Release of oxytocin, prolactin and cortisol in response to extraordinary suckling

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ABSTRACT: The aim of this trial was to clarify whether suckling after several weeks of only machine milking can affect the release of oxytocin, cortisol and prolactin in dairy cows. In total twelve Brown Swiss cows on their first to third lactation were used. Pre-experimental period: all cows were suckled three times daily (9.00, 14.00 and 20.00) and milked twice daily (7.00 and 17.30) in tie housing during the first 5 days postpartum. Afterwards the calves were separated and moved to another building. The cows were moved to loose housing and milked in the dairy parlour within the same stable. Experimental period: after four weeks of only machine milking twice daily, cows were relocated from the herd to the same place as they were housed and milked during their postpartum period. The cows were again suckled on day 3 and 4 after relocation at the same time as above. Oxytocin, cortisol and prolactin levels were evaluated during the first suckling on day 3 (9.00) and during suckling at the same time next day (4th suckling) after relocation. Oxytocin secretion was clearly inhibited in all cows during the first five minutes of suckling with tendency of slow increase during the next minutes of suckling (suckling lasted about 10 min) except for two primiparous cows showing an increase of oxytocin release from the first minute of suckling. The release of oxytocin in response to the 4th suckling significantly increased immediately after start of suckling. Both, cortisol and prolactin significantly increased in response to all sucklings. There were significantly higher prolactin and lower cortisol values during the first suckling as compared with the fourth suckling in primiparous but not in multiparous cows.

Keywords: dairy cows; suckling; oxytocin; prolactin; cortisol

INTRODUCTION

Release of oxytocin in response to teat stimulation is important for fast and complete milk removal (Bruckmaier and Blum, 1998). In addition, release of prolactin and cortisol is induced by machine milking (Bruckmaier *et al.,* 1993, Gorewit *et al*., 1992, Tančin *et al*., 1995) or suckling (Williams *et al*., 1993).

Under certain conditions the release of all hormones can be influenced. Lack of oxytocin release and delayed cortisol and augmented prolactin release were observed during milking of primiparous cows with inhibited milk ejection after parturition (Bruckmaier *et al*., 1992). On the other hand, milking of dairy cows in unfamiliar conditions caused a lack of oxytocin release but cortisol and prolactin were increased before milking resulting from the relocation stress (Bruckmaier *et al*., 1993). It was found that suckling by an alien calf has no stimulatory effect on oxytocin release and reduced the prolactin release as well (Silveira *et al*., 1993). Furthermore, the first two sucklings of dairy cows in lactation conditioned only to machine milking did not induce oxytocin release (Kraetzl *et al*., 2000). These examples show that the lack of oxytocin release is under different control. Lacking oxytocin and prolactin release in response to suckling by an alien calf can be related to maternal bonds. However, lacking oxytocin release during the first suckling of cows conditioned only to machine milking (Kraetzl *et al*., 2000) is supposed to be probably caused by lack of information from the udder at a central level. Prolactin seems to be suitable for indicating the transfer of stimuli from udder to brain (Williams *et al*., 1993).

The aim of this work was to determine the effect of the first suckling of dairy cows conditioned to machine milking on the release of oxytocin, cortisol and prolactin.

MATERIAL AND METHODS

Animal and experimental procedures

In total, twelve Brown Swiss cows in their first (6 primiparous cows) to third lactation (6 cows) were used. The cows had free access to a mixed ration and received concentrate according to their individual milk production.

Pre-experimental period: All cows were suckled three times daily (9.00, 14.00 and 20.00) and milked twice daily (7.00 and 17.30) in tie housing during the first five days postpartum. Afterwards (at 18.00) the calves were separated and moved to another building. The cows were moved to the loose housing and milked in the parlour within the same stable for the next four weeks.

Experimental period: After four weeks of only machine milking, cows were again relocated from the herd to the same place as they were housed and milked during their postpartum period. The cows were suckled on day 3 and 4 after relocation at the same time as in postpartum period. On day 3, for the first (9.00) and second (14.00) suckling calves were brought to their mothers only for suckling and were relocated to calf barn afterwards. After evening milking calves were moved to their mothers and left in their presence.

Blood collection

Blood samples (10 ml) were taken via permanent jugular cannula. Oxytocin was determined in samples taken at –5 and –1 min, and 0.5, 1, 1.5, 2, 2.5, 3 min, and than in 1 min interval until the end of suckling. Suckling started at 0 min. Cortisol and prolactin were evaluated at –5, and –1 min, before start of suckling, 2, 4, 6 and 8 min during suckling related to the start of suckling, and 0, 5, 10, 20, 30 min after suckling. Sampling tubes contained 200 μl of a solution with EDTA (300 μmol/l, Merck, Darmstadt, Germany) and acetylsalicylate (1%, Serva, Heidelberg, Germany) for anticoagulation. The samples were immediately cooled on ice, centrifuged for 15 min at 3 000 g, and the plasma stored at –20° C until analysed.

Hormone determination

Oxytocin was determined radioimmunologically as described by Schams *et al.* (1979) after the extraction with SEP-PAK C18 cartridges (Water Assoc., Inc., USA). The within-assay coefficient of variation (CV) ranged from 5.9 to 7.8% and the between-assay CV from 11.2 to 16.9% in samples with high (17.2 ± 1.9 pmol/l) and low (1.6 ± 0.3 pmol/l) oxytocin concentration, respectively.

Plasma cortisol concentration was measured by a competitive enzymeimmunoassay previously characterised (Sauerwein *et al*., 1991). The mean recovery after extraction was 77.5 + 8.6% (n = 18 assays). Interassay coefficient of variation was 12.5%.

Prolactin was determined by a homologous bovine radioimmunoassay as originally described by Schams and Karg (1969). The antiserum produced in a rabbit exhibited no cross reactivity to other pituitary hormones or oxytocin. The reference preparation was NIH-P-B4 (biological activity 24.1 IU/mg). Validation experiments showed a mean recovery of added bovine prolactin to bovine plasma of 97.6% ± 4.1 and the dilution curves of bovine plasma run parallel to the standard. The sensitivity was 0.1 ng/ml plasma, intra-assay CV was between 6–8% and inter-assay CV was 7.5–12.8%, respectively.

Statistical analysis

Results are presented as means ± sem. Absolute values of oxytocin, cortisol and prolactin were evaluated by analysis of variance using the General Linear Model (GLM) procedure of the SAS program package (SAS 6. 11, 1995).

The effect of treatment and parity on cortisol and prolactin release was further evaluated for time periods related to suckling. The five phases represent:

Phase I: 5 and 0 min before suckling;
Phase II: 2, 4 min during suckling;
Phase III: 6, 8 min during suckling;
Phase IV: 0, 5 and 10 min after suckling;
Phase V: 20 and 30 min after suckling.

Oxytocin data were also evaluated for time periods. The four phases represent: 0.5–2 min, 2.5–4 min during suckling, end of suckling, and 5 min after the end of suckling. For statistics the same General Linear Model procedure was used. Differences between treatments were post hoc localized by Bonferroni's *t*-test. Differences were assumed as significant if $P < 0.05$.

RESULTS

The intensity of suckling was very high in all suckling events from the beginning of udder introduction to the calf and lasted about 9–11 min. Basal levels were not influenced by treatment, parity and age. The concentrations of oxytocin, prolactin and cortisol significantly increased in response to all sucklings measured ($P < 0.05$) except one multiparous cow that did not release oxytocin in response to both sucklings in contrast to cortisol and prolactin (Figure 1).

The release of oxytocin in response to the first suckling differed between the cows. Two primiparous cows released oxytocin (around 35 ng/l) within the first minute of suckling and no difference between first and fourth suckling was seen. These two cows were excluded from statistical evaluation because of this specific reaction to suckling. The first suckling resulted in delayed and reduced release of oxytocin in both primiparous and multiparous cows as compared with suckling on the following day at the same time (fourth suckling). During the first four minutes of suckling there was a significantly lower oxytocin concentration during first suckling as compared with fourth suckling (Figure 2).

The effect of first and fourth sucklings on cortisol and prolactin concentration in relation to parity is shown in Figure 2. The effect of suckling more influenced the reaction of primiparous than multiparous cows. The

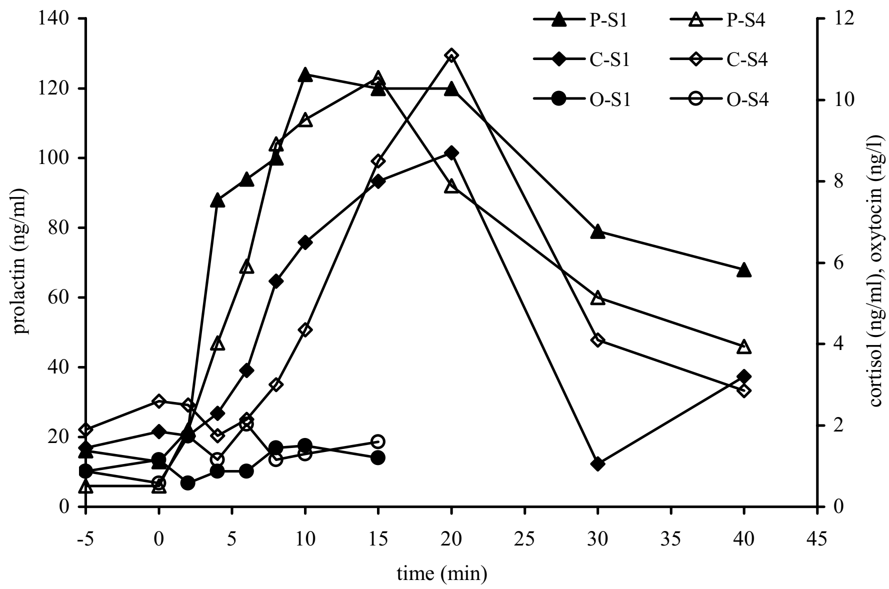

Figure 1. The example of prolactin (P), cortisol (C) and oxytocin (O) levels in one cow with total lack of oxytocin release in response to both the first (1) and fourth (4) suckling (S) events. In 10 min suckling was ended

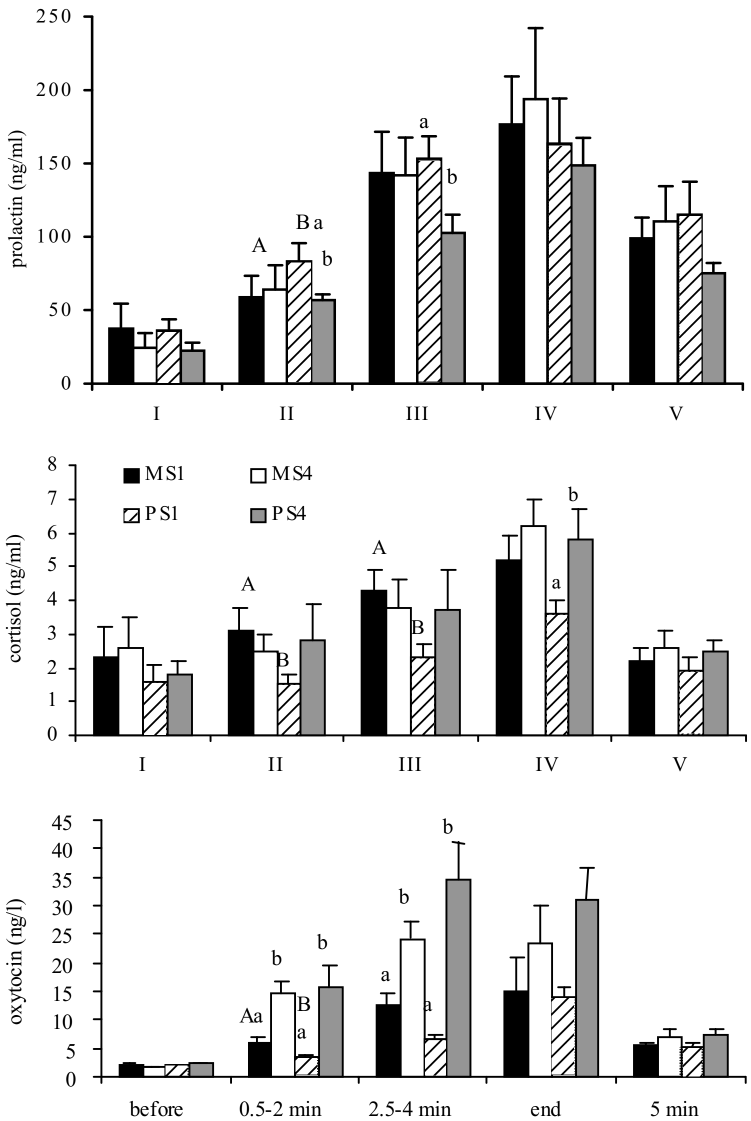

Figure 2. The effect of both sucklings (S1,4) related to parity of cows (M – multiparous, P – primiparous cows) on prolactin, cortisol and oxytocin release. Phases for prolactin and cortisol: I before suckling (–5 to 0 min), II (2 to 4 min) and III (6 to 8 min) during suckling, IV (0, 5, 10 min) and V (20 and 30 min) after suckling. Phases for oxytocin: before suckling (–5 to 0 min), during suckling from 0.5 to 2 min and from 2.5 to 4 min, end – immediately after the end of suckling, 5 min after the end of suckling. Different superscripts between parity (A, B) and within parity (a, b) indicate significant differences $P < 0.05$

primiparous cows released less cortisol and more prolactin during the first suckling as compared with the fourth one. Within the parity there was no effect of cow on prolactin and cortisol release.

DISCUSSION

The first suckling of dairy cows after four weeks of only machine milking caused a lack of oxytocin release. The effect was more variable in primiparous than in multiparous cows. In two primiparous cows even the first suckling induced comparable oxytocin release to fourth suckling. The high individual variability of oxytocin secretion in response to the first suckling can be due to the effect of previous experience of these cows with suckling. The possible relationships of suckling induced lack of oxytocin release to maternal bonds (Silveira *et al*., 1993), previous experiences (Kraetzl *et al*., 2000) or unknown stimulus (Bruckmaier *et al*., 1992) were recently described in greater detail (Tančin *et al*., 2000b). Also prolactin release was inhibited during suckling by alien calves (Perez *et al*., 1985). In suckling-milking management system, oxytocin and prolactin release in ewes are not under the similar central regulation (Marnet and Negrao, 2000).

Bruckmaier and Blum (1998) reviewed the central inhibition or lack of the release of oxytocin in response to teat stimulus under various conditions. The endogenous opioid mechanism seems to be the most potent regulator of oxytocin release at the brain level. This was several times shown in the rat (Russell *et al*., 1993) and pig (Lawrence *et al*., 1992) under stress conditions. However the lack of oxytocin release in response to milking in dairy cows under stress conditions is still unclear (Wellnitz *et al*., 1997). There is evidence that under physiological conditions the endogenous opiods have a tonic effect on oxytocin release (Tančin *et al*., 2000a). Endogenous opioids also suppress cortisol (Tančin *et al*., 2000c) and stimulate prolactin secretion (Peck *et al*., 1988; Schams *et al*., 1998)

The basal levels of prolactin and cortisol signal that animals were not stressed before suckling. Thus, the release of these hormones was considered to be influenced only by suckling. In multiparous cows no effect of both the first and fourth sucklings on prolactin and cortisol values was observed though oxytocin release was reduced during the first suckling. In primiparous cows the lower oxytocin concentrations during the first suckling were concomitant with higher prolactin and lower cortisol levels. There is evidence about delayed cortisol and higher prolactin release in primiparous cows with inhibited release of oxytocin during milking (Bruckmaier *et al*., 1992). The first suckling can be considered as a kind of emotional response resulting in the increase of endogenous beta-endorphine (Bishop *et al*., 1999). Probably the endogenous opioid system could play some role in the release of measured hormones during first sucklings of the cows conditioned to machine milking. Recently using naloxone, an opioid antagonist, Kraetzl *et al*. (2000) were not able to overcome the inhibition of oxytocin release during the first suckling of lactating cows conditioned only to machine milking. Probably no or only low release of oxytocin during the first suckling seems to be the result of information lack. If so, only the oxytocin release seems to be affected in contrast to increased levels of prolactin and cortisol. Whether release of oxytocin in response to the first suckling is a lack of information or actively inhibited is not clear now. Further studies are needed to find out the possible mechanisms involved in the regulation of oxytocin release in response to disturbed milk removal in dairy cows.

REFERENCES

Bishop J.D., Malven P.V., Singleton W.L , Weesner G.D. (1999): Hormonal and behavioral correlates of emotional states in sexually trained boars. J. Anim. Sci., *77*, 3339–3345.

Bruckmaier R.M., Blum J.W. (1998): Oxytocin release and milk removal in ruminants. J. Dairy Sci., *81*, 939–949.

Bruckmaier R., Schams D., Blum J.W. (1992): Aetiology of disturbed milk ejection in parturient primiparous cows. J. Dairy Res., *59*, 479–489.

Bruckmaier R., Schams D., Blum J.W. (1993): Milk removal in familiar and unfamiliar surroundings: concentrations of oxytocin, prolactin, cortisol and β-endorphin. J. Dairy Res., *60*, 449– 456.

Gorewit R.C., Svennersten K., Butler W.R., Uvnas-Moberg K. (1992): Endocrine responses in cows milked by hand and machine. J. Dairy Sci., 75, 443–448.

Kraetzl W.-D., Tančin V., Schams D., Bruckmaier R.M. (2000): The inhibition of oxytocin release in response to first suckling of dairy cows in the middle of lactation without suckling experiences is not opioid dependent. Appl. Anim. Behav. Sci., in press.

Lawrence A.B., Petherick J.C., McLean K., Gilbert C.L., Chapman C., Russell J.A. (1992): Naloxone prevents interruption of parturition and increases plasma oxytocin following environmental disturbance in parturient sows. Physiol. Behav., *52*, 917– 923.

Marnet P.-G., Negrao J.A. (2000): The effect of a mixed, management system on the release of oxytocin, prolactin, and cortisol in ewes during suckling and machine milking. Reprod. Nutr. Dev., *40*: 271–281.

Peck D.D., Thompson F.N., Jernigan A., Kiser T.E. (1988): Effect of morphine on serum gonadotropin concentrations in postpartum beef cows. J. Anim. Sci., *66*, 2930–2936.

Perez O., Jimenez De Perez N., Poindron P., Le Neindre P., Ravault J.P. (1985): Mother-young relations and prolactin response to mammary stimulation in the cow: influence of milking and free or fettered suckling. Reprod. Nutr. Dev., *25*, 605–618.

Russell J.A., Coombes J.E., Leng G., Bicknell R.J. (1993): Morphine tolerance and inhibition of oxytocin secretion by $_{\kappa}$-opioids acting on the rat neurohypophysis. J. Physiol., *469*, 365–386.

SAS (1995) SAS/STAT User's Guide, release 6.11. Cary, NC, SAS Institute.

Sauerwein H., Dursch I., Meyer H H.D. (1991): Quantitation of glucocorticoid receptors in bovine skeletal muscle: topographical distribution, sex effect and breed comparisons. J. Steroid Biochem. Mol. Biol., *39*, 941–945.

Schams D., Karg H. (1969): Radioimmunologische Bestimmung von Prolactin im Blutsaum vom Rind. Milchwissenschaft, *24*, 262–265.

Schams D., Schmidt-Polex B., Kruse V. (1979): Oxytocin determination by radioimmunoassay in cattle. I. Method and preliminary physiological data. Acta Endocrinol., *92*, 258–270.

Schams D., Tančin V., Kraetzl W. (1998): The effect of morphine and naloxone on the release of oxytocin, cortisol and prolactin and on milk ejection in dairy cows. J. Anim. Sci., *76* (Suppl. 1), J. Dairy Sci., *81* (Suppl. 1), 213.

Silveira P.A., Spoon R.A., Ryan D.P., Williams G.L. (1993): Evidence for maternal behavior as a requisite link in suckling-mediated anovulation in cows. Biology of Reproduction, *49*, 1338–1346.

Tančin V., Harcek L., Brouček J., Uhrinčať M., Mihina Š. (1995): Effect of suckling during early lactation and change over to machine milking on plasma oxytocin and cortisol levels and milk characteristics in Holstein cows. J. Dairy Res., *62*, 249–256.

Tančin V., Kraetzl W. D., Schams D. (2000a): The effect of morphine and naloxone on the release of oxytocin and on milk ejection in dairy cows. J. Dairy. Res., *67*, 13–20.

Tančin V., Kraetzl W.-D., Schams D., Bruckmaier R. (2000b): The effect of conditioning to suckling and milking in dairy cows and calf presence on the release of oxytocin. Appl. Anim. Behav. Sci., in press.

Tančin V., Schams D., Kraetzl W.-D. (2000c): Cortisol and ACTH release in dairy cows in response to machine milking after pretreatment with morphine and naloxone. J. Dairy Res., *67*, 467–474.

Wellnitz O., Bruckmaier R.M., Blum J.W. (1997): Naloxone and adrenergic blocking agents fail to abolish central inhibition of milk ejection in cow. J. Dairy Res., *64*, 627–631.

Williams G.L., McVey W.R., Hunter J.F. (1993): Mammary somatosensory pathways are not required for suckling-mediated inhibition of luteinizing hormones secretion and delay of ovulation in cows. Biol. Reprod., *49*, 1328–1337.

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