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Inhibition of oxytocin release during repeated milking in unfamiliar surroundings: the importance of opioids and adrenal cortex sensitivity

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Summary. The aim of this study was to test if the opioid antagonist naloxone has a beneficial effect on normalization of oxytocin (OT) release during repeated milking of cows in unfamiliar surroundings. One control milking without naloxone treatment in all cows was performed in the familiar parlour. For four successive evening milkings, cows were transported to, and milked in, the operating theatre of the research station without (control group) or with naloxone administration (1 mg/kg BW) (naloxone group) before milking. After cessation of spontaneous milk flow, but not before 3 min of milking, vaginal stimulation was applied for 2 min. After milk flow ceased again, 10 IU of OT was injected intravenously to remove the remaining milk including residual milk. Milk flow was recorded continuously and blood samples were collected via a jugular vein cannula at 1-min intervals from 1 min before the start of milking until i.v. injection of OT. The inhibition of milk ejection and its normalization during repeated milking in unfamiliar surroundings was not influenced by naloxone treatment. Concentrations of cortisol and β-endorphin during control milking and all relocations were similar in the naloxone and control groups, although their concentrations were higher after relocations than in the control. Therefore, a role of endogenous opioids in the inhibition of milk ejection in unfamiliar surroundings could not be demonstrated. In addition, the effect of exogenous $ACTH_{1-24}$ (8 IU, i.v.) on the release of cortisol related to the response of cows milked in unfamiliar surroundings was studied. Cows with totally inhibited milk ejection in response to vaginal stimulation during milking after first relocation had numerically, but not significantly lower cortisol levels ($8{\cdot}8 \pm 3{\cdot}4$ ng/ml; AUC/min) in response to ACTH than did cows with at least partial milk ejection ($38{\cdot}7 \pm 12{\cdot}9$ ng/ml). Thus animals with a higher adrenal response to ACTH seemed to have less severe inhibition of milk ejection.

Keywords: Oxytocin, milking in unfamiliar surroundings, opioids, adrenal cortex, cows

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In practical dairying, cows may be exposed to various emotionally stressful situations (such as calf removal, transport, relocation to new surroundings). During milking in unfamiliar surroundings, the milk ejection reflex can be lacking owing to insufficient oxytocin (OT) release accompanied by increased levels of cortisol and β-endorphin (Bruckmaier *et al.* 1993). But with repeated milking in unfamiliar surroundings, OT release and milk ejection gradually normalized, with simultaneously decreasing β-endorphin and cortisol concentrations (Bruckmaier *et al.* 1996).

Exogenous cortisol has been shown to exert no inhibiting effect on milk ejection in cows (Mayer & Lefcourt, 1987). However, endogenous opioids were shown to inhibit OT secretion in response to various stimuli in rats and pigs (Bicknell & Leng, 1982; Leng *et al.* 1988; Lawrence *et al.* 1992) and exogenous morphine inhibited milking-related OT release in dairy cows (Tančin *et al.* 2000*a*). Administration of the opioid antagonist naloxone abolished inhibition of OT release in the rat (Pumford *et al.* 1991, Russell *et al.* 1993) and also abolished the inhibitory effect of morphine during milking in cows (Tančin *et al.* 2000*a*). The repeated administration of naloxone to rats resulted in a large release of OT (Caron *et al.* 1998). However, naloxone did not abolish inhibition of OT release during milking in unfamiliar surroundings (Wellnitz *et al.* 1997). Nevertheless, a partial stimulatory effect of naloxone on OT release during milking in familiar surroundings indicates a role of endogenous opioids in the regulation of OT release (Tančin *et al.* 2000*a*). Thus the importance of the opioid system in the central inhibition of oxytocin release during milking under stress conditions in dairy cows is still unclear.

The response of the hypothalamic-pituitary-adrenal (HPA) axis to its stimulation can characterize the individual sensitivity of animals to stress (von Borell & Ladewig, 1992; Anisman *et al.* 1998). Therefore, a possible relationship between the reaction of individual cows to novel surroundings and the sensitivity of the adrenal cortex to exogenous ACTH could help to explain the mechanisms of central inhibition of OT release.

The aim of this study was to test if naloxone can accelerate the normalization of OT release in cows repeatedly milked in unfamiliar surroundings. In addition, we tested the hypothesis that cortisol response to ACTH challenge is related to the severity of inhibition of milk ejection in unfamiliar surroundings.

Materials and methods

Animals

Twelve Brown Swiss dairy cows were used. They were in weeks 10–52 of their second to sixth lactation with a daily milk production from 12 to 31 kg. The cows were kept in loose housing and had free access to a mixed ration providing energy and nutrients for the production of 22 kg milk and received additional concentrate according to their individual production levels.

Experimental protocol

Cows were milked twice daily at 05·00 and 16·00. Experiments were performed only during the evening milking time, whereas morning milkings were performed in the milking parlour without experimental treatment. On the first day of the experiment all cows were milked without naloxone treatment in the familiar milking parlour (control milking). On the next 4 successive days, cows were milked in the operating theatre of the research station. Six cows were milked without treatment

(control group) and the six cows in the naloxone group were given naloxone (1 mg/kg BW, i.v.) 10 min before the start of all milkings in the operating theatre. Prior to milking, cows were transported from the stable to the operating theatre in a truck. Naloxone was administered in the truck before unloading. Cows were moved from the truck to the operating theatre immediately before milking. Milking started after a 1-min manual prestimulation and milk flow was recorded using a mobile system (Lactocorder®). When milk flow had decreased below 0·2 kg/min, but not before 3 min of milking (period of normal milking), vaginal stimulation was applied (by blowing air repeatedly into the vagina) for 2 min (period of vaginal stimulation). After milk flow again decreased below 0·2 kg/min, 10 IU of OT was injected intravenously to remove the remaining milk, including residual milk. After the end of each milking, cows were returned to the herd.

Cows were fitted with permanent catheters in one jugular vein at least 6 h before the first experimental milking. Blood samples were collected at 1-min intervals from 1 min before start of milking until i.v. injection of OT. Blood samples were anticoagulated with EDTA, cooled on ice, centrifuged at 1500 ***g*** for 15 min and the plasma was stored at −20 °C until analysed.

The day after the end of milking experiments, ten of the cows were given 8 IU (i.v.) of synthetic $ACTH_{1-24}$ 2–3 h after morning milking. Blood was collected at −60, −45, −30, −15, 0, 15, 30, 45, 60, 75 and 90 min before and after ACTH injection for cortisol determination. For technical reasons, the jugular vein catheters could not be used in two cows (one from each group).

Hormone analyses

Plasma OT concentration was determined by radioimmunoassay (Schams, 1983). Cortisol concentration was measured by a competitive enzyme immunoassay (Sauerwein *et al.* 1991). β-endorphin was measured by a competitive enzyme immunoassay using a kit (EIAH-8609; Peninsula Laboratories, Inc., St Helens, Merseyside, UK).

Statistical analysis

For statistical evaluation of OT concentrations, mean values for basal levels, for the change during prestimulation (Δ −1 to 0 min), for the period from start of prestimulation until 2 min of milking (Δ AUC/min −1 to 2 min), for levels of OT at the start of vaginal stimulation (V0) and for a period of 3 min from the start of vaginal stimulation (Δ AUC/min V0 to V3 min) were calculated. Milk yields were divided into three fractions: milk obtained before the start of vaginal stimulation (normal milking), in response to vaginal stimulation (vaginal stimulation) and in response to OT injection (10 IU OT, i.v.). Cortisol levels were determined in blood samples taken at −1 min (start of prestimulation), at 0 min (start of milking) and at 3 and 6 min of milking. Calculations were performed for basal mean values and for the period from −1 min to 6 min of milking (AUC −1 to 6 min). β-Endorphin levels were determined in one blood sample taken at −1 min and calculations were performed for the pre-milking mean value. Data are presented as means with SEM. A repeated measures analysis of variance was calculated using the MIXED procedure of the SAS program package (SAS 6.11, 1995). The animal was the repeated subject. Significant differences ($P < 0{\cdot}05$) within groups between relocation numbers and between groups within relocation numbers were localized by using Bonferroni's t test based on least square means. Student's test was used to compare pre-milking β-endorphin levels

and cortisol levels in response to ACTH challenge (AUC/min 0–60 min) between two groups of cows with either total inhibition of milk ejection (< 10 % of total milk obtained) or with only partial or no inhibition of milk ejection during normal milking or in response to vaginal stimulation during the first relocation. Pearson's coefficient of correlation was calculated between various traits for each relocation separately and also between cortisol release in response to ACTH (AUC/min 0–60 min) and OT release in response to vaginal stimulation during milking after first relocation by using a SAS CORR procedure (SAS 6.11, 1995).

RESULTS

Milk yield

Milk yields are shown in Table 1. During control milking total milk yield was not different between control and naloxone groups. Most of the milk, 79 ± 3 %, was obtained during normal milking. Only a small percentage, or no milk (0–8 %), was obtained in response to subsequent vaginal stimulation in ten animals. In two animals, vaginal stimulation induced removal of higher amounts of milk (17 and 20 % of total milk respectively). The amount of milk removed in response to OT injection ranged from 6 to 26 %.

During the first milking in unfamiliar surroundings, the fraction of milk removed during normal milking was significantly and similarly reduced in both groups compared with control milking. During subsequent relocations, the fraction of milk yield obtained during normal milking slowly increased, but the increases were significant only during the last two relocations compared with the first relocation in the control group. In the control and naloxone groups, the fractions of milk removed during normal milking were similar but with considerable variation between individual cows.

The fractions of milk obtained in response to vaginal stimulation were higher during all relocations compared with control milkings in both groups, but the differences were significant only for the control group during the first two relocations compared with control milking in the parlour. No differences were observed between groups within relocation number.

The main fraction of milk, during all relocations, was removed by exogenous OT, in both the control and naloxone groups.

Plasma concentration of oxytocin

Plasma OT concentrations are shown in Table 2. Basal plasma OT concentrations were similar during all relocations and control milkings with no significant differences between the control and naloxone groups. The increment of plasma OT concentrations from the start of prestimulation until 2 min of milking (Δ AUC/min −1 to 2 min) was not significantly lower at first milking in unfamiliar surroundings for both groups and remained low during subsequent relocations in the control group. The significant differences in OT release between the naloxone and control groups observed at normal milking during the last relocation, and on the start of vaginal stimulation during the last two relocations, were mainly due to one cow in the naloxone group that released more OT (V0 min 43 pg/ml during the third relocation; V0 min 21·8 pg/ml, Δ −1 to 0 min 125·5 pg/ml, Δ AUC/min -1 to 2 min 88·2 pg/ml during the last relocation) than the other animals. The increments of OT release in

Table 1. *Milk fractions during milking in familiar and unfamiliar surroundings*

(Values are means ± SEM for $n = 6$)

	Naloxone group					Control group				
		Relocation no.					Relocation no.			
Period	Control	1	2	3	4	Control	1	2	3	4
Normal milking (%)	77 ± 4^{a}	20 ± 12^{b}	30 ± 15^{b}	38 ± 16^{b}	38 ± 16^{b}	80 ± 4^{a}	7 ± 3^{b}	16 ± 7^{bc}	27 ± 11^{c}	29 ± 14^{c}
Vag. stimulation (%)	6 ± 3	21 ± 11	21 ± 10	11 ± 8	17 ± 8	5 ± 3^{a}	37 ± 14^{b}	31 ± 12^{bc}	19 ± 11^{ca}	18 ± 12^{ca}
10 i.u. oxytocin (%)	17 ± 2^{a}	59 ± 14^{b}	49 ± 16^{b}	51 ± 18^{b}	45 ± 16^{b}	15 ± 3^{a}	56 ± 14^{b}	53 ± 12^{b}	54 ± 11^{b}	53 ± 14^{b}
Total milk yield (kg)	$10{\cdot}7 \pm 1{\cdot}3$	$10{\cdot}3 \pm 1{\cdot}1$	$10{\cdot}3 \pm 1{\cdot}2$	$9{\cdot}6 \pm 0{\cdot}9$	$8{\cdot}8 \pm 0{\cdot}8$	$10{\cdot}7 \pm 1{\cdot}9$	$10{\cdot}0 \pm 2{\cdot}1$	$9{\cdot}3 \pm 2{\cdot}0$	$8{\cdot}9 \pm 1{\cdot}7$	$9{\cdot}3 \pm 1{\cdot}7$

[a,b,c] Means of control milkings and relocations within treatment without a common superscript letter were significantly different ($P < 0{\cdot}05$).
No significant differences were found between groups.

Table 2. *Oxytocin, cortisol and β-endorphin concentrations during milkings in familiar and unfamiliar surroundings*

(Values are means ± SEM for $n = 6$)

	Naloxone group					Control group				
	Relocation no.					Relocation no.				
	Control	1	2	3	4	Control	1	2	3	4
Oxytocin pg/ml										
−1 min	2·6 ± 0·7	2·8 ± 0·9	2·9 ± 0·6	3·1 ± 0·4	3·5 ± 0·8	2·0 ± 0·3	1·7 ± 0·4	2·2 ± 0·2	2·0 ± 0·5	2·5 ± 0·7
Δ −1 to 0 min	7·2 ± 1·4^{a}	1·6 ± 0·5^{a}	5·6 ± 1·8^{a}	4·3 ± 1·9^{a}	24·9 ± 20·2^{b}*	1·2 ± 0·6	1·4 ± 0·6	0·6 ± 0·4	2·3 ± 0·9	3·0 ± 2·0*
Δ AUC/min −1 to 2 min	6·9 ± 1·7^{a}	3·2 ± 2·0^{a}	6·2 ± 2·6^{ab}	6·5 ± 3·1^{ab}	18·2 ± 14·1^{b}*	9·3 ± 6·7	1·1 ± 0·3	0·8 ± 0·5	2·0 ± 0·9	1·9 ± 1·1*
V0 min	8·6 ± 2·3^{ac}	5·4 ± 2·5^{a}	9·0 ± 3·0^{a}	15·3 ± 6·9^{b}*	12·9 ± 3·8^{bc}*	10·6 ± 3·1^{a}	2·3 ± 0·4^{b}	2·6 ± 0·4^{b}	3·9 ± 1·0^{b}*	4·2 ± 0·7^{b}*
Δ AUC/min V0 to V3 min	1·6 ± 0·7^{a}	17·3 ± 9·2^{b}	10·3 ± 5·6^{ba}	9·4 ± 8·3^{ba}	58 ± 3·1^{a}	3·4 ± 1·8	5·0 ± 1·7	9·4 ± 4·3	6·8 ± 1·8	3·9 ± 1·5
Cortisol ng/ml										
−1 min	2·4 ± 0·9^{a}	17·1 ± 2·9^{b}	16·8 ± 2·3^{b}	14·8 ± 4·1^{b}	16·8 ± 4·1^{b}	3·7 ± 0·8^{a}	22·6 ± 5·4^{b}	22·9 ± 6·1^{b}	20·4 ± 5·3^{bc}	14·5 ± 4·1^{c}
AUC −1 to 6 min	19·9 ± 5·5^{a}	142·7 ± 20·2^{b}	126·7 ± 20·1^{b}	119·0 ± 22·7^{b}	118·9 ± 29·9^{b}	29·5 ± 7·9^{a}	172·8 ± 44·0^{b}	178·8 ± 46·5^{b}	157·2 ± 46·1^{b}	106·4 ± 24·2^{c}
β-endorphin pg/ml										
−1 min	117·1 ± 18·3^{a}	184·1 ± 28·6^{b}	141·2 ± 18·0^{ac}	153·2 ± 16·7^{bc}	136·1 ± 15·9^{ac}	110·5 ± 21·7^{a}	140·1 ± 13·9^{ab}	139·9 ± 33·1^{ab}	159·4 ± 30·6^{b}	115·7 ± 33·1^{a}

−1 start of manual teat stimulation.
0 start of milking.
V0 start of vaginal stimulation.
^{a,b,c} Means of control milkings and relocations within treatment without a common superscript letter were significantly different ($P < 0·05$).
* Mean of the naloxone group is significantly ($P < 0·05$) different from mean of control group within relocation number.

response to vaginal stimulation after normal milking tended to be higher during all relocations compared with control milking in the parlour in both groups; the difference was significant during the first relocation for the naloxone group. There were no significant differences in OT release in response to vaginal stimulation between groups within relocation number.

Plasma concentration of cortisol

Plasma cortisol concentrations are shown in Table 2. Basal (−1 min) and milking (AUC −1 to 6 min) cortisol concentrations were significantly elevated in all relocations compared with control milkings. The increase of cortisol release significantly diminished during the last relocation compared with the first two (basal level) and three relocations (AUC −1 to 6 min) in the control group, but there were no significant differences between relocations in the naloxone group. Release of cortisol did not differ between treatments within relocation number.

Plasma concentration of β*-Endorphin*

Plasma pre-milking β-endorphin levels are shown in Table 2. β-Endorphin levels were elevated during relocations compared with controls, the differences being significant during the first relocation for the naloxone group and in the last two relocations for the control group. β-Endorphin release did not differ between the naloxone and control groups during control milking, and at all relocations.

β-Endorphin level during the first milking in unfamiliar surroundings was significantly lower ($P < 0{\cdot}05$) in cows with inhibited milk ejection during normal milking or in response to vaginal stimulation (only $6{\cdot}6 \pm 0{\cdot}7$ % of total milk yield before OT administration) during the first relocation ($114{\cdot}0 \pm 3{\cdot}7$ pg/ml), than in cows that released more milk ($60{\cdot}8 \pm 8{\cdot}3$ % of total milk) ($186{\cdot}2 \pm 19{\cdot}9$ pg/ml). β-Endorphin levels tended to be already lower ($P = 0{\cdot}075$) during control milking in the parlour in cows with total inhibition of milk ejection during subsequent relocation ($80{\cdot}0 \pm 10{\cdot}0$ pg/ml) than in cows with more milk spontaneously available ($130{\cdot}7 \pm 17{\cdot}0$ pg/ml).

Correlations

A positive correlation was found during the first relocation between the pre-milking β-endorphin level and OT released after prestimulation (at 0 min) ($r = 0{\cdot}59$, $P < 0{\cdot}05$), during normal milking (AUC/min −1 to 2 min) ($r = 0{\cdot}54$, $P = 0{\cdot}07$) and in response to vaginal stimulation (AUC/min V0 to V3 min) ($r = 0{\cdot}86$, $P < 0{\cdot}01$). A positive correlation during the first relocation was also found between cortisol (AUC −1 to 6 min) and the amounts of milk obtained before OT injection ($r = 0{\cdot}57$, $P < 0{\cdot}05$).

ACTH challenge

Peak cortisol concentrations were reached at 30 min after ACTH administration. Cortisol concentrations (AUC/min 0–60 min) were positively correlated only with the OT release in response to vaginal stimulation during the first milking in unfamiliar surroundings ($r = 0{\cdot}58$, $P = 0{\cdot}076$). The cows with inhibited milk ejection during normal milking, and in response to vaginal stimulation (only $7{\cdot}1 \pm 0{\cdot}8$ % of milk available in response to vaginal stimulation) during milking, after first the relocation, had nonsignificantly ($P = 0{\cdot}179$) lower cortisol levels (AUC/min 0–60 min) ($8{\cdot}8 \pm 3{\cdot}4$ ng/ml) in response to ACTH than did cows that released more milk ($58{\cdot}1 \pm 9{\cdot}1$ % of milk) ($38{\cdot}7 \pm 12{\cdot}9$ ng/ml).

discussion

Naloxone had no effect on basal OT concentrations before milking as was previously reported in cows (Wellnitz *et al.* 1997; Tančin *et al.* 2000*a*), goats (Seckl & Lightman, 1987) and mares (Aurich *et al.* 1996).

During normal milking in the parlour, prestimulation and milking induced continued OT release during the entire milking, as expected (Bruckmaier *et al.* 1994). Therefore the main portion of milk stored within the udder could be removed during the period before vaginal stimulation. Subsequent vaginal stimulation slightly increased OT release, but no milk, or only a small percentage, could be removed additionally (except in two cows), because the udder was already emptied.

During the first milking in unfamiliar surroundings, plasma concentrations of OT only slightly increased or remained at baseline levels during normal milking in both groups, as reported previously (Bruckmaier *et al.* 1993, 1996; Wellnitz *et al.* 1997). Therefore only a part of stored, or even no milk, was available. One animal in the naloxone group showed undisturbed milk ejection during the first milking in unfamiliar surroundings.

OT release and, hence, milk ejection also did not differ between groups during the second relocation. There were significant differences of OT release during the third (in V0 min) and fourth relocations (during normal milking and V0 min) but these differences were due to high OT values in one cow in the naloxone group. In this cow, during the first relocation most of the milk was obtained in response to vaginal stimulation, but was obtained during normal milking in subsequent relocations.

Vaginal stimulation has been found to be a more potent stimulus for OT release than teat stimulation (Schams *et al.* 1982; Bruckmaier *et al.* 1992). In response to vaginal stimulation, sufficient OT was released to induce milk ejection in primiparous cows with disturbed milk removal (Bruckmaier *et al.* 1992). But only a moderate and transient release of OT, or none at all, was observed in response to vaginal stimulation during milking in unfamiliar surroundings (Bruckmaier *et al.* 1993). Naloxone administration augmented OT release in response to vagino-cervical stimulation in goats (Seckl & Lightman, 1987) and in mares (Aurich *et al.* 1996). In our experiment, individually different reactions of cows were observed in response to vaginal stimulation. The increment of OT release did not differ in response to vaginal stimulation between the naloxone and control groups. Therefore, no effect of naloxone on OT release in response to vaginal stimulation during repeated milking in unfamiliar surroundings could be demonstrated.

During our experiment, most cows had disturbed milk ejection until the fourth relocation. In a previous investigation, OT release and milk yield had already normalized after the second relocation and were normal after six relocations when every milking was performed in the operating theatre (Bruckmaier *et al.* 1996). Whether cows are relocated for each milking, or only once daily as in this experiment, may be important for the course of normalization of milk ejection, i.e. adaptation to a novel milking environment is delayed if milking is partly performed in the familiar surroundings.

Milking in unfamiliar surroundings, accompanied with elevated concentrations of cortisol and β-endorphin, was a considerable emotional stress for the animals (Bruckmaier *et al.* 1996). Elevated cortisol concentrations in cows were previously observed after relocation (Varner *et al.* 1983) and during transport (Bremel & Gangwer, 1978) as a reaction to, and as an indicator of, stress. β-Endorphin and ACTH are derived from a common precursor in the pituitary (propriomelanocortin)

(Eipper & Mains, 1980) and they were shown to be released concomitantly in stressed rats (Guillemain *et al*, 1977). Elevated β-endorphin levels at the start of milking in unfamiliar surroundings were also observed in this study, with no differences between the naloxone and control groups. Surprisingly, β-endorphin levels during the first milking in unfamiliar surroundings were lower in cows with total inhibition of milk ejection during all relocations compared with cows that showed OT release in response to milking or after vaginal stimulation.

Basal and milking levels of cortisol were significantly elevated during all relocations for both treatments in our experiment. Cortisol levels decreased significantly during the fourth relocation in the control group, but not in the naloxone group. Cortisol release in cows has been shown to be under opioid control. Naloxone stimulated the release of cortisol in cows under normal (Nanda *et al*. 1992) or stress conditions (Nanda *et al*. 1989) and during milking in cows (Tančin *et al*. 2000*b*). No effect of naloxone could be demonstrated in this study. However, a clear stimulatory effect of naloxone on cortisol release in transported cows was observed 30–45 min after administration. Therefore, nonsignificant changes of cortisol levels during all relocations in the naloxone group could be considered to be an effect of naloxone.

Administration of naloxone was not able to reduce inhibition and accelerate normalization of milk ejection during normal milking or in response to vaginal stimulation of cows, after several days of repeated relocation to unfamiliar surroundings. Possibly, naloxone could not reach the OT neurones (blood-brain barrier). However, naloxone potentiated OT release in cows during normal milking (Tančin *et al*. 2000*a*). The positive correlation found between β-endorphin levels and OT released (after prestimulation, during normal milking and in response to vaginal stimulation) during the first relocation is surprising. Therefore, it is questionable whether the peripheral concentration of endogenous opioids was related to the inhibition of OT release (Wellnitz *et al*. 1997, Tančin *et al*. 2000*a*). It is more likely that the inhibition of milk ejection in unfamiliar surroundings was not mediated by endogenous opioids. Possibly, elevated β-endorphin concentrations are just observed concomitantly with inhibition of milk ejection without having a causal relationship. This hypothesis is also supported by the lack of effect of naloxone on the inhibition of milk ejection.

ACTH challenge

Peak cortisol concentration after ACTH injection was reached at 30 min, as reported for a similar dose by Verkerk *et al*. (1994). The adrenal responses to ACTH challenge tended to be higher in those cows with more milk released during normal milking, or in response to vaginal stimulation, during the first relocation. Furthermore, the increase of cortisol levels during the first milking in unfamiliar surroundings, compared with values during control milkings, tended to be lower in cows with total inhibition of milk ejection during all relocations compared with cows that showed similar positive reaction in OT release in response to milking or after vaginal stimulation, in both groups (data not shown). Thus animals with a higher adrenal response to ACTH or to stress had less pronounced inhibition of milk ejection as a consequence of more OT released. It has been demonstrated that the adrenal sensitivity to stress or ACTH correlates with behavioural activities in cattle (Redbo, 1998; Stelwagen *et al*. 2000) in response to new surroundings.

In conclusion, the inhibition of milk ejection and its normalization during repeated milkings in unfamiliar surroundings is hardly, or not at all, influenced by

naloxone. The variation between individuals seems to be related to the adrenal cortex sensitivity.

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