THE SCIENCE BEHIND MILK EJECTION

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Introduction

The physiology of milk ejection has been thoroughly studied. In 1910, Ott and Scott (1910) discovered that extracts from the posterior pituitary could cause an increase in milk flow from a cannulated teat and some years later it was observed that the extracts from the posterior pituitary caused an increase in intra mammary pressure (Gaines, 1915). In 1941, it was observed that milk ejection was a neuroendocrine reflex (Ely and Peterson, 1941), and a year later it was observed that a factor called oxytocin, detected in the blood, caused contraction of the myoepithelial cells surrounding the alveoli (Peterson and Ludwick, 1942). The role of oxytocin was then further demonstrated by Andersson, (1951), who discovered that the reflex included a terminal hormonal component. Numerous experiments on dairy cows have since then been done in order to find out how stimulation of the reflex should be performed for optimal milk production.

Suckling not only provides the young with nutrients but is also a way to establish social contact between the mother and young. The biology of the milk ejection reflex has therefore been of considerable scientific interest both in farm and wild animals. It is well known that species differences exist when it comes to the biology of milk ejection, such as hormonal release during suckling. In some species milk let down can only occur if the young is present. In pigs, for instance, the oxytocin release during suckling is related to frequency of the sow's grunting. Furthermore, the significance of the anatomy of the mammary gland for milk ejection and milk removal has been observed. However, in the following the milk ejection in dairy cows will be discussed.

Anatomical Arrangements of the Udder of Importance For Milk Ejection

The udder of the dairy cow consists of four separate quarters, each with a teat. During normal circumstances milk produced in one gland can never pass over to the other glands and during milking the milk is released from each gland independently. Usually, milk flow from the front and rear quarters respectively is equal. However, milk flow from the different quarters can also be quiet different, where one gland might have long milking time and low milk flow rates, while another gland within the same udder might have a short milking time with high milk flow rates (Seeman, 1997), where the difference probably indicates some kind of disturbances in the udder.

The milk is synthesised in the epithelial cells of the alveoli and is stored in the alveolus, ducts and cisterns. In an udder filled with milk, usually less than 20 % of the milk is stored in the cisternal compartment (udder and teat cisterns and the bigger milk ducts) (Bruckmaier et al., 1994b), while the rest of the milk is stored in the smaller ducts and the alveolus. From a milking point of view this has to be considered, since removal of alveolar milk is only possible through activation of the milk ejection reflex, while the cisternal milk can be removed by overcoming the teat sphincter barrier. The importance of activation of the milk ejection reflex for optimal milk removal from alveolar compartments was demonstrated by Pfeilsticker et al., (1996), who observed that cisternal milk yield and fraction were significantly higher in teat-stimulated cows than in unstimulated controls.

Oxytocin – The Hormone Involved in Milk Ejection

Different types of touch of the teats, such as suckling by the calf, hand or machine milking, stimulates pressure and touch-sensitive receptors in the teat skin. Stimulation of these receptors induces nerve impulses which travel via segmental pathways in the CNS (central nervous system) to the PVN (paraventricular nulei) and SON (supraoptic nuclei) in the hypothalamus resulting in a release of the pituitary hormone oxytocin. Oxytocin is a nonapeptide consisting of nine amino acids and it is produced in the SON and PVN in the hypothalamus. Via carrier proteins (neurophysin I) oxytocin is transported from the cell bodies PVN and SON through the pituitary stalk. From the pituitary it is released into the blood and transported to the udder where it attaches to the receptors at the myoepithelial cells surrounding the alveoli. As a result the myoepithelial cells contract to expel the milk (Crowley & Armstrong, 1992; Findlay, 1966; Lincoln & Paisley, 1982; Linzell, 1955; Soloff et al, 1980).

Oxytocin - Importance Beyond Milk Let Down

In recent decades it has been discovered that oxytocin neurons also project from the PVN to other regulatory sites in the brain, such as the hypothalamus, striatium, the raphe nuclei, the locus coeruleus, vagal centres in the brain stem and sensory neurons and the sympathetic chain in the spinal cord (Buijs et al, 1978; Hawthorn et al. 1985; Sawchenko & Swanson 1982; Sofroniew, 1983). This anatomical arrangement allows oxytocin release e.g. in the milking-suckling situation to integrate hormonal and neurogenic effects, and the hormone may be involved in regulation of other physiological functions beyond the milk ejection reflex. In mono-gastric animals it has been observed that oxytocin is involved in metabolism (Björkstrand, 1995; Stock, 1989) and behaviour (Kendric et al., 1987; Pedersen & Prange, 1979; Petersson, 1999; Richard et al., 1991; Uvnäs-Moberg, et al., 1992; Witt et al., 1992). Moreover, it has been suggested that the hormone regulates the anti-stress system (Uvnäs-Moberg, 1998).

It has been observed that oxytocin may enhance positive social interactions, such as maternal behaviour and promote the bonding between individuals (for review, see Uvnäs-Moberg et al., 2001). Indeed it has been observed that cows, having higher milking related oxytocin levels due to simultaneously milking and feeding, also had lowered cortisol levels. The cows with elevated oxytocin showed a higher frequency of social interactions and were ruminating more (Johansson et al., 1999b). However, many more studies are needed for a better understanding the importance of oxytocin's effect on behaviour in dairy cattle.

Milking Related Release of Other Hormones

Besides the milking related release of oxytocin, the hormones prolactin and cortisol are released in dairy cows (Gorewit et al., 1992). The biological significance of this release is unclear. However it has been indicated that prolactin can influence milk synthesis and together with growth hormone it might play an active role in maintaining milk secretion (Knight & Flint, 1995). Cortisol is, among others, a catabolic hormone making amino acids and other fatty acids available for milk production (Tucker, 1988). That suckling/milking is influencing the metabolism has further been indicated in monogastric animals, where a suckling induced release of the GI (gastrointestinal) hormones gastrin, somatostatin and CCK (cholecystokinin) has been observed (Uvnäs-Moberg, 1983; Lindén, 1989; Eriksson et al., 1994). In dairy cows milking (Svennersten et al, 1989) and the routine milking and feeding simultaneously influenced the GI hormones (Samuelsson et al., 1996). Probably stimulation of teats (during suckling or milking) activates the vagal nerves in order to adapt the food intake and metabolism in the lactating animal. Hormones like gastrin and CCK have a trophic effect on the gastric mucosa, whereby the suckling-induced release of these hormones affects the capacity in the GI tract during lactation (Uvnäs-Moberg, 1989).

Stimulation of the Milk Ejection Reflex.

That the cow needs stimulation of different senses and also an environment that is not stressful during milking was described already in a 4000-year old mural painting from Egypt (Amoroso & Jewell, 1963). The picture shows a calf standing next to the cow, and both the calf and a child are suckling while a man is titillating the vagina of the cow. Thereby, touch receptors are activated by the stimulation on the teats and in the vagina. In addition, the senses of hearing, smell and sight are stimulated by the presence of the calf.

To evoke the milk ejection reflex the most important stimulus is to activate the receptors in the teat which are sensitive to touch and warmth. This is usually done during the so-called pre-stimulation. However, it has been observed in beef cattle that the suckling includes three main activities; pre-stimulation, milk intake and post-stimulation (Lidfors et al., 1994). It is therefore most likely, from a philosophical point of view, that the different suckling activities have special physiological purposes.

Pre-stimulation.

The pre-stimulation procedure includes cleaning of the teats, drawing some control milk and giving massage to the teats, all done before the milking unit is attached. The biological significance of the pre-stimulation phase is to evoke the milk ejection reflex. When a proper pre-stimulation is practised, alveolar milk is already flowing to the cisternal cavities when the milking machine is attached, whereby the milking time and milk flow are influenced. It has been reported that the time it takes from the start of teat stimulation until the onset of milk ejection is about 1 - 2 minutes (Bruckmaier et al., 1994; Mayer et al, 1991).

Several milking experiments have been performed to investigate the importance of pre-milking stimulation, and different types of mechanical pre-stimulation have been tested. The early findings reported that pre-stimulation of the udder facilitated the milk ejection reflex (Phillips, 1965; Whittlestone, 1968). In complete lactation experiments it was found that manually stimulated cows increased milk production by nearly 30% and the lactation period was prolonged (Phillips, 1984; Phillips 1986). However, the relative effect of pre-stimulation on milk production has decreased in the course of time when experiments from the 1950s are compared to later studies, which is probably due to the progress in breeding for milkability (Phillips, 1986).

In later short-term studies manual pre-stimulation has been compared to milking without pre-stimulation, and it was observed that manual pre-stimulation resulted in shorter machine on time (Gorewit & Gassman, 1984; Momongan & Schmidt,1970). The time delay between start of teat stimulation and until teat cups are attached also seems to have significant influence on milk yield and milking performance (Rasmussen et al., 1992). Milk yield decreased and residual milk increased when the delay was prolonged from 1 to 5 minutes (Brandsma and Maatje, 1987).

The effects of pre-stimulation could be due to oxytocin release. However, in experiments where the effects of manual pre-milking stimulation measured milking related release of oxytocin as well, it was observed that the differences in mean peak concentrations of oxytocin were small, despite that shorter machine on time and higher peak and average milk flow rates were observed. It was concluded that it is the timing of oxytocin release prior to commencement of milking rather than the maximal concentration that was important for an efficient milking (Mayer et al., 1984; Sagi et al., 1980). In a later short-term study, it was further observed that oxytocin concentrations increased very similarly in response to manual pre-stimulation or liner stimulation. But if the cows were milked without pre-stimulation intra mammary pressure did not reached its maximum until the start of milking, with increased risk for bimodal milk flow curves (Bruckmaier & Blum, 1996). In conclusion it is the stimulation of the receptors in the teats per se that it is important for activation of milk ejection and causing the release of oxytocin.

Practical experience indicates that there is a lactation effect regarding the need for stimulation. Earlier studies have reported that the magnitude of oxytocin release in response to milking decreased as lactation advanced (Momongan & Schmidt, 1970; Wachs et al., 1984). However, it has also been reported that the milking related oxytocin concentrations are increasing during lactation. A high degree of variation of oxytocin concentrations within and between individual animals at a given lactation stage was also observed (Mayer et al., 1991). Therefore the increased demand of stimulation might be due to other factors than oxytocin. Bruckmaier and Hilger (2001) reported a significant relationship between the degree of udder filling and the delay from the start of milking until commencement of milk ejection. Furthermore, Bruckmaier et al., (1994b) found that milk ejection in response to oxytocin was delayed when less milk was stored in the alveolar tissue. Increased stimulatory requirements at the end of lactation could therefore be a consequence of low amounts of milk stored in the udder i.e. the degree of udder-fill. The practical consequence is that the need for pre-stimulation is especially important when milking intervals are short and during late lactation when the udder-fill is low, in order to prevent milking on empty teats.

Most of the studies regarding pre-stimulation have been done as short-term studies and the effects have been observed mainly on milk flow and milking time rather than milk yield. However, in a study performed during a complete lactation with both first calvers and older cows a significantly higher lactation yield in kg 4 % FCM (fat corrected milk) was observed when the cows were milked with a standard routine compared to a control routine. The standard routine included a proper manual pre-stimulation and the teat cups were attached within 1 minute after pre-stimulation started. The control routine included pre-stimulation which varied in time as well as the time between start of stimulation until the teat cups were attached (Rasmussen et al., 1990). This finding is in agreement with Merrill et al., (1987) who found a tendency for enhanced lactation yield due to full stimulation. It is likely that this long-term effect could be due to a more efficient udder emptying, where the results only might be detected in the longer perspective.

A more efficient udder emptying probably results in a more efficient removal of the inhibitor FIL (feed back inhibitor of lactation). This substance is locally produced in the udder and acts as a regulatory mechanism for milk synthesis (Wilde & Peaker, 1990; Wilde et al., 1995). As milk accumulates in the udder between milkings, the milk secretion rate gradually decreases because of the action of FIL on the milk secreting cells. Therefore, frequent removal of milk, as well as efficient emptying of the alveoli are important for the maintenance of lactation.

Automatic milking systems are the latest advancements in machine milking. These have the advantage that the cows can be milked without the direct involvement of the farmer during milking, the cows can be milked more frequently than twice a day and the cow can enter the milking unit voluntarily. The pre-stimulation therefore must be done mechanically. In some AM-systems the teats are washed and prepared one by one and the teat cups are attached individually. So far, no negative effects have been observed regarding the stimulation by the AM-system. It has been observed that the teat cleaning devices are suitable for pre-stimulation since oxytocin is released in proper amounts irrespective the number of teats stimulated. However, the pre-stimulation in AMS is even more important than in conventional twice daily milking systems because of the shorter milking intervals. It was also observed that sequentially delayed attachment of teat cups and removal of teat cups at quarter level at the end of milking seem to have no negative consequences for milk ejection (Bruckmaier et al., 2001).

Milking with an AMS compared to parlour milking reached the same degree of udder emptying, measured as strip yield and fat content in strip milk, which indicates that the pre-stimulation in AMS was sufficient. It was also observed that milk yield increased by about 6 % and the milking frequency was on average 2.4 times per day (Svennersten et al., 2000). However, there are also published data showing that an AMS with daily milking frequencies in a range of 2,4 - 2,8 did not respond with a related milk yield increase compared to conventional milking units with twice-daily milking (De Koning 2002). This was probably due to irregular short and long milking intervals. (Hamann et al., 2003).

Stimulation during the entire milking

Firstly, for a complete milk removal the circulating oxytocin needs to reach a threshold level (Gorewit & Sagi, 1984; Schams et al., 1984). Secondly, continuously elevated oxytocin concentrations are necessary. Therefore appropriate teat stimulation throughout the milking is important for the milking process (Bruckmaier et al., 1994). In addition to the importance of the circulating levels of oxytocin it has been observed that anatomical changes occur in the structure of the PVN during lactation, giving increased possibilities for contact between the oxytocinergic neurons (Theodosis et al., 1986). Moreover, oxytocin itself mediates a positive feedback on its own release from the magnocellular neurons in the PVN and SON (Moos et al., 1984; Freund-Mercier & Richard 1981, 1984). Together, all these findings are indicative of the importance of appropriate stimulation during milking.

The type of tactile teat stimulation can influence the milking related release of hormones. During hand milking the release of both oxytocin and prolactin was significantly higher compared to machine milking (Gorewit et al., 1992). Suckling by the calf also resulted in an elevated release of oxytocin compared to machine milking (Bar-Peled et al., 1995; Lupoli et al.,2001). However, the opposite response has also been observed (Akers & Lefcourt, 1982), which might be due to a behaviour-related effect where the cow prefers giving milk to the calf.

Whether the tactile stimulation as such during milking/suckling influences milk yield is not fully investigated. There are indications that both suckling by the calf and hand milking increase milk yield (Bar-Peled et al., 1995; Hamann & Tolle, 1980). These production responses can be due to more efficient udder emptying or an indirect effect of oxytocin. Some observations indicate that oxytocin levels are positively correlated to milk yield (Nissen et al., 1996; Samuelsson et al., 1994). Oxytocin has been administrated to the cows between milkings, which has resulted in increased yield (Morag, 1968; Nostrand 1991). However, experiments have also been published where the oxytocin did not produce any effect on milk yield (Knight, 1994). Noteworthy is the finding in monogastric animals that oxytocin influence growth hormone secretion (Björkstrand et al., 1997), as well as being an important releasing factor for prolactin in rats and pigs (Mori et al., 1990). Growth hormone has been claimed to be the main galactopoietic hormone in ruminants (e.g. Mepham, 1987). Recent results have indicated that prolactin can influence milk synthesis also in ruminants, and that prolactin and GH together play interactive roles in maintaining milk secretion (Knight & Flint, 1995).

The tactile teat stimulation also seems to influence local mechanisms in the mammary gland. When milking two adjacent udder quarters with different types of tactile teat stimulation (hand milking versus machine milking) there was a difference in the production capacity (Svennersten et al., 1990; Svennersten et al., 1991). That the tactile stimulation can affect production has also been observed in kangaroos (Tyndale-Biscoe, 1973). The kangaroo are breast feeding two different young with different ages. The young are suckling each a gland and despite that the hormonal and nutritional environment are the same for the two adjacent glands they produce different yields with different composition, adapted to the needs of the different young. The only thing that differs is the type of tactile teat stimulation. It has been hypothesised that local neurogenic mechanisms might be activated during teat stimulation, which might directly or indirectly influence the milk-secreting cells (Eriksson, 1994).

Post-stimulation

Hand-stripping was practised in the old days when the dairy farmer was paid according to milk fat content. In some experiments the effect of stripping or post-stimulation has been studied. Post-stimulation (Svennersten, 1992) or incomplete stripping (Ebendorff et al., 1987) have been shown to influence milk production. It is likely that the positive effects seen in experiments with stripping are due to the more efficient udder emptying and removal of the inhibitor substance FIL. That post-stimulation is of biological significance has been indicated in pigs, where it was demonstrated that the more the piglet was suckling after milk intake the more milk was produced in that specific teat at the next meal (Algers, 1989). The phenomenon has been called the restaurant hypothesis. Similar findings have been observed also in Bos indicus (Jung, 2001). However, it must be emphasised that it is not practical in modern farming to strip the cows after machine milking is completed, but it could be worth-while to consider this when developing new milking techniques.

Stimuli Other Than Milking Such as Feeding During Milking

Tactile teat stimulation can be considered as the most efficient for stimulation of milk ejection. However, actual observations indicate that milk ejection might occur without the tactile teat stimulation but rather stimulation of other senses. It has been indicated that the milk ejection reflex is activated by visual or auditory stimuli of the calf (Peeters et al., 1973; Pollock & Hurnik, 1978). It is noteworthy that it has been observed that milk leakage before milking was not related to increased oxytocin levels (Bruckmaier, 1988).

It has been observed that feeding induces a release of oxytocin in monogastric animals (Uvnäs-Moberg et al., 1985). In dairy cows just a small release has been detected (Svennersten et al., 1990b), while in dairy calves, the feeding-related release was more pronounced, in particular when the calves were suckling (Lupoli et al,. 2001). In dairy cows feeding during milking potentiates the release of oxytocin (Svennersten et al., 1995). Two mechanisms may be involved in the feeding –induced oxytocin secretion. Firstly, it could be due to increased activation of sensory nerves in the oral mucosa. Since these fibres project directly to the nucleus of the solitary tract (NTS) that is linked to the PVN, oxytocin may be released. Secondly, an afferent neural vagal link between the stomach, the NTS and PVN has been demonstrated in the rat (Verbalis et al., 1986; Renaud et al., 1987). The vagal influence on oxytocin secretion has been validated in experiments where electrical afferent vagal nerve stimulation increased plasma levels of oxytocin (Stock and Uvnäs-Moberg 1988). In some experiments it has also been observed that the nutritional status as such influences the milking-related release of oxytocin. During food deprivation basal as well as milking-related oxytocin release was decreased (Svennersten et al., 1995). Feeding 1.5 hours before milking gave higher milking-related release than when the cows were fed 1.5 hours after milking (Johansson et al., 1999). The practical importance of these findings is that incremental feeding during milking in conventional milking systems as well as in an AMS has a positive influence on milking parameters such as milking time, milk flow and amount of residual milk (Samuelsson et al., 1993; Johansson et al., 1998; Brandsma, 1978; Kokorina & Krasnoperova, 1979).

Inhibition of Milk Ejection

Milk ejection can also be inhibited. The disturbance of milk removal can be a consequence of peripheral inhibition of the reflex and inhibition at the level of the central nervous system. In practise inhibition can have enormous effects on milk production both in the short-term and long-term perspective.

Peripheral inhibition of the milk ejection reflex is characterized by the lack of an oxytocin effect at the udder level under conditions of normal milking related release of oxytocin from the pituitary. The inhibition occurs in response to catecholamines and as a result of a blockade of oxytocin receptors, both demonstrated under experimental conditions in response to administration of catecholamines and during oxytocin blockade. Catecholamines stimulate α-adrenergic receptors, causing a contraction of the teat and cisternal area whereby the milk removal is inhibited in spite of a normal milking-related release of oxytocin. As long as milk is available in the cisterns the milk flow is not reduced. However, the effect of the inhibition of the ejection occurs when the milk travels from the alveolar area into the cistern through the contraction of milk ducts (Blum et al., 1989, Bruckmaier et al., 1991; Bruckmaier et al., 1997; Gorewit & Aromondo,1985).

During central inhibition, the disturbed milk ejection reflex was a lack of oxytocin release in response to pre-stimulation and milking. After injections of physiological doses of oxytocin normal milk ejection occurred. Disturbance of milk removal has been observed in primiparous cows immediately after parturition, during oestrus and during milking in unfamiliar surroundings. Milking-related release of prolactin was present, indicating the afferent pathways from the mammary gland to the hypothalamus were intact. The basal concentrations of cortisol and β-endorphin were higher in the cows milked in unfamiliar surroundings compared to when milked in familiar. The elevated concentrations of these substances indicate that the cows were subjected to some kind of emotional stress. Elevated cortisol levels can be considered as a stress reaction in cows (Bruckmaier et al., 1992; Bruckmaier et al., 1996; Bruckmaier et al., 1993; for review see Bruckmaier & Blum 1998).

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