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Milk removal in familiar and unfamiliar surroundings: concentrations of oxytocin, prolactin, cortisol and *β*-endorphin

By RUPERT M. BRUCKMAIER, DIETER SCHAMS* and JÜRG W. BLUM

Institut für Tierzucht der Universität Bern, CH-3012 Bern, Schweiz
**Institut für Physiologie der Technischen Universität München,*
W-8050 Freising-Weihenstephan, Deutschland

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Summary. Eight cows were machine milked either in an operating theatre or in their familiar barn. During the experiments, milk flow curves were recorded and blood samples were taken for determination of concentrations of oxytocin, prolactin, cortisol and *β*-endorphin. The milking cluster was attached without udder preparation. After cessation of milk flow, air was blown into the vagina for 2 min. When milk flow had stopped again, 1 i.u. oxytocin and finally 10 i.u. oxytocin were injected to remove the remaining milk. After the start of milking, oxytocin remained basal in unfamiliar, but increased in familiar surroundings. Therefore, during normal milking only 9% of total milk was removed in unfamiliar, whereas 79% was available in familiar surroundings. In response to subsequent vaginal stimulation in the operating theatre, oxytocin increased transiently in five cows and 15–71% of the milk was removed in these animals. In the other three cows in the operating theatre, oxytocin remained basal during vaginal stimulation, and no more milk was available. After injection of 1 i.u. oxytocin, 56 and 11%, and after injection of 10 i.u. oxytocin, 13 and 8% of milk was removed in unfamiliar and familiar surroundings respectively. Concentrations of prolactin increased during the course of milking in both treatments. Premilking concentrations of cortisol and *β*-endorphin were elevated in unfamiliar as compared with familiar surroundings. During the course of milking, cortisol increased slightly and *β*-endorphin decreased in unfamiliar, whereas both hormones increased markedly during milking in familiar surroundings. We conclude that disturbed milk removal in unfamiliar surroundings is due to central inhibition of oxytocin release during normal milking and partly also to a response to vaginal stimulation. This blockade is possibly associated with elevated concentrations of *β*-endorphin.

The cisternal milk, which is stored within teat and gland cistern of the mammary gland, can be passively removed by overcoming the teat sphincter barrier. In contrast, the alveolar milk, i.e. the main portion of the stored milk, is fixed by adhesive and capillary forces within alveoli and small ducts, and must be shifted actively into the cisternal cavity during oxytocin (OT)-induced milk ejection. The importance of milk ejection for machine milking has been demonstrated (Schams *et al.* 1984; Gorewit & Gassman, 1985; Mayer *et al.* 1991).

Milk removal can be disturbed under various conditions. Peripheral inhibition of milk ejection, i.e. disturbed action of OT, which is released from the pituitary, was experimentally induced by electroshock and administration of catecholamines and α-adrenergic agonists (Mielke, 1981; Lefcourt & Akers, 1984; Gorewit & Aromando, 1985; Blum *et al.* 1989; Bruckmaier *et al.* 1991), whereas the milking-related OT release was not reduced by electroshock and catecholamine treatments (Blum *et al.* 1989). Exogenous OT could not induce milk ejection after α-adrenergic agonist administration (Bruckmaier *et al.* 1991). In contrast, disturbed milk removal, which sometimes appears in primiparous parturient cows (Bruckmaier *et al.* 1992) and sporadically in older cows without obvious reasons (R. M. Bruckmaier, D. Schams & J. W. Blum, unpublished results), was shown to be due to central inhibition of milk ejection, i.e. lack of or reduced release of OT in response to teat stimulation and milking. This central inhibition of milk ejection can be totally abolished by exogenous OT in physiological amounts (Bruckmaier *et al.* 1992).

A transient reduction of milk yield of dairy cows, presumably due to disturbed milk ejection, was also demonstrated after relocation to new housing systems (Varner *et al.* 1983). Relocations to unfamiliar surroundings often occur in practical farming if animals change their owner. Economic losses and udder health problems due to incomplete milk removal must be expected. The goal of this work was therefore to investigate the existence and the aetiology of disturbed milk removal in unfamiliar surroundings. In addition, the patterns of several hormones during milking, including β-endorphin, which has to our knowledge not been studied before in dairy cows, were recorded in familiar and unfamiliar surroundings.

MATERIALS AND METHODS

Eight cows (five Simmental × Red Holstein, three Swiss Braunvieh) in month 4 of their second to sixth lactation were milked on two succeeding days at 16.00 (10 h after morning milking) in their familiar barn, where they were housed attached in groups of 24 animals, and in an operating theatre. The operating theatre was characterized by bright lighting and tiled walls, whereas in the barn lighting was dim. One cow at a time was taken to the operating theatre only for milking so that the animals could not get accustomed to the new environment. In both locations the same milking equipment was used, incorporating a strain gauge unit permitting continuous milk flow recordings (Schams *et al.* 1984). The milk flow signal (weight gain differentiated by time) was continuously conveyed to a strip chart recorder. In both treatments, the milking cluster was attached without any udder preparation. After milk flow had ceased for 3 min, vaginal stimulation was applied by blowing air repeatedly into the vagina for 2 min. After flow had stopped again, a physiological dosage (1 i.u.) and finally a supraphysiological dosage (10 i.u.) of OT was injected intravenously (i.v.) to remove the remaining milk.

Before the first experiment, an indwelling catheter was inserted into the left jugular vein for collection of blood samples (10 ml) at 1 min intervals from 2 min before the start of milking until the i.v. injection of 10 i.u. OT at the end of milking. Blood samples were treated with heparin (50 USP units/ml blood) to prevent coagulation, cooled on ice and centrifuged immediately after the experiment at 1500 ***g*** for 20 min. The plasma was stored at −20 °C in several portions for determination of hormones.

Concentrations of oxytocin, prolactin (PRL) and cortisol were determined by radioimmunoassay as described previously by Schams (1983), Bruckmaier *et al.* (1992) and Blum *et al.* (1985) respectively. Concentrations of β-endorphin were determined radioimmunologically using a kit (Incstar Corporation 46065, Stillwater,

Table 1. *Absolute and relative milk yields in unfamiliar and familiar surroundings during normal milking, and in response to vaginal stimulation and exogenous oxytocin*

(Values are means ± SEM for $n = 8$)

Unfamiliar surroundings					
Period...	Normal milking	Vaginal stimulation	1 i.u. oxytocin	10 i.u. oxytocin	Total
Milk yield, kg	1·2±0·3	2·8±1·3	7·2±0·8	1·6±0·2	12·8±0·5
Relative milk yield, %	9±2	22±9	56±8	13±2	100

Familiar surroundings					
Period...	Normal milking	Vaginal stimulation	1 i.u. oxytocin	10 i.u. oxytocin	Total
Milk yield, kg	10·3±0·7	0·3±0·2	1·4±0·3	1·0±0·4	13·0±0·6
Relative milk yield, %	79±3	2±2	11±3	8±1	100

MN 55082, USA). The method has been shown to be suitable for the determination of β-endorphin in cattle (Bruckmaier *et al.* 1992). Within-assay coefficients of variation were < 10% for all radioimmunoassays employed.

Statistical analyses

For statistical evaluation and presentation of hormone results, mean values for periods of 2 min were calculated before the start of milking (two blood samples, phase I), from 1 min after the start of milking (three blood samples, phase II), from 1 min after the start of vaginal stimulation (three blood samples, phase III) and 2 min before the injection of 10 i.u. OT at the end of milking (three blood samples, phase IV). Values are presented as means ± SEM. Changes during the course of experiment and differences between treatments were tested for significance ($P < 0·05$) by means of Wilcoxon's signed rank test employing the SAS program package (release 6.04) (SAS, 1990).

RESULTS

Milk yields

Mean total yields (Table 1) at experimental milkings were similar in unfamiliar and familiar surroundings. As shown in Table 1 and Fig. 1, in the operating theatre only a small amount of the total milk was obtained during normal milking, whereas most of the milk was removed in the barn. In response to subsequent vaginal stimulation, in the operating theatre no milk was obtained in three animals and 15–71% could be removed in five animals (mean 22±9%). In the barn, small amounts of the total milk were obtained in response to vaginal stimulation. After injection of 1 i.u. OT, the main portion of milk was removed in the operating theatre and a little more milk was obtained in the barn. After injection of 10 i.u. OT, another small amount of milk was removed in both treatments.

Oxytocin concentrations

Plasma concentrations of OT (Fig. 1, Table 2) before milking (phase I) were similarly low in unfamiliar and familiar surroundings. After the start of milking (phase II), concentrations of OT remained basal in unfamiliar but increased markedly in familiar surroundings. During vaginal stimulation (phase III), OT increased only moderately and transiently in five cows and not at all in three cows (from which no milk was removed during this period) in the operating theatre. In the

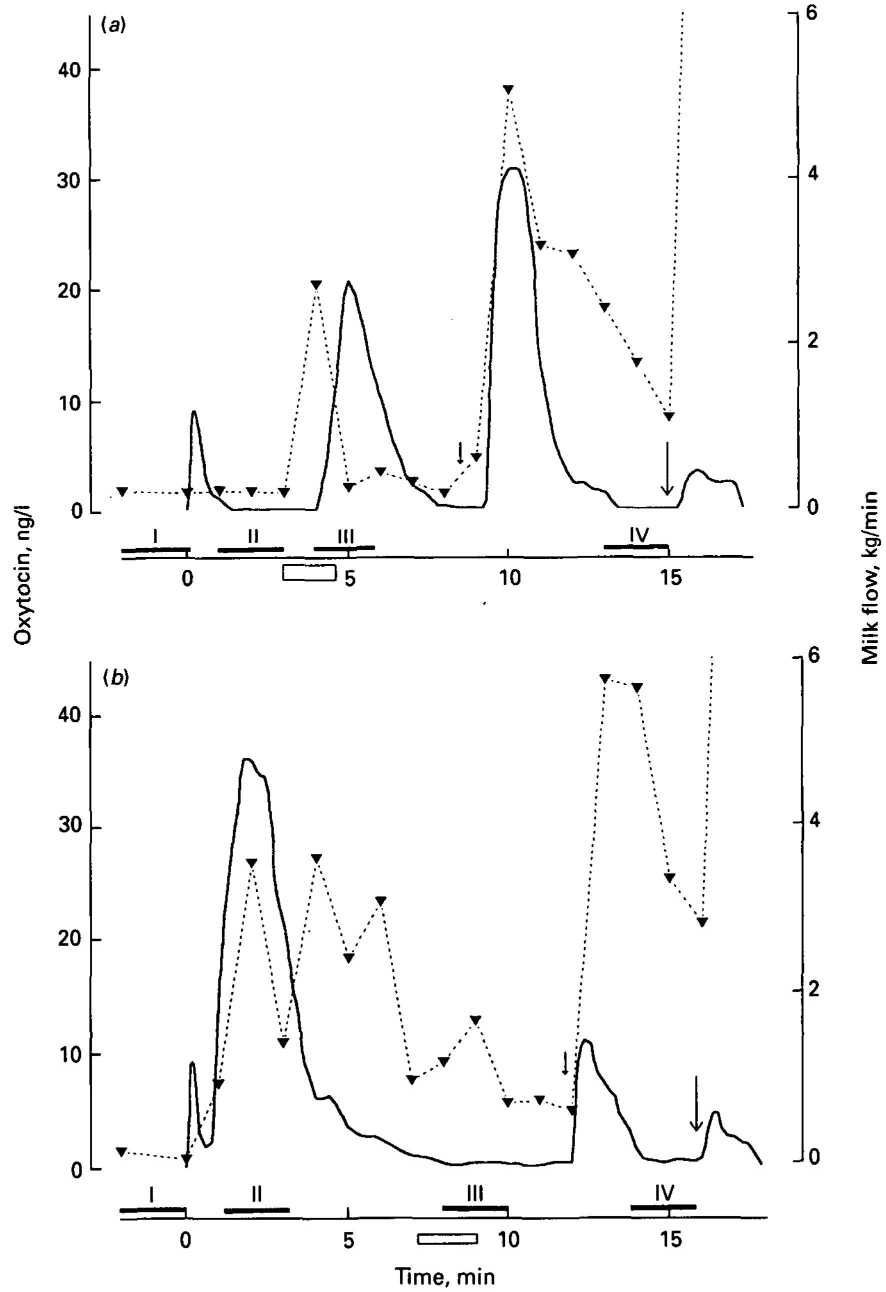

Fig. 1. Concentration of oxytocin (▼) and milk flow rate (——) in one cow before and during milking in (*a*) unfamiliar and (*b*) familiar surroundings. ▭, Vaginal stimulation; 0, start of milking; small arrow, i.v. injection of 1 i.u. oxytocin; large arrow, i.v. injection of 10 i.u. oxytocin. ▬, Phases I–IV (see text).

barn, OT remained elevated or increased slightly during vaginal stimulation. After injection of 1 i.u. OT (phase IV), blood concentrations of OT were similarly high in unfamiliar and familiar surroundings. After injection of 10 i.u. OT, plasma concentrations of OT increased beyond the range of the standard curve of the radioimmunoassay (to 400–500 ng/l) in both groups.

Table 2. *Hormone levels in blood plasma before milking (phase I), after the start of milking (phase II), in response to vaginal stimulation (phase III) and at the end of milking (phase IV) in unfamiliar and familiar surroundings*

(Values are means ± SEM for $n = 8$)

Unfamiliar surroundings

Phase…	I	II	III	IV
Oxytocin, ng/l	1·5±0·3^{a}	2·1±0·4^{a}	3·7±0·7^{a}	27·1±0·8^{b}
Prolactin, μg/l	84±17^{a}	83±15^{a}	118±33^{b}	206±49^{c}
Cortisol, μg/l	8·7±0·7^{a}*	8·9±0·8^{a}	8·4±1·0^{a}	11·2±1·1^{b}
β-Endorphin, ng/l	16·1±1·9^{a}*	13·7±1·6^{ab}	13·3±1·3^{b}	11·7±1·1^{b}

Familiar surroundings

Phase…	I	II	III	IV
Oxytocin, ng/l	1·5±0·2^{a}	20·9±5·1^{b}	26·3±10·6^{b}	30·9±3·3^{c}
Prolactin, μg/l	68±17^{a}	106±28^{b}	286±49^{c}	297±36^{c}
Cortisol, μg/l	3·8±0·6^{a}	4·5±0·6^{a}	6·2±0·9^{b}	7·7±0·9^{b}
β-Endorphin, ng/l	8·3±0·8^{a}	8·3±1·0^{a}	8·6±1·0^{a}	10·4±1·3^{b}

[a,b,c] Means of phases without common subscript letters are significantly different within row and treatment ($P < 0{\cdot}05$).

* Means of phase I within trait are significantly different between treatments ($P < 0{\cdot}05$).

Prolactin concentrations

Concentrations of PRL (Table 2) before milking (phase I) were slightly but not significantly higher in unfamiliar than in familiar surroundings. During phase II, PRL remained basal or increased slightly in some animals in unfamiliar surroundings, whereas in familiar surroundings, PRL increased markedly during phase II. In response to vaginal stimulation (phase III), PRL increased moderately in the operating theatre and markedly in the barn, and increased further in both treatments until the end of milking (phase IV).

Cortisol concentrations

Basal concentrations of cortisol (phase I) were significantly elevated in unfamiliar as compared with familiar surroundings (Table 2). During the course of milking, cortisol increased continuously in familiar surroundings, whereas in unfamiliar surroundings the concentrations remained high and unchanged until phase III and finally increased further to the end of milking (phase IV).

β-Endorphin concentrations

Basal concentrations of β-endorphin (phase I) were significantly higher in unfamiliar than in familiar surroundings (Table 2). β-Endorphin values in the barn remained stable until phase III and increased slightly ($P < 0{\cdot}05$) at the end of milking. In contrast, β-endorphin in the operating theatre decreased during the whole course of milking.

DISCUSSION

Basal concentrations (phase I) of OT were similar in unfamiliar and familiar surroundings and in a range found previously (Schams *et al.* 1984; Mayer *et al.* 1991; Bruckmaier *et al.* 1992). Basal concentrations of PRL were relatively high in both treatments, probably because the experiments were conducted during the summer (June) (Schams & Reinhardt, 1974). Basal concentrations of cortisol and β-endorphin were significantly higher and those of PRL tended to be higher in unfamiliar than in familiar surroundings. Obviously, relocation to unfamiliar surroundings was a considerable emotional stress for the animals. Elevated cortisol concentrations in cows were previously observed after relocation (Varner *et al.* 1983), during transportation (Bremel & Gangwer, 1978) and during isolation from companion animals (Willett & Erb, 1972) as a reaction to and an indicator for stress conditions. Adrenocorticotropin (ACTH), i.e. the releasing factor for cortisol, and β-endorphin are derived from a common precursor in the pituitary (Eipper & Mains, 1980) and were shown to be released concomitantly in stressed rats (Guillemain *et al.* 1977). Furthermore, the administration of opioid peptides produced negative feedback for the release of cortisol in cows (Nanda *et al.* 1992). Thus it is likely that there is also a common regulation of ACTH and β-endorphin release in cows, and the simultaneously elevated premilking concentrations of β-endorphin and cortisol in unfamiliar surroundings are therefore not surprising. Furthermore, it has been suggested that endogenous opioid concentrations modulate PRL release in several species (Barb *et al.* 1991). It is possible that the elevated basal concentrations of β-endorphin were responsible for higher concentrations of PRL in unfamiliar surroundings. This is consistent with the finding that administration of the opioid antagonist naloxone suppresses PRL concentrations in cows (Gregg *et al.* 1986).

During normal milking, i.e. tactile stimulation by the milking cluster, OT was released in a normal manner in the barn and was succeeded by alveolar milk ejection (Schams *et al.* 1984; Mayer *et al.* 1991; Bruckmaier *et al.* 1992). Therefore the main portion of milk stored within the udder was removed during this period. Owing to lack of previous stimulation, the milk flow curve in the barn followed a bimodal pattern, demonstrating delayed milk ejection after the start of milking (Mayer *et al.* 1984). The advantage of milking without premilking stimulation in this experiment was the possibility of estimating the 'pre-ejection' milk fraction (cisternal milk) from the first peak of the bimodal milk flow curve. This fraction could be compared with the milk fraction removed during normal milking in unfamiliar surroundings. Indeed, OT release during normal milking in the operating theatre was inhibited, milk ejection did not occur, and only a fraction roughly similar to that of the first peak in the barn was removed. This fraction is thought to be the cisternal milk. Whereas an inhibitory action of elevated concentrations of cortisol on OT release is not likely (Mayer & Lefcourt, 1987), inhibitory effects of endogenous opioid peptides on OT release and milk ejection have been reported previously in rats (Bicknell *et al.* 1988), rabbits (Tindal & Blake, 1986), and mice (Haldar & Bade, 1981). It is possible that the lack of oxytocin release during normal milking in unfamiliar surroundings was also due to high β-endorphin concentrations within the hypothalamus–pituitary system, which are reflected by elevated β-endorphin concentrations in the peripheral blood.

In response to vaginal stimulation, sufficient OT was released to induce milk ejection in primiparous cows with disturbed milk removal and more OT than in response to teat stimulation was released in normal cows (Bruckmaier *et al.* 1992). In this investigation, only a moderate and transient amount of OT or none at all was released during vaginal stimulation in the operating theatre, demonstrating a much stronger inhibition of OT release in unfamiliar surroundings than in primiparous parturient cows. Therefore, only a small amount or no milk was removed in response to vaginal stimulation.

After injection of OT, milk removal was normal in the operating theatre, and the milk flow rates were comparable to those in the barn. Peripheral inhibition of milk removal, which occurs after catecholamine administration together with normal release of OT and lack of mammary action of endogenous and exogenous OT (Blum *et al.* 1989; Bruckmaier *et al.* 1991), could therefore be excluded in this investigation. Milk yield at the end of milking, i.e. after injections of OT, was similar in unfamiliar and familiar surroundings in this study. A reduction in milk synthesis and secretion, as was observed during long-term elevated corticoid concentrations such as those that occur in *Escherichia coli* endotoxin mastitis (Varner *et al.* 1983; Bruckmaier *et al.* 1993) could be excluded.

During the course of milking, concentrations of PRL and cortisol increased only moderately in unfamiliar surroundings, whereas those of β-endorphin actually decreased. This was possibly due to the already elevated concentrations of PRL, cortisol and β-endorphin before milking. Nevertheless, because there is some PRL and cortisol released during milking, the afferent pathways responsible for the release of PRL and cortisol seem to be still intact, although the missing or reduced release of PRL during early milking indicates reduced action of the milking stimulus. In familiar surroundings, the release of PRL and cortisol during milking occurred as previously demonstrated (Koprowski & Tucker, 1973; Reinhardt & Schams, 1974; Bruckmaier *et al.* 1992). Increasing concentrations of β-endorphin during milking in the barn were to the best of our knowledge demonstrated for the first time in dairy cows. Increasing β-endorphin concentrations, probably of hypothalamic origin, were demonstrated during suckling in ewes (Gordon *et al.* 1987). β-Endorphin is thought to be responsible for delayed cyclic activity in humans (Quigley & Yen, 1980; Ellingboe *et al.* 1982) and in cattle (Myers *et al.* 1989; Barb *et al.* 1991). There may be an association of increasing β-endorphin concentration during milk removal and suckling- or milking-induced suppression of cyclic activity in cattle (Williams, 1990).

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