrenaline, serotonin, GABA and others for the regulation of the OT release from the neurohypophysis (Parker & Crowley, 1992; Blasquez *et al*. 1994; Leshin *et al*. 1995), and finally, a spinal opioid block of the afferent milk ejection reflex arc, as described by Wright (1985) for the rat, is also possible but seems to be unlikely by the i.v. route of the morphine injection. All these speculations merit further investigation.

In conclusion, opiates can also inhibit the central OT release in a very early stage immediately after parturition, but opioids are obviously not dominantly responsible for a disturbed milk ejection in post-partum primiparous cows, as can be seen from the failure of naloxone to restore an inhibited OT release.

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