SYMPOSIUM: MILK EJECTION

Neuroendocrine Control of the Milk Ejection Reflex

GORDON T. GOODMAN and CLARK E. GROSVENOR
Department of Physiology and Biophysics
University of Tennessee Center for the Health Sciences
894 Union Avenue
Memphis 38163

ABSTRACT
The rate and extent to which milk is ejected from the mammary gland during suckling or milking depend upon the relative functioning of a complex array of facilitatory and inhibitory factors which are intertwined and capable of adapting. Some of these operate to regulate the release of oxytocin from the neurohypophysis; others function peripherally to regulate the contractile effectiveness of the oxytocin-sensitive myoepithelium within the mammary gland.

INTRODUCTION
Stimulation of a lactating mother's mammary gland by the young or by machine or hand milking initiates a neuroendocrine reflex that ultimately results in expulsion of milk from the gland. The mechanism by which milk is ejected from the gland involves mechanical stimulation of teats of the mammary gland activating pressure sensitive receptors in the dermis of the areola, nipple, or teat (1, 56) which transform suckling stimuli into nerve impulses. These travel via the spinothalamic tract and dorsal longitudinal fasciculus (27) to the paraventricular and supraoptic nuclei in the hypothalamus. The neurons within these two nuclei synthesize and package oxytocin in vesicles which are transported from the cell body down the axons and stored within the posterior pituitary. Upon stimulation by suckling, the axon terminals discharge oxytocin into the circulation where it travels to the mammary gland and binds to specific receptors located on myoepithelial cells (54). Oxytocin-receptor coupling stimulates contraction of the myo-

epithelium which surrounds the alveoli and the ductules; this raises the intraalveolar pressure and results in expulsion of milk from the alveoli into the larger ducts, or for the cow into the gland cistern.

The foundation of the milk ejection reflex was proposed in 1910 by Ott and Scott (46). Since then few details have been added; furthermore, relatively few investigations have dealt with manipulation of the milk ejection reflex. Because of recent investigations (detailed below), we are beginning to realize that milk ejection is a Sherringtonian phenomenon—a result of effects of various facilitatory and inhibitory components and their interactions. Facilitatory components include those factors that influence the synthesis, release, transport, and binding of oxytocin to the mammary myoepithelium and those factors that facilitate expulsion of milk from the gland. Inhibitory components of the milk ejection reflex involve mainly components of the sympathetic-adrenal system, which may oppose the release, transport, and binding of oxytocin to the mammary myoepithelium and which interfere with the expulsion of milk from the gland (27). The objective of this report is to define and integrate the various facilitatory and inhibitory factors regulating the milk ejection reflex.

Factors Affecting Oxytocin Secretion
It generally is believed that oxytocin is the primary contractile agent of the milk ejection reflex and that the motor innervation of the mammary gland plays no direct part in contracting the myoepithelium (13, 19). However, myoepithelial cells also are sensitive to mechanical stimulation; milk ejection may be produced by mechanical stimulation in the presence of general anesthesia and of local anesthesia of the mammary gland (18, 19). Milk ejection probably is most efficient when mechanical stimuli and oxytocin combine to contract the myoepithelium.
Measurements of oxytocin concentrations in blood of several species reveal that the release of oxytocin following a milking stimulus is fairly rapid and that the elevated concentrations of oxytocin in serum are rather shortlived (4, 17, 22, 32, 42). Momongan and Schmidt (42) showed that concentrations of oxytocin in plasma of Holstein cows increased from undetectable to approximately 80 μU/ml plasma 2 min after the application of the milking machine to the teats of the cow. Within 5 min after initiation of milking, concentrations of plasma oxytocin had returned to undetectable. Animals given a premilking stimulus (udder washing) exhibited higher concentrations of oxytocin in serum with a more rapid release (approximately 120 μU/ml) within 1 min after initiation of milking) than animals given no premilking stimulus. Although cows milked with or without the premilking stimulus gave approximately the same yields of milk, this study indicated that other factors influence the amount as well as the time of oxytocin secretion from the pituitary.

The amount of oxytocin released following milking is dependent upon the number of offspring suckling simultaneously (frequency) and age of the offspring (presumably intensity of the suckling stimulus) and is independent of the quantity of milk within the mammary gland, milk flow, or duration of suckling (9, 20, 21, 35, 36, 37).

As the pregnant animal approaches parturition, there is an increase of the number of myoepithelial cells (48, 49), number of myofibrils per myoepithelial cell, and of the number of myoepithelial cell processes (54) within the gland. Soloff et al. (55) demonstrated that binding of oxytocin to particulate fractions of rat mammary gland increased approximately fourfold throughout pregnancy reaching a maximum approximately 5 days after parturition. Although the mechanism by which the number of oxytocin receptor sites increases is not known, it is possible that estrogen may be involved because estrogens regulate the concentration of oxytocin receptors in the myometrium and serum concentrations of estrogen increase as the animal approaches parturition (45, 53). The role estrogen and progesterone play in the milk ejection reflex is not known, nor is clear whether manipulation of these steroid concentrations would alter the ability of oxytocin to stimulate myoepithelial contraction during early versus late lactation with or without concomitant pregnancy.

The milk ejection reflex may become conditioned in the Pavlovian sense as the result of feeding, presence of the milker prior to milking, crying of the baby, etc. Peeters et al. (47) demonstrated that visual or auditory cues normally associated with milk withdrawal, such as presentation of the calf and washing the udder of a lactating cow, initiate milk ejection.

DeNuccio and Grosvenor (15) demonstrated that intramammary gland pressure (IMP) to a given dose of oxytocin increased with increased glandular volume and that progressively smaller doses of oxytocin were needed to elicit a given response as mammary gland volume increased. These authors also observed that when volumes of milk in the mammary gland are large, milk is refluxed from the ducts back into the alveoli, thereby causing sufficient distension of the alveoli to stimulate the alveolar myoepithelium, causing intramammary pressure to rise again. It would be of interest to determine if the spurious release of oxytocin from the pituitary into the blood between milkings is the result of oscillations of alveolar contraction-relaxation.

Inhibitory Components of Milk Ejection Reflex

Various auditory, visual, physical, or emotional stresses inhibit the milk ejection reflex (11, 16, 44). Inhibition of this reflex is of either central or peripheral origin; central inhibition involves interference with the release of oxytocin from the posterior pituitary (11) and peripheral inhibition is concerned with interference with the expulsion of milk from the mammary gland. Both central and peripheral inhibition are consequences of activation of the sympathetic-adrenal system.

The mechanism by which the sympathetic adrenal system exerts a central inhibition upon milk ejection initially was studied by Cross (12). He showed that electrical stimulation of the supraoptic-hypophyseal tract in the hypothalamus stimulates milk ejection in anesthetized lactating rabbits whereas stimulation of the paraventricular nuclei, also known to synthesize oxytocin, produced signs of sympathetic activity with no milk ejection. However, if the rabbit was adrenalectomized acutely, milk
ejection responses could be elicited from stimulation of the paraventricular nucleus. This was the first indication that sympathetic activation inhibits oxytocin release from the pituitary. In the same study Cross (12) demonstrated that inhibition of the milk ejection reflex occurred following an injection of epinephrine and was abolished by bilateral adrenalectomy. It is now known that the neurological control of oxytocin release is also governed, in part, by dopaminergic (8, 50), cholinergic (6), and noradrenergic (7, 43) components.

Peripheral inhibition of the milk ejection reflex may occur via three possible mechanisms: 1) inhibition of binding of oxytocin directly at the myoepithelium; 2) vasoconstriction, thereby reducing access of oxytocin to the mammary myoepithelium; or 3) increased resistance of the ducts within the mammary gland, thereby inhibiting milk flow to the exterior (19, 41).

In many species, including the cow, milk ejection occurs soon after the suckling or milking stimulus is applied. Gorewit (22) showed that within 1.5 min after washing the udder of a cow, intramammary pressure increased from approximately 19 to 25 mm Hg to approximately 30 to 40 mm Hg. The increase of intrammary pressure occurred concomitantly with increases of serum concentrations of oxytocin. In the rat, however, milk ejection does not occur immediately after onset of suckling but after a prolonged latency period which varies from 10 to 20 min (14, 29, 34). It is not known whether the hiatus between onset of suckling in the rat and ejection of milk is due to impaired release of oxytocin from the pituitary or to peripheral inhibition of the milk ejection reflex. And why is this hiatus masked in cows, humans, and pigs?

Grosvenor et al. (24, 25) showed that the central nervous system and sympathetic system probably maintain a degree of duetal and arteriolar smooth muscle tone within the mammary gland. Based upon the effect of dorsal nerve root sectioning, the authors proposed...
that the afferent mammary nerves might function to help maintain smooth muscle tone within the mammary gland.

To determine effects of mechanical stimulation of the mammary gland upon the sympathetic-adrenal inhibitory system, Grosvenor and Mena (28) applied manual compression for 10 to 15 s to two thoracic mammary glands in anesthetized lactating rats while recording the IMP response of an abdominal mammary gland to intravenous injection of a given dose of oxytocin (Figure 1). This stimulation, designed to mimic the keading action of offspring during suckling, resulted in immediate and significant depression of the oxytocin-induced increase of IMP. The IMP response gradually recovered over the next few minutes, followed by a period of increased contractile responsiveness to the oxytocin injection. During the increased responsiveness phase, the amplitude of the oxytocin-induced increase of IMP was dramatically greater than the IMP obtained during manual compression and slightly greater than the IMP recorded before manual compression. That a similar pattern of depression, recovery, and increased responsiveness to an oxytocin injection was obtained with a single injection of epinephrine, coupled with pretreatment of the rat with an adrenergic antagonist, which blocked the initial depression phase of the IMP response following mammary stimulation, indicates that the sympathetic nervous system was involved in the depressed and increased sensitivity phases of the contractile response of the mammary gland to an oxytocin injection. These data also indicate that the sympathetic nervous system normally may be activated in an acute or "phasic" manner by mechanical stimulation of the mammary gland (28). Parenthetically, in goats and cows the motor inhibitory system of the mammary gland also can be aroused in a reflex manner by activation of mammary receptors (10, 23). Plasma catecholamine concentrations increase following electrical stimulation of the teats of goats (31) and during machine milking of sheep (2). However, the phase of increased responsiveness to oxytocin injection has not been reported in these species, and whether the motor inhibitory system is activated consistently with initiation of a milking stimulus is not apparent.

Under what physiological conditions is the mammary gland preferentially released from the tonic inhibitory influences of the sympathetic-adrenal system? Mena et al. (38) demonstrated that brief periods (5 to 10 s) of electrical stimulation of an isolated mammary nerve induced a reproducible release of approximately 40 to 400 µU (depending upon stimulus strength) of oxytocin from the neurohypophysis as evidenced by subsequent increases of IMP of a contralateral mammary gland (Figure 2). This effect was blocked by spinal cord section and hypophysectomy (data not shown). Prolonged (60 to 120 s) electrical stimulation of an isolated mammary nerve, however, depressed oxytocin-induced increases of IMP (see Figure 4). Therefore, depending upon the duration and frequency of stimulation of the mammary gland, the sympathetic-adrenal system may or may not be activated at the same time that the neuroendocrine reflex for oxytocin release is activated. Because little milk is ejected from the rat during the first 10 to 15 min of suckling, it is probable that activation of the motor inhibitory system occurs during this period, and this phase then is followed by a phase of increased responsiveness to oxytocin (28), during which time the sympathetic system is depressed and milk is ejected rapidly. Milk is obtained more quickly by the pups if the mother's sympathetic system is aroused and allowed to progress to the increased responsiveness phase prior to the onset of the milk ejection test (29).
The mechanism by which the mammary gland changes from a phase of decreased contractile responsiveness to oxytocin to a phase of increased responsiveness following sympathetic activation has not yet been elucidated fully. However, four possible explanations currently are being examined. The first is that activation of the motor inhibitory system inhibits oxytocin secretion, and the increased responsiveness phase is merely the delayed secretion of oxytocin from the posterior pituitary. Another possibility is a gradual increase in or masking of oxytocin receptors on the mammary myoepithelium, thereby allowing a given concentration of oxytocin gradually to increase its ability to stimulate milk ejection with time. A third possibility is that the mammary gland may become progressively resistant or insensitive to circulating catecholamines, i.e., the catecholamine receptors may adapt. A fourth possibility is that facilitatory factors overcome or turn off the phasic inhibitory component of the sympathetic-adrenal system.

Concentration of oxytocin in plasma increases soon after initiation of suckling in the rat though the pups receive relatively little milk within the first 15 min (Figure 3). These data, coupled with recent data from Clapp et al. (5) demonstrating that plasma epinephrine is released reflexly in the rat within the first few minutes following mammary nerve stimulation, indicate that epinephrine is released at the same time as oxytocin. Mena et al. (5, 39) introduced milk into an abdominal mammary gland whose pressure was being monitored and found that rapid increases of glandular volume immediately blocked the depressant effect of prolonged mammary nerve electrical stimulation on the oxytocin-induced increase of IMP comparable to that of adrenalectomy or $\beta$-adrenergic blockade (Figure 4, upper part) and blocked the normal rise of catecholamines in plasma which follows such stimulation (5). Because introduction of milk into the mammary gland increased alveolar diameter with concomitant increases in myoepithelial tension (15), it was suggested that increased myoepithelial tension may have overridden activation of the sympathetic-adrenal system. Introduction of much smaller quantities of milk into thoracic mammary glands (insufficient to produce the above effect) resulted in gradual disappearance of the depressant action of electrical stimulation of an isolated mammary nerve upon the oxytocin-induced IMP response of an abdominal mammary gland. This effect started between 5 and 10 min after increasing glandular volume and resulted in a complete blockade of the depressant effects of mammary nerve stimulation within 40 min (Figure 4, lower part). These data indicate that there is a second mechanism to shut off the sympathetic-adrenal inhibitory system, one which involves reflex activation of mammary baroreceptors. With inactivation of the sympathetic-inhibitory system of the mammary gland, ejection of milk occurs more rapidly and completely. To what extent, if any, activation of the sympathetic nervous system occurs in response to milking in cows and the degree to which it influences the rate and extent of milk ejection is not known.

The mechanism by which the sympathetic nervous system becomes refractory to additional stimuli that normally would activate it once the initial inhibitory phase is over is unknown. It is possible that sensory receptors
Figure 4. Effect of electrical stimulation (5–30 V, 1-ms pulses, 10/s) of a mammary nerve upon IMP responses to oxytocin (Oxy) injected 60 and 120 s after onset of stimulation of lactating rats. Solid dots indicate oxytocin injections. Top: Responses obtained before and after bilateral adrenal ligature (ADX), propranolol injection, or increasing the volume of milk (vol. increase) into an abdominal gland whose pressure is being recorded. Bottom: Effect of rapidly introducing .04 ml of milk intraductally into a thoracic gland on the IMP response to oxytocin of an abdominal gland after mammary nerve stimulation (39).
on the teats of the mammary gland become refractory to further stimulation, or that mammary afferents inhibit sympathetic efferents within the mammary gland (40), or that adrenergic receptors on the ductal or alveolar myoepithelium become desensitized because of previous exposure to catecholamines (33).

In 1971, Grosvener and Mené (26) reported that frequency of suckling declined in rats as lactation progressed, yet pup growth continued to increase. This may be explained by increasing efficiency of both suckling by pups and ejection of milk by the mother as lactation progresses. The mother could eject her milk more rapidly and completely either through a damping of the inhibitory phase of milk ejection or by hastening the onset of the increased responsive phase of milk ejection following sympathetic activation. Based upon this hypothesis we decided to test the effect of stimuli (noise) upon the rate of milk ejection in rats during early and late lactation (Figure 5). We found that an exteroceptive stimulus (noise) given during suckling had little effect upon milk ejection in early lactation, but both rate and amount of milk yield were decreased during late lactation. The mechanism for the greater responsiveness of the rat to noxious stimuli during late lactation is not known. Possibly the duration of the inhibitory phase of milk ejection is lengthened, and the sympathetic system is aroused more easily than in early lactating animals.

As lactation progresses, the offspring no longer require nourishment solely from the dam; mother and offspring progressively become competitors for a single food supply. To examine the relationship between food intake and rate of milk ejection of the lactating rat, Grosvenor and Mena (30) restricted food intake to one group of early to mid-lactating rats (Table 1). They found that pups of underfed mothers obtained a much greater percentage of the total milk after 15 min (84% vs. 32%) and after 45 min (100% vs. 57%) of sucking than did animals fed ad libitum. Thus, underfed animals ejected their milk more rapidly and completely than did mothers fed ad libitum, although the total milk available to the pups of the underfed mothers was only approximately one-half to two-thirds that available to pups of mothers fed ad libitum.

When pups of underfed and fed mothers were exchanged for the milk ejection test, faster milk ejection occurred with pups suckling the underfed mothers. This ruled out the possibility that the faster rate was from increases of frequency or intensity of sucking or that because of being chronically underfed the pups had become more proficient at extracting the milk. Instead the faster milk ejection appeared to be due to more effective contraction of the glands of the underfed mothers. The mechanisms responsible are currently under investigation.

Although there are obvious anatomical differences between the rat and other species, e.g., cow or human, the mechanism controlling the milk ejection reflex of these species may be similar to that of the rat in spite of the fact that man imposes restraints or otherwise regulates the milking patterns of humans or of domestic animals. For example, injection of epinephrine inhibits milk ejection or oxytocin-induced elevation of IMP in rats (28), rabbits (11), cows (16), and sows (3, 57). In the rat the inhibitory effect of epinephrine upon milk ejection may be abolished by preinjection mammary compression (28). Similarly, in the cow, the inhibitory effect of epinephrine upon milk ejection may be abolished partially (51) or completely (52) if udder washing precedes injection of epinephrine.

Thus, milk ejection involves not only the presence or absence of oxytocin but an intricate array of facilitatory and inhibitory influences which are intertwined and capable of adapting. Unraveling the components remains one of the fascinating problems for investigation in the lactating animal.

REFERENCES


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