The importance of increased levels of oxytocin induced by naloxone to milk removal in dairy cows

V. Tancin[1], J. Macuhova[2], D. Schams[2], R.M. Bruckmaier[2]

[1]Research Institute for Animal Production, Nitra, Slovak Republic
[2]Physiology Weihenstephan, Technical University Munich, Freising, Germany

ABSTRACT: The aim of this study was to test whether a more pronounced oxytocin release induced by naloxone during milking causes higher efficiency of milk removal. Eight pregnant multiparous Holstein cows from second to fifth lactation were used for this experiment. The experiment was carried out during three consecutive days, i.e. six milkings (three morning and three evening milkings). During first and third evening milking in cross over design (four and four animals) 250 mg of naloxone or 10 ml saline was injected 5 min before the start of udder preparation. During these milkings 2 IU of oxytocin was injected i.v. after stripping and the amount of milk obtained in response to oxytocin injection was measured. Pre-milking naloxone treatment increased the milking-related release of oxytocin, however, only in six of eight cows. The stimulatory effect of naloxone on oxytocin release in the mentioned six cows differed individually from 4 ng/l to 132 ng/l. Naloxone treatment did not influence milk yield before stripping and stripping milk yield. However, naloxone treatment significantly reduced the amount of milk obtained in response to oxytocin injection. Peak flow rate tended to be higher after naloxone treatment. In conclusion, oxytocin release seems to be very important for the evaluation of different milking routines and milk removal environment with respect to the welfare of dairy cows.

Keywords: dairy cows; milking; oxytocin; naloxone

Fast and complete milk removal is related to the release of oxytocin and milk ejection occurrence during the whole milking process (Bruckmaier et al., 1994). Maximum milk ejection occurs in response to tactile teat stimulation when oxytocin blood levels reach concentrations above a certain threshold level (Schams et al., 1984). The right timing of oxytocin release and milk ejection during milking seems to be more important for efficient milk removal than absolute oxytocin levels (Schams et al., 1984). However, several studies showed that oxytocin concentrations during milking could be related to the conditions of milk removal and production in cows (Tancin and Bruckmaier, 2001) or ewes (Marnet and McKusick, 2001). Already stereotype of milking routine was shown to be an important factor to increase milkability, to reduce the amount of residual milk in the udder (Velitok, 1977) or to increase milk production in cows (Rasmussen et al., 1990). Probably the stereotype of milking routine allows the cows to be more ready (better welfare) to milking conditions that could result in enhanced oxytocin release and efficiency of milk removal. Thus more oxytocin in blood during milking could be a result of better welfare of cows (Tancin et al., 2000b; Hopster et al., 2002). On the contrary, stress or discomfort during milking can reduce oxytocin release and milk yield (Rushen et al., 2001; Tancin et al., 2001; Macuhova et al., 2002).

Under normal conditions naloxone (opioid antagonist) can stimulate the release of oxytocin during milking (Tancin et al., 2000a). Naloxone in combination with morphine (exogenous opioid agonist) also resulted in higher milking related oxytocin release that occurred simultaneously with reduced milk volume obtained after 1 IU of i.v. oxytocin (Kraetzl et al., 2001). However, naloxone was not able to reduce the negative effect of stress on oxytocin release during suckling and milking (Kraetzl et al., 2001; Macuhova et al., 2002). Thus, the administration of naloxone during normal milking could be a good approach to see the effect of increased endogenous oxytocin on milking performance. The aim of this study was to test whether an enhanced oxytocin release in response to naloxone treatment results in higher efficiency of milk removal.

MATERIAL AND METHODS

Animals, milking and housing

Eight pregnant multiparous Holstein cows from second to fifth lactation were used for this experiment. They were in week ten to twenty of lactation with a morning milk yield of 11.18 ± 1.08 kg and an evening milk yield of 9.24 ± 1.21 kg. The cows had free access to a mixed ration and were housed in free stall housing.

Cows were routinely milked in the 2 × 5 herringbone milking parlour twice a day at 05.00 and 16.00 h using Bou-Matic milking equipment. Milking was performed at a vacuum of 42 kPa, a pulsation rate of 55 cycles/min and pulsation ratio of 60:40. The pre-milking udder preparation consisted of fore stripping and cleaning of the udder with wet towels for 1 min. After milk flow decreased below 0.2 kg/min machine stripping was performed at the end of milking. During milking milk flow was continuously recorded by a Lactocorder® (Bruckmaier and Blum, 1996).

Experimental design

Cows were habituated to the experimental conditions during milking three days before the start of the experiment.

The experiment was carried out during three consecutive days, i.e. six milkings (three morning and three evening milkings). During the first and third evening milking in cross over design (four and four animals) 250 mg of naloxone (naloxone hydrochloride, Tocris, Bristol, UK, dissolved in 10 ml sterile saline) or 10 ml saline was injected 5 min before the start of udder preparation. During these milkings after stripping 2 IU of oxytocin was injected i.v. to cows and the amount of milk after oxytocin injection was measured. During other milkings, i.e. milkings without saline or naloxone treatment, usual milking routine was applied without oxytocin injection.

Blood sample collection and hormone analysis

One day before the start of the experiment, cows were fitted with a permanent catheter (Cavafix Cetro Splittocan 335, length 32 cm, diameter 1.8 × 2.35 mm, Braun, Melsungen, Germany) in one jugular vein after morning milking to allow frequent blood collection during milking. Blood samples were collected at –5, –1 and 0 min before the start of pre-milking udder preparation and thereafter at every minute until the end of milking. Teat cups were attached at 1 min according to blood sampling scheme. During blood collection, 10 ml samples were collected into tubes containing EDTA, cooled in an icebath, centrifuged at 3 000 g for 15 min, aliquoted and stored at –20°C until assayed. Plasma oxytocin concentrations were determined by radioimmunoassay as originally described for cattle (Schams, 1983) after extraction with SEP-PAK C18 cartridges (Waters Assoc., Inc., USA). The antiserum showed no cross-reaction 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 on average 76 ± 10%, (mean ± sd, n = 9 assays). The intra-assay coefficient of variation (CV) varied from 5.9 to 7.8% and the inter-assay CV from 11.2 to 16.9 % in samples with high (17.2 ± 1.9 ng/l) or low (1.6 ± 0.3 ng/l) oxytocin concentration.

Statistical analysis

Calculations for oxytocin concentrations were performed as mean values for an area under the curve over the basal values (Δ AUC/min) for the period from 1st min until the end of machine milking, during first and last two minutes of milking. For statistical evaluation, a repeated measures analysis of variance was calculated using the MIXED procedure of the SAS program package (SAS, 2001) for morning and evening milkings and Pair-t test for two evening milking with saline and naloxone treatments. The data are presented as means ± S.E.M.

RESULTS

Effect of naloxone administration

The effect of naloxone administration on oxytocin release and characteristics of milk removal efficiency is shown in Table 1. Pre-milking naloxone treatment significantly stimulated the release of oxytocin in response to milking procedure, however, only in six of eight cows. The stimulatory effect of naloxone on oxytocin release in six cows was individually variable and ranged from 4 ng/l to 132 ng/l. Naloxone treatment did not influence milk yield before stripping and stripping milk yield. However, naloxone treatment significantly reduced amount of milk obtained after 2 IU of i.v. oxytocin (Table 1). Peak flow rate tended to be higher after naloxone treatment.

Two above-mentioned cows without response to naloxone were not involved in the statistical analysis comparing the effect of naloxone and saline treatments. There were technical problems with permanent catheters and blood collection during milking with naloxone treatment in these cows. Therefore, it was necessary to move frequently but gently with the neck of cows during blood collection. The volume of milk obtained after 2 IU of i.v. oxytocin at naloxone treatment was 1.21 kg and 4.25 kg, respectively in these two cows, and at saline treatment 0.34 kg and 1.02 kg, respectively. Oxytocin levels were also lower at naloxone treatment (8.32 and 16.56 ng/l) as at saline one (50.56 and 51.45 ng/l).

Effect of the day of experiment

The effect of the day of experiment on oxytocin levels and milking parameters from three continuous morning and evening milkings is shown in Table 2. The effect of naloxone was not considered. There was a significant reduction of milk yield during second morning milkings as compared to first one, but without difference during evening milkings. There were no differences observed among days between oxytocin levels and peak flow rate means during both morning and evening milking. However, there was high variability in oxytocin release among cows and also in cow among milkings.

DISCUSSION

Mechanisms involved in the regulation of oxytocin release under normal or disturbed milking condition related to the opioid importance are not clear in dairy cows at present (Tancin and Bruckmaier, 2001) as compared to rat (Leng et al., 1988) or pig (Lawrence et al., 1992). The high individual response of dairy cows to naloxone could be a result of their individual sensitivity to stress. We have re-

Table 1. The effect of saline and naloxone treatment on oxytocin and milking removal during evening milking

n = 6	Treatments			
	saline		naloxone	
	mean	SEM	mean	SEM
Milk yield before stripping (kg)	8.32	0.74	8.66	0.78
Stripping milk yield (kg)	0.62	0.15	0.65	0.21
Milk yield after 2 IU of i.v. oxytocin (kg)	0.56^{a}	0.15	0.47^{b}	0.12
Milk yield after 2 IU of i.v. oxytocin (%)	5.81^{c}	1.21	4.86^{d}	1.11
Peak flow rate (kg/min)	3.47	0.55	3.61	0.63
Oxytocin during entire milking, Δ AUC/min (ng/l)	17.42^{a}	3.34	64.45^{b}	25.34
Oxytocin of first 2 min, Δ AUC/min (ng/l)	21.02^{a}	4.72	72.39^{b}	21.08
Oxytocin of last 2 min, Δ AUC/min (ng/l)	11.34^{a}	3.48	48.17^{b}	17.73

$^{ab}P < 0.05$; $^{cd}P < 0.1$

Table 2. Oxytocin release and milking parameters during three continuous morning and evening milkings

	The order of milking/morning					
n = 8	first		second		third	
	mean	SEM	mean	SEM	mean	SEM
Milk yield before stripping (kg)	11.11[a]	0.87	9.71[b]	0.74	10.56[ab]	0.71
Stripping milk yield (kg)	0.69	0.13	0.60	0.13	0.53	0.07
Peak flow rate (kg/min)	3.80	0.52	3.74	0.62	3.69	0.57
Oxytocin during entire milking, Δ AUC/min (ng/l)	55.591	28.55	20.61	3.51	42.37	19.78
Oxytocin of first 2 min, Δ AUC/min (ng/l)l	34.15	22.43	15.32	2.91	38.22	21.18
Oxytocin of last 2 min, Δ AUC/min (ng/l)	59.97	28.68	25.46	6.08	54.52	23.24
n = 6	The order of milking/evening					
	first		second		third	
Milk yield before stripping (kg)	8.89	0.61	8.24	0.45	8.31	0.68
Stripping milk yield (kg)	0.49	0.22	0.50	0.22	0.32	0.15
Peak flow rate (kg/min)	3.36	0.49	3.29	0.42	3.35	0.47
Oxytocin during entire milking, Δ AUC/min (ng/l)	37.66	25.73	19.44	7.43	31.61	13.66
Oxytocin of first 2 min, Δ AUC/min (ng/l)	29.06	15.13	15.52	6.32	22.11	14.58
Oxytocin of last 2 min, Δ AUC/min (ng/l)l	40.74	6.83	25.81	11.94	38.84	21.62

$^{ab}P < 0.05$

cently shown that cows with higher adrenal cortex sensitivity to ACTH had fewer problems with milk ejection reflex during milking in new milking place (Macuhova et al., 2002; Weiss et al., 2004). Even in two cows we could not demonstrate the ability of naloxone to potentionate oxytocin release in dairy cows due to small disturbances during milking.

Already after saline milking low amounts of milk volume were removed after 2 IU of i.v. oxytocin, showing good milking conditions where cows were already at usual physiological oxytocin levels well milked out. Therefore we could not find more clear effect of naloxone on milk yield. However, in spite of good milking conditions naloxone treatment significantly reduced amount of milk volume obtained after milking by 2 IU of i.v. oxytocin in this study. Because we do not expect that naloxone could have other effect on the axis of the regulation of milk removal in dairy cows this effect seems to be a result of higher oxytocin released during milking induced by naloxone. Higher oxytocin levels in naloxone treatment were kept until the end of milking. Possibly these higher oxytocin levels especially towards the end of milking could have positive effect on udder emptying and so reducing amount of milk removed from udder in response to 2 IU of i.v. oxytocin. It could be suggested that the more oxytocin concentration in blood the more effective is milk removed from udder. This supports also negative relationship between oxytocin release and milk remained in the udder observed during milking with naloxone treatment in combination with morphine (Kraetzl et al., 2001). Therefore, it is important to measure the release of oxytocin if two or more treatments affecting milk removal are expected to have effect. For example, there was a positive correlation between oxytocin levels and increment of milk yield after the change of milking conditions (Tancin et al., 2000b). Also when cows are well adapted to milking procedures they have higher oxytocin release in response to milking (Hopster et al., 2002). Furthermore, there was a negative correlation between oxytocin levels and residual milk volume in primiparous cows early postpartum (individual adaptation sensitivity to milking routine) but not in the middle of lactation (Van Reenen et al., 2002).

It is difficult to conclude whether sudden reducing of oxytocin release during milking due to environmental disturbances could be the main reason of increasing volume of remained milk in the udder as naloxone was not able to influence oxytocin release. Probably other mechanisms like sympathetic nervous system are involved. Activation of sympathetic nervous system reduced oxytocin release and increased the volume of residual milk (Lefcourt et al., 1997; Van Reenen et al., 2002; Tancin et al., 2003). More study is needed for the better understanding of importance of oxytocin release and sympathetic activation in milk removal efficiency under normal or disturbed milk removal.

In conclusion, the higher oxytocin levels induced by naloxone reduced amount of milk in udder after milking. Therefore, oxytocin release seems to be very important and useful parameter involved in the evaluation of the effect of different milking routines and milk removal environment on the welfare of dairy cows.

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Corresponding Author:

Doc. Ing. Vladimir Tancin, DrSc., Slovak Agricultural Research Centre, Hlohovska 2, SK-949 92 Nitra, Slovak Republic
Tel. +421 376 546 153, e-mail: tancin@scpv.sk