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Factors affecting milk ejection and removal during milking and suckling of dairy cows

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ABSTRACT: The release of oxytocin and milk ejection occurrence in response to teat stimulation are crucial for fast and complete milk removal during milking or suckling. The milk ejection reflex can be disturbed at central or peripheral level under different experimental and practical conditions. The central disturbance results in the lack or insufficient ejection of the alveolar milk into the cistern due to inhibited oxytocin release from pituitary into the blood circulation. The important role in the pathophysiological regulation of the inhibited release of oxytocin is played by an opioid system. Endogenous opioids have suppressive effects on oxytocin release under the normal conditions of milk removal. However under the conditions of disturbed milk ejection their role in dairy cows was not confirmed. Other possible mechanisms involved in the central inhibition of oxytocin release are discussed. In dairy cows as compared with rats the mechanisms involved in the regulation of oxytocin release at the central level remain unclear. The central inhibition of oxytocin release has often been observed in dairy practice during milking of primiparous cows after parturition, suckling by alien calf, calf removal before milking, milking of cows in the presence of own calf, relocation and milking in an unknown milking place. If sufficient released oxytocin cannot induce the transfer of milk from alveoli to cistern, peripheral mechanisms are involved. Peripheral mechanisms are related to the increased levels of catecholamines and/or activation sympathetic nervous system at the udder level. In conclusion, the release of oxytocin and milk ejection efficiency can be very easily suppressed by many factors. The effect of milking conditions on regulation of milk ejection has to be considered. Thus the physiological requirements of dairy cows have to be respected.

Keywords: dairy cows; milking; suckling; hormones

CONTENTS

1. INTRODUCTION

A good milking process in dairy cows requires optimisation of management, technological and physiological processes. For fast and complete milk removal, the active role of dairy cows must be considered. The active role means that the milk ejection reflex must be induced to shift milk from the alveolar tissue to lower parts of the udder – cistern from which milk is available for mechanical removal. Before milk ejection occurs, only very small amounts of milk in cistern are available for removal (ca. 20%). Complete milk ejection during each milk removal is necessary for the maintenance of high production level and udder health.

Although the basic elements of the milk ejection reflex have been known for more than 40 years, our understanding of this fascinating neuroendocrine mechanism in dairy cows stems mainly from the results obtained in laboratory animals. Recent advances in farm animal research have demonstrated that the knowledge from laboratory animals is often specific to these species only. It is clear from the animal science and dairy practice requirements that milk yield, time of milking and udder health are the most important parameters of good relationships between machine and cow. Thus the knowledge of the physiology of normal milk removal and pathophysiology of disturbed milk removal should be pointed out and taken into consideration as it was recently reviewed in dairy cows (Bruckmaier and Blum, 1998). The knowledge is needed to create the optimal adaptation of the milking environment to the requirements of the cow. The objective of this review is to show the effect of milking conditions on regulation of milk ejection and to present the importance of intrinsic mechanisms involved in this regulation.

2. OXYTOCIN AND THE MILK EJECTION REFLEX

The milk ejection reflex is an innate reflex that is not under conscious control of the animal and occurs in response to tactile stimulation of the mammary gland. The neuroendocrine milk ejection reflex arc consists of two components: a neural one and a hormonal one (Crowley and Armstrong, 1992). The neural component starts by the stimulation of the neural receptors primarily located in the tip of the teats (Lefcourt and Akers, 1984). The neural system carries the impulses from these receptors to the brain, especially to the supraoptic (SON) and paraventricular (PVN) nuclei of the hypothalamus (Richard, 1972). These nuclei are clusters of the nerve cell bodies that are able to synthesize the nonapeptide hormone oxytocin, which moves, attached to a carrier protein (neurophysin I), through the pituitary stalk via the nerve cell axons. Then oxytocin is stored in the neurosecretory terminals of the neurohypophysis (Crowley and Armstrong, 1992). The release of oxytocin into blood circulation represents the beginning of the endocrine way. After arriving via the systemic circulation, oxytocin binds at specific receptors in the mammary gland to influence the myoepithelial cell activity (Soloff *et al*., 1980; Zavizion *et al*., 1992). Myoepithelial cells are located between the basement membrane and the epithelial cells of the mammary alveoli and along the mammary duct system. Due to the binding of oxytocin, the myoepithelial cells contract and the intraalveolar pressure is increased, leading to the expulsion of milk from the alveoli through shortened ducts into the cisternal system (Crowley and Armstrong, 1992). Alveolar milk ejection causes a rapid increase of pressure within the cistern up to an individual maximum (Bruckmaier and Blum, 1996; Mayer *et al*., 1991). The release of oxytocin not only in response to prestimulation but also throughout whole milking is necessary for fast and complete milk removal (Bruckmaier *et al*., 1994).

3. DIFFERENT KINDS OF TEAT STIMULI – MILK EJECTION

The milk ejection reflex and oxytocin release can be influenced by different kinds of tactile stimulation and environmental conditions at milk removal.

3.1. Suckling and machine milking

Domestication of cattle has changed milk removal significantly. Due to the use of milk for human consumption suckling was replaced by hand or machine milking. This change probably influenced also the regulation of milk ejection in dairy cows as compared with less domesticated breeds. The two new kinds of milk removal are not natural and certain breeds do not release oxytocin in response to these stimuli, and the milk ejection does not occur (Pegram *et al*., 1991). Although dairy cows are accustomed to hand and machine stimuli, it has been shown several times that different kinds of stimuli result in a different intensity of oxytocin release in high producing cows.

Both suckling and machine milking have a comparable stimulatory effect on oxytocin release (Tančin *et al*., 1993, 2001a; de Passillé *et al*., 1997), even though Samuelsson and Svennersten (1996) documented a stronger effect of the suckling stimulus. When milking is performed in the presence of the calf, suckling induces higher oxytocin release than machine milking (Akers and Lefcourt, 1982; Tančin *et al*., 1994). A failure of oxytocin release was demonstrated in ewes during machine milking in a mixed system (Marnet *et al*., 1999a). Probably the release of oxytocin by milking in the presence of the calf is centrally suppressed and it is not related to the kind of stimulus. We assume that the mother-young interactions are of main importance.

The presence of the calf influences the regulatory mechanisms in the whole organism of cows, which was mainly studied in relation to reproductive functions (Williams, 1990; Stevenson *et al*., 1997). In literature there is also evidence that suckling evokes the release of oxytocin and milk ejection even in beef cows with denervated mammary glands causing interruption of the neural pathways from the udder to the brain (Williams *et al*., 1993). The mother-young bonds also affect the release of oxytocin during suckling if the calf is own or alien (Silveira *et al*., 1993). It was pointed out that suckling induced stimulation traverses the lower brainstem through diffuse pathways in the reticular formation with many effects in the brain and cannot be ascribed to any compact somatosensory pathway related only to milk ejection (Wakerley *et al*., 1994) as probably induced by milking.

The behavioural activity of calves, and thus udder massage during suckling, is higher in beef than in dairy cows (Lidfors, 1996). The effect is induced by the available amount of milk in the mammary cistern in cows (Mayntz and Costa, 1998) or rats (Yamamuro and Sensui, 1994). It has not been studied in dairy cows whether the mentioned factor – intensity of suckling affects the oxytocin release. However, it cannot be excluded that there is an indirect influence on oxytocin release and milk ejection. The reduction of the suckling stimulus by removing two or three pups from nipples produced a proportional fall in the magnitude of the burst of firing in rats (Lincoln and Wakerley, 1975). The burst is known as one of the two different modes – burst and tonic, as thalamic cells respond to excitatory inputs (Sherman, 2001). When compared the milk amount obtained by 3× daily milking with 3× suckling (6× daily milk removal) the amount of milk obtained by calf (two calves per cow) represents 59% and 76% of the total milk yield in 2nd and 6th week lactation, respectively (Bar-Peled *et al*., 1995). The calf presence also increases the residual milk volume (de Passille *et al*., 1997). It was supposed that higher volumes of residual milk might be due to reduced oxytocin release and milk ejection during milking of the nursed cows. Taking into consideration the threshold of oxytocin effect (Schams *et al*., 1984; Bruckmaier *et al*., 1994), reduced oxytocin is still significant to evoke milk ejection during milking in the calf presence (Tančin *et al*., 2001a). The above-mentioned distribution of released milk between calf and machine or increased volume of residual milk can probably be ascribed to voluntary keeping the milk for the needs of the calf (Bar-Peled *et al*., 1995). However voluntariness is a very wide explanation. Further investigations will be necessary to know whether peripheral or central mechanisms are involved.

The reduced oxytocin release during milking in the presence of the calf seems to be actively regulated. It can be pointed out from the oxytocin data that the amount of milk in the udder could be an important factor in oxytocin release during machine milking in the calf presence but not during suckling (Tančin *et al*., 2001a). We have found significantly lower oxytocin levels during milking cows under conditions of relatively empty udder due to suckling. But the oxytocin release was not negatively influenced during suckling if the udder was relatively empty due to previous milking. We suggest that the lower oxytocin release during milking in the presence of calves is caused by psychological disturbance on a central level although mechanisms in the udder cannot be excluded.

In post-partum and early lactation periods oxytocin secretion in response to suckling was more pronounced in primiparous than in multiparous cows (Tančin *et al*., 2001a). Oxytocin, released within the central nervous system, is an important factor to stimulate maternal behaviour in sheep (Keverne and Kendrick, 1994; Levy *et al*., 1995) and to decrease the response to stress in rats (Petersson *et al*., 1996). Primiparous dams showed a higher incidence of abnormal maternal behaviour after calving than multiparous cows (Edwards and Broom, 1982). Thus in primiparous cows higher activity of oxytocin neurones possibly causes simultaneously an elevated release of oxytocin within the CNS and to the peripheral circulation and could be important to establish maternal behaviour. Oxytocin and milk removal are also required for post-partum alveolar proliferation and mammary gland function of mice (Wagner *et al*., 1997), which could also be important to establish the lactation for primiparous cows.

3.2. Hand and machine milking

Hand milking induced a more pronounced release of oxytocin than did machine milking (Sagi *et al*., 1980; Gorewit *et al*., 1992) although this was not confirmed by other authors (McFadden *et al*., 1987). The latter authors found higher oxytocin levels during milking in parlour than during hand milking. Mechanical stimulation with the milking machine is usually adequate, but machine milking can lead to a lower oxytocin response than manual stimulation indicating the importance of hand preparation of the udder for fast and complete milking (Mayer *et al*., 1984; Bruckmaier and Blum, 1998). In any case, under ordinary conditions, mechanical stimulation is sufficient to induce normal oxytocin release and milk ejection (Bruckmaier and Blum, 1996; Mayer *et al*., 1984).

The induction of stress (Blum *et al*., 1989) or adrenaline administration (Gorewit and Aromando, 1985; Bruckmaier *et al*., 1991) result in the inhibition of milk ejection. On the other hand, adrenaline is not released in response to milking (Blum *et al*., 1989). Adrenocorticotropin (ACTH), the main stimulator of cortisol release, suppressed milk secretion (Thomas and Fell, 1985). Also the administration of corticoliberin (CRH) which stimulates ACTH and cortisol release induced the inhibition of milk production in rat (Almeida *et al*., 1994). On the other hand, not even supraphysiological doses of cortisol influenced milk ejection (Mayer and Lefcourt, 1987). However, machine and hand milking or suckling stimulate the release of cortisol (Gorewit *et al*., 1992; McFadden *et al*., 1987; Tančin *et al*., 1995a, b, 2001b). The physiological importance why milking induces the release of cortisol in dairy cows is unknown (Gorewit *et al*., 1992).

4. THE RELEASE AND ROLE OF CORTISOL DURING MILK REMOVAL

We have shown recently that the mechanisms regulating the release of cortisol during milking are unclear (Tančin *et al*., 2000c). The increase of cortisol during milking was not likely induced by ACTH because there were no changes in ACTH concentrations. Even naloxone, an opioid antagonist, further increased cortisol levels in response to milking but had no effect on ACTH. The cortisol increase during milking under normal conditions seems to be regulated by other mechanisms than stress (Tančin *et al*., 2000c). It seems that the hypothalamo-pituitary-adrenal axis is not activated in response to machine milking. Although the control of the adrenal glucocorticoid secretion has generally been supposed to exclusively depend on the release of ACTH (Jones, 1979), under certain conditions the plasma glucocorticoid concentrations have been found to be inappropriate to the existing ACTH status (Krieger, 1979). There is evidence that the autonomic nervous system can influence adrenocortical steroidogenesis by splanchnic sympathetic nerve activation (Ehrhart-Bornstein *et al*., 1995; Edwards, 1997). Splanchnic nerve stimulation increases the blood flow through the adrenal gland, which might lead to an increase in steroidogenesis by increasing the rate at which ACTH is delivered to the gland, even though the concentration in the plasma does not change (Urquart, 1965; Edwards, 1997). Machine milking induces the heart rate in dairy cows (Johansson *et al*., 1998; Hopster *et al*., 2000).

Though the importance of increased levels of cortisol in response to milking is not clear there is some evidence about the relationship between cortisol and the function of the mammary gland in literature. Glucocorticoids play an important role in the formation and maintenance of mammary tight junctions *in vitro* in mouse (Zettl *et al*., 1992). Tight junctions may be involved in the mammary gland secretion and regulation of milk yield in dairy cows (Allen, 1990). As recently shown, cortisol seems to be important in the reduction of mammary epithelial cell tight junction leakiness in the udder of dairy cows (Stelwagen *et al*., 1998). But this effect seems to be effective only under normal conditions because under social isolation the tight junction permeability was negatively influenced and even to a larger extent in cows with higher release of cortisol (Stelwagen *et al*., 2000).

5. DISTURBED MILK EJECTION

5.1. Post-partum period

In primiparous cows immediately or during several days after parturition the inhibition of milk ejection is often observed, caused by a failure of oxytocin release (Bruckmaier *et al*., 1992; Kraetzl *et al*., 1999; Tančin *et al*., 2001a). The delayed release of oxytocin in response to first milking after parturition was also observed in primiparous ewes (Marnet *et al*., 1999b). Mechanisms responsible for the inhibition of milk ejection in primiparous cows immediately after parturition probably arise from the unknown mechanical stimulus of hand and machine for animals that needs to be conditioned. In pregnant heifers the first hand contact with the udder evoked only a small increase in oxytocin release over the basal level compared with older ones, probably as a result of novel experience (Lefcourt and Akers, 1991). The adaptation to milking (hand, machine) is typical of dairy but not of primitive cows (Le Neidre, 1992).

The failure of oxytocin release during milking in the early post-partum period cannot be ascribed to the non-functional neuroendocrine arc. Normal release of oxytocin was observed in response to suckling in primiparous cows with inhibited oxytocin release during milking in the post-partum period (Tančin *et al*., 2001a). However, the functionality of the neuroendocrine arc of milk ejection is not probably always essential for oxytocin release in response to suckling as it was mentioned above (Williams *et al*., 1993). Thus two questions could be important. Is not the neuroendocrine reflex developed, or is milk ejection actively blocked in primiparous cows in the post-partum period?

5.2. Calf removal

Post-partum calf removal before milking did not influence oxytocin release, milk ejection and milk yield despite the high behavioural activity of cows until the start of milking (calling, vocalisation, looking for) (Tančin *et al*., 2001a). From the stress point of view post-partum calf separation did not influence blood cortisol levels (Hopster *et al*., 1995). However, if the calf is separated later, there is a negative effect on milk yield (Bar-Peled *et al*., 1995). A stronger negative effect of calf separation on the oxytocin release in early lactation could be due to the higher milk demands of older calves, i.e. also higher intensity of suckling and lower amount of milk stored in the udder (Bar-Peled *et al*., 1995; Tančin *et al*., 2001a). Even total lack of oxytocin secretion during milking occurred in some cows (Tančin *et al*., 2001a).

5.3. Suckling

Suckling under the same conditions can result in an inhibition of oxytocin release. Removal of the own calf and suckling by an alien calf caused a failure of oxytocin release (Tančin *et al*., 2001a). Silveira *et al*. (1993) also reported that suckling by the own calf on day 2 after calving stimulated oxytocin release in 100% of beef cows but suckling by alien calves stimulated oxytocin release in 36.4% of the cows only.

Another situation with inhibited release of oxytocin during suckling occurs in the first suckling of dairy cows after several weeks of only machine milking. The failure of oxytocin release is observed less frequently in cows with previous suckling experience (Tančin *et al.*, 2001a) than in cows without such experience in which also the endogenous opioid β-endorphin increases (Kraetzl *et al.*, 2001). We have recently shown that under the inhibited oxytocin release during extraordinary suckling the signal reaches the brain because of prolactin and cortisol increase (Tančin *et al.*, 2001). However, the regulation of reduced oxytocin release in response to the first suckling is not clear yet.

5.4. Relocation

The inhibition of milk ejection as a consequence of inhibited oxytocin secretion can be seen to be related to milking after the milking surroundings under experimental (Bruckmaier *et al.*, 1993, 1997; Wellnitz *et al.*, 1997) and practical conditions (Tančin *et al.*, 1993, 1995a) have changed. Also relocation even to familiar surroundings reduces the secretion of oxytocin and milk yield (Tančin *et al.*, 2000b). The relocation results in the release of endogenous opioid β-endorphin (Bruckmaier *et al.*, 1993).

The sensitivity to emotional stress induced by relocation could also be influenced by calf presence. We have recently demonstrated that suckling of primiparous cows in new surroundings (parlour) caused a reduction in oxytocin release, but the amount of oxytocin increment was still sufficient to activate the milk ejection reflex (Tančin *et al.*, 2001a). When primiparous cows were first milked in the parlour, oxytocin release was more reduced, and in some animals the total inhibition of oxytocin release and milk ejection was observed as compared with suckling in the same place. Suckling is probably a stronger stimulus to release oxytocin under stress conditions than machine milking.

6. POSSIBLE MECHANISMS

Inhibition of milk ejection can be induced at a central (brain) or peripheral (mammary gland) level (Goodman and Grosvenor, 1983; Bruckmaier and Blum, 1998), but only central inhibition of oxytocin release is observed in dairy practice (Bruckmaier and Blum, 1998).

6.1. Central inhibition

There are several neural systems in the brain inhibiting the release of oxytocin or activity of oxytocin neurones in PVN and SON (Crowley and Armstrong, 1992). The main attention to understand the possible mechanisms involved in the central inhibition of the release of oxytocin is paid to the importance of endogenous opioid system – EOP (Bicknell and Leng, 1982; Bicknell, 1985). The opioid system may have a physiological role in controlling oxytocin release at three levels – from the neuronal terminals in the neurohypophysis, supraoptic and paraventricular cells in the hypothalamus and inputs to oxytocin neurones (Douglas *et al.*, 1995). The presence of opioid receptors and opioids in the bovine hypothalamus and neurohypophysis (Pesce *et al.*, 1987; Leshin *et al.*, 1992; Zadina *et al.*, 1997) supports the assumption that endogenous opioids could influence oxytocin secretion during milking in dairy cows as it is well observed in rats (Russell *et al.*, 1993).

The release of oxytocin can be affected by the noradrenergic and dopamine systems. The main attention is paid to the noradrenergic cells (A2 cell group) of the nucleus *tractus solitarii* in medullary structures and to the dopaminergic cells of the posterior and periventricular hypothalamus acting directly at the hypothalamus (Crowley and Armstrong, 1992). Oxytocin release may be influenced at the level of neurosecretory terminals by noradrenaline-containing fibres of the sympathetic nervous system controlling the neurohypophysial blood flow (Holzbauer *et al.*, 1983).

6.1.1. Opioid control

The direct effect of opioids on the release of oxytocin into blood circulation from neurosecretory terminals within the neurohypophysis is mediated via κ-receptors, for which dynorphins are endogenous ligands (Pesce *et al.*, 1987). At the level of the hypothalamus the μ-opiate receptors for β-endorphin are most important to inhibit the activity of oxytocin neurones in rats (Wakerley *et al.*, 1994).

The central inhibition of milk ejection means a reduction of oxytocin release in response to stimuli but the mechanisms responsible for this inhibition are not clearly understood in dairy cows. As we pointed out previously, during emotional stress evoked by social isolation oxytocin release and milk ejection are suppressed while β-endorphin increases (Bruckmaier *et al.*, 1993; Kraetzl *et al.*, 2001). β-Endorphin together with ACTH are parts of the precursor proopiomelanocortin (Eipper and Mains, 1980). Both hormones are possibly released concomitantly during stress (Guillemain *et al.*, 1977).

Central failure of oxytocin release under stress conditions can be abolished by *i.v.* administration of naloxone – general EOP antagonist, as it is documented in rats (Leng *et al.*, 1988; Douglas *et al.*, 1998) or sows (Lawrence *et al.*, 1992). These data suggest that endogenous opioids are involved in the central inhibition of oxytocin release in response to mechanical stimuli. The question why naloxone could not overcome the central inhibition of oxytocin release during milking under stress conditions (Wellnitz *et al*., 1997) or during suckling by alien calf (Kraetzl *et al*., 2001) or during milking of primiparous cows with inhibited milk ejection early post partum (Kraetzl *et al*., 1999) is still unresolved in dairy cows. Not even naloxone could influence oxytocin release in response to normal milking (Wellnitz *et al*., 1997) or during parturition in cows (Aurich *et al*., 1993). A possible explanation is that naloxone could not reach the oxytocin neurones (blood-brain barrier) or naloxone is not able to block EOP receptors in cows (Bruckmaier and Blum, 1998).

We have recently published clear evidence about the inhibitory effect of intravenous administration of morphine (μ-receptor agonist) on oxytocin secretion in response to hand stimulation and machine milking of dairy cows (Tančin *et al*., 2000a). Morphine suppressed the release of oxytocin during milking in a dose dependent manner. We have confirmed the same inhibitory effect of morphine administration on oxytocin secretion in response to teat stimuli as it was described in rats (Pumford *et al*., 1991) in a dose-dependent manner (Wright and Clarke, 1984), however using much lower doses than generally used in rats. The inhibitory effect of morphine administration on milk ejection was eliminated by exogenous oxytocin. It seems that morphine did not influence the sensitivity of the mammary gland to oxytocin, as observed in rats (Russell *et al*., 1993). It is clear from our data that morphine suppressed the milk ejection reflex in dairy cows during milking, as a consequence of the central inhibition of oxytocin release and not at the udder level. Obviously μ-receptors for opioids are active and can inhibit the release of oxytocin in dairy cows in response to machine milking.

When morphine was injected together with naloxone, naloxone surmounted the inhibitory effect of morphine and even potentiated the release of oxytocin in response to machine milking (Schams *et al*., 1998; Kraetzl *et al*., 1999; Tančin *et al*., 2000a). Naloxone in combination with morphine can reverse its inhibitory effect on oxytocin release (Pumford *et al*., 1991) in potentiated manner in the rat as well (Sumner *et al*., 1989). Naloxone, administered even alone, stimulated and potentiated the release of oxytocin in response to machine milking (Tančin *et al*., 2000a). A similar effect of naloxone on oxytocin release was documented during vaginal stimulation in goats (Seckl and Lightman, 1987) and mares (Aurich *et al*., 1996). Thus the endogenous opioid system seems to exert an inhibitory effect on oxytocin release during machine milking in dairy cows under the normal conditions. Also naloxone is able to reach the brain areas important in the regulation of oxytocin activity. Naloxone is also able to increase the volume of morphine distribution in the brain and thus through changed morphine kinetics induce the inhibition of morphine action in addition to the competitive antagonism at opiate receptors in rats (Miyamoto *et al*., 1993).

Further studies are needed to specify the mechanisms responsible for the central inhibition of oxytocin release during machine milking under stress conditions. There are some interesting relationships between released β-endorphin and cortisol in different situations. For example, emotional stress induced the release of β-endorphin and not of cortisol in sheep but physical stress stimulated cortisol only (Hashizume *et al*., 1994). β-Endorphin and motor activity, not cortisol, seem to be indicators of the negative emotional state (Bishop *et al*., 1999). Also during transport naloxone was able to potentiate the release of cortisol (Nanda *et al*., 1989) but not during social isolation in dairy cows (Rushen *et al*., 1999). Different effects of various stressors on milk release were confirmed in rats (Lau, 1992). Reduced milk ejection during stress was not a direct consequence of increased corticosterone. The increased levels of β-endorphin with simultaneous normal cortisol levels suggest that another source of β-endorphin, which could be more effective in oxytocin inhibition, is to be involved. During emotional stress β-endorphin did not probably derive from the anterior lobe but it came from the intermediate lobe, where β-endorphin would not be co-secreted with ACTH (Bishop *et al*., 1999). Thus increased β-endorphin in the intermediate pituitary can reach the terminals of axons of oxytocin neurones in neurohypophysis by local diffusion and influence the oxytocin release (O'Donohue and Dorsa, 1982). Based on the anatomical localization of immunoreactive oxytocin and β-endorphin in the bovine pituitary, it was concluded that the distribution of oxytocin neurosecretory terminals was denser in that part of the *lobus nervosus* bordering the pars intermediata where β-endorphin staining was confined than in the centre of the lobe (McDonnell *et al*., 1994). Possibly β-endorphin secreted by the intermediate lobe reflects the amount of β-endorphin released within the brain to act as a neurotransmitter (Bishop *et al*., 1999).

6.1.2. Noradrenergic control

Noradrenaline derived from the A2 cells has predominantly excitatory effects on oxytocin neurones (Crowley and Armstrong, 1992). The excitatory effect is mediated via α1-adrenergic receptors in PVN and SON (Clarke *et al*., 1979). The activation of β-adrenergic receptors has inhibitory effects on oxytocin release in rats (Song *et al*., 1988). β-Adrenergic receptors are activated by adrenaline released from the adrenal medulla (Song *et al*., 1988). The authors found that adrenal demedullation of rats in midlactation did not alter the basal levels of oxytocin, but markedly enhanced the suckling-induced release of oxytocin, suggesting an inhibitory action of adrenal catecholamine at the central level and/or neurohypophysis. It was also documented that the hypophysial portal epinephrine plasma concentration is highly dependent on an adrenal source (Pesce *et al*., 1990).

In sheep and heifers oxytocin release during milking or first hand contact with the udder was suppressed while noradrenaline increased (Lefcourt and Akers, 1991; Lefcourt *et al*., 1997,). However, machine milking did not induce the release of adrenaline (Blum *et al*., 1989). Intravenous administration of adrenaline reduced the release of oxytocin during milking (Gorewit and Aromando, 1985), which was not confirmed in other works where even the stimulatory effect of catecholamine and oxytocin release were found (Blum *et al*., 1989; Bruckmaier *et al*., 1997; Lefcourt and Akers, 1984). One could expect that catecholamines are involved in the central inhibition of oxytocin release under stress conditions. But in dairy cows, the administration of propranolol (β-adrenergic antagonist) was not able to abolish or influence the central inhibition of oxytocin release during milking in unfamiliar surroundings (Wellnitz *et al*., 1997). But more work is needed to specify the effect of exogenous and endogenous catecholamines on milk ejection and milk removal in dairy cows.

6.2. Peripheral inhibition

Peripheral inhibition of the milk removal is characterised by the failure of an oxytocin effect at the level of the mammary gland under conditions of normal oxytocin release from the pituitary gland in response to teat stimulation and milking (Bruckmaier and Blum, 1998). This kind of inhibition occurred in response to catecholamines (Lefcourt and Akers, 1984; Gorewit and Aromando, 1985; Blum *et al*., 1989; Bruckmaier *et al*., 1991) and as a result of oxytocin receptor blockade (Bruckmaier *et al*., 1997). The mechanism by which catecholamines inhibit milk ejection was described (Hammon *et al*., 1994). The latter authors confirmed the presence of α-adrenergic receptors in milk ducts. Activation of these receptors inhibits milk ejection through the inhibition of milk transfer from alveoli to cistern (Bruckmaier and Blum, 1998) and not as it was previously thought by the inhibition of blood flow through the udder and thus reduced presence of oxytocin in myoepithelial cells (Gorewit and Aromando, 1985).

7. CONCLUSION

From the milk secreted in the udder only a small amount representing cisternal milk is available for mechanical removal. Thus the alveolar milk ejection induced by the release of oxytocin in response to machine milking and during the entire milking procedure is an essential factor for fast and complete milk removal in dairy cows. The milk removal can be disturbed at central or peripheral level under different conditions. The central disturbance represents a failure of ejection or insufficient ejection of the alveolar milk into the cistern due to inhibited oxytocin release from pituitary into the blood circulation. If the sufficient oxytocin release cannot induce the transfer of milk from alveoli to cistern, peripheral mechanisms are involved. Incomplete milking represents an economic loss and milk remaining in the mammary gland is a propitious medium for micro-organisms, potentially causing increased incidence of mastitis. Optimal conditions in practical dairy farming should be respected and the biological requirements of dairy cows should be followed to maintain high production in combination with animal's good health.

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