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Autocrine control in milk production and mammary development

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ABSTRACT

In dairy animals, the development and secretory activity of each mammary gland in the udder is regulated independently by local mechanisms sensitive to the frequency and efficiency of milk removal. Acute regulation of milk secretion occurs through autocrine inhibition by a secreted milk constituent, which may act by modulating the sensitivity of the tissue to circulating hormones. Sustained changes in milking frequency or efficiency are associated with modulation of secretory cell differentiation and, ultimately, the number of secretory cells. Elucidation of these local intra-mammary mechanisms offers the prospect of controlling milk secretion in a novel and more specific manner than has hitherto been possible.

Keywords Milk secretion, autocrine control, mammary development.

INTRODUCTION

Changing the frequency of milking has been used for many years as a method for manipulating milk production in dairy animals. This response was assumed, falsely, to occur either through a change in the pattern of release of galactopoietic hormones, or through alteration of the gland's physical distension by stored milk. It is only recently that the physiological mechanism underlying this response has begun to be understood, as evidence has accumulated for local intramammary control of milk secretion through a chemical mechanism. In this paper, we review evidence for local feedback inhibition by a milk constituent produced by, and acting on, the secretory cells of the mammary gland, assess its role in the gland's adaptation to inefficient or infrequent milking, and discuss its possible use as a tool for controlling milk production.

EVIDENCE FOR AUTOCRINE CONTROL

Local regulation of mammary function was demonstrated in a series of studies using lactating goats and cows. When more frequent milking was applied to just one gland of goats (Blatchford and Peaker, 1982; Henderson *et al.*, 1983) or two quarters of cows (Hillerton *et al.*, 1990), its resultant effect on milk secretion was observed only in the treated gland(s). Clearly, this local response could not be attributed to an effect of systemic hormones. Additional evidence that hormones are not involved came from hourly milking of autotransplanted goat mammary glands, which responded to frequent milking despite being denervated, and therefore incapable of stimulating the release of prolactational hormones (Linzell and Peaker, 1971).

The local effect of manipulating milking frequency was also not due to a change in the pattern of physical distension of the gland by accumulated milk. When glands were milked thrice instead of twice daily, and milk removed at the extra milking was replaced by an equal volume of inert solution (thereby maintaining the same pattern of gland distension), there was nevertheless a stimulation of milk secretion (Henderson and Peaker, 1984). Therefore, the effect of milking frequency or efficiency on secretory rate was attributable directly to removal of milk from the gland.

These results suggested that the stimulation of milk secretion by frequent milking was elicited by more frequent removal of a milk constituent which limits secretion by negative feedback on the secretory cells. The presence of a feedback inhibitor in milk has been demonstrated using rabbit mammary explant cultures as a bioassay. A milk fraction containing whey constituents with a molecular mass of 10-30 kiloDaltons inhibited synthesis of lactose and caseins in these cultures in a dose-dependent manner (Wilde *et al.*, 1987a). Inhibition by the whey fraction was rapid, consistent with the acute response elicited by changes in milking frequency, and was readily reversible. When explants exposed to the inhibitory fraction for 6 hours were washed and cultured again, rates of lactose and casein synthesis returned to control levels (Wilde *et al.*, 1987a). Moreover, the 10-30kDa fraction that was active *in vitro* inhibited milk secretion when injected into the glands of lactating goats via the teat duct (Wilde *et al.*, 1988a). As in tissue culture, the effect of the whey fraction was rapid, reversible, dose-dependent and specific to the 10-30kDa fraction of whey proteins. A second fraction containing proteins of >30 kDa, which was inactive in the bioassay, also did not affect milk secretion *in vivo* (Wilde *et al.*, 1988a). The inhibitory goat whey fraction also reduced milk accumulation in rabbit mammary glands when injected into the gland through the teat duct (Wilde *et al.*, 1988b). The dose-dependence of the effect exerted by the 10-30kDa fraction on milk secretory rate in goats, and the demonstration that this fraction was active when introduced via the teat canal, together suggest that the degree of feedback inhibition during normal lactation is determined by the concentration of inhibitor in the alveolar lumen. Moreover, since the alveolar lumen is lined solely by secretory epithelial cells, this indicates that these cells are producing an inhibitor of their own activity. Therefore, the term autocrine control seems an appropriate one to describe this local control mechanism.

The chemical nature of the inhibitor and its mechanism of action on the secretory cell is currently under investigation. Recent evidence suggests that the inhibitor may act by changing the sensitivity of secretory epithelial cells to circulating hormones through modulation of the number (or distribution) of receptors for galactopoietic hormones. In short term cultures of lactating mouse mammary cells, partially-purified goat inhibitor reduced specific binding of prolactin to cell-surface receptors (Bennett *et al.*, 1990). Manipulation of autocrine inhibition *in vivo* was also accompanied by an effect on prolactin binding: in glands stimulated by four weeks of thrice daily milking, the total number of prolactin receptors increased significantly, whereas long term incomplete milking of one gland reduced milk secretion and prolactin binding (McKinnon *et al.*, 1988). Autocrine modulation of endocrine control offers an efficient method for integrating the strategic control of the gland by galactopoietic hormones and the tactical control afforded by this local mechanism. An action exerted early in the prolactin signal transduction pathway would also provide a convenient method for regulating synthesis of milk constituents coordinately. This was the case in all experiments using lactating goats: milking frequency regulated milk yield, but had no effect on gross milk composition. Relative increases in milk protein and lactose concentrations in glands of cows that were milked four times instead of twice daily were significant but very small (Hillerton *et al.*, 1990). Autocrine regulation mediated by an effect on prolactin receptors in ruminant mammary tissue might seem unlikely, given the traditional view that this hormone is relatively unimportant for maintenance of established lactation (Cowie *et al.*, 1980). On the other hand, depletion of circulating prolactin by daily bromocriptine treatment reduced goat milk yield by 20% (Knight *et al.*, 1990a). Moreover, in bromocriptine- treated goats milked three times a day in one gland, there was a larger differential in the milk yield of treated and untreated glands than when the same treatment was applied to normo- prolactinaemic goats (Knight *et al.*, 1990a). This does suggest that a frequent milking- induced increase in prolactin receptor number assumes even greater importance when the hormone's circulating concentration is low. It remains to be determined how a secreted milk protein acting on the apical cell membrane can change hormone receptor distribution on the baso-lateral cell surface.

AUTOCRINE CONTROL AND MILKING EFFICIENCY

The dose-dependent effects of the autocrine inhibitor *in vivo* and *in vitro* suggests a mechanism in which the concentration of inhibitor in milk increases with time. In this scheme, frequent milking stimulates milk secretion by preventing the achievement of a high inhibitor concentration, and milking reduces the inhibitor's concentration to a low level, in order to re-establish an optimal rate of milk secretion. However, this hypothesis must also take account of the presence of residual milk in the gland after milking. If feedback inhibition is to be relieved, then inhibitor present at a high, inhibitory concentration in the residual milk must either be diluted by lower levels in the first milk secreted after milking, or else it must be neutralized in some way. How changes in inhibitor concentration are elicited is not clear, but it is unlikely that they occur through a change in inhibitor secretion: this would require an additional mechanism by which milking frequency or efficiency could affect the synthesis rate of the inhibitor. An alternative possibility is that the inhibitor, once secreted, is processed in the alveolar lumen, either from an active form to an inactive one, or from an inactive pro-inhibitor to an active form. In either case, first order metabolism of inhibitor in the alveolar lumen would bring about an increasing concentration with time during milk accumulation, even though the inhibitor was being secreted at a constant rate. Inhibitor inactivation or pro- inhibitor activation would respectively act to neutralise or dilute the active inhibitor present in residual milk.

Whatever the mechanism by which inhibition is relieved, it would be predicted that release from inhibition after milking should be slower in glands which contain more residual milk. If residual volume is too large, feedback inhibition is relieved only slightly or not at all, which explains why frequent milking by catheter (which removes only cisternal milk, hence maximising the residual fraction) did not increase milk yield in goats (Henderson and Peaker, 1987). Therefore, completeness of milking is likely to be an important determinant of the degree of autocrine inhibition in each gland, and should have a major influence on the rate of milk secretion. This prediction was confirmed in lactating goats: the rate of milk secretion in individual glands was inversely related to the fraction of milk left after milking, irrespective of the actual volume of milk in the gland at the time of milk removal (Peaker and Blatchford, 1988).

Since the inhibitor acts directly on the secretory cells, for it to be effective it must be present within the secretory alveolar lumen. In other words, cisternal milk exerts no feedback inhibition. It follows that animals with relatively large cisterns should be less subject to feedback inhibition than those which, by dint of a small cistern, are forced to store a high proportion of their milk within the alveolar space. This prediction has also been tested through the use of catheter milking. It emerged that the goat has an especially large cisternal: alveolar milk storage ratio in comparison to other species, even to the extent that alveolar milk and residual milk were effectively indistinguishable (Peaker and Blatchford, 1988). Therefore, we must offer an alternative explanation for the relationship between residual milk volume and secretion rate; it may be that the cisternal:alveolar storage ratio is the important factor, rather than, or as well as, residual volume. The storage ratio also influences the degree of response obtained to frequent milking. Goats with a relatively high cisternal:alveolar ratio respond less well than those with a low ratio, because they are less subject to feedback inhibition during normal twice-daily milking (Knight *et al.*, 1989). Gland anatomy is genetically determined. Therefore, breeding for high-yielding dairy animals may have been, at least in part, through unwitting selection for a high cisternal:alveolar storage ratio. The use of this value as a selection criterion to improve productivity is currently under investigation.

Efficiency of milking also depends on the technique and technology employed in the milking parlour. Glands of cows milked by machine alone produced less milk on a tissue weight basis than those which were subsequently hand-milked to minimise the volume of residual milk (Michel *et al.*, 1986; Singh and Dave, 1985). The proportion of residual milk in the gland after milking has been found to increase in the second and subsequent lactations (Barnes *et al.*, 1986; Ebendorff *et al.*, 1987). Therefore, although yield tends to increase in successive lactations, milk secretion may become more inefficient, and autocrine inhibition may be an increasingly important factor. It is notable that omission of hand milking after machine milking had a greater effect on milk yield in the second lactation than in the first (Ebendorff *et al.*, 1984).

LONG TERM EFFECTS ON MAMMARY DEVELOPMENT

The response of milk secretion to a change in milking frequency is rapid, occurring within hours, and is maintained for as long as that milking regimen is applied. Thus, when one gland of goats was milked three times daily for one week, yield increased unilaterally for that period, and then returned to its pre-treatment value (Henderson *et al.*, 1983). However, a sustained change in milking frequency or efficiency elicits a sequence of local adaptations in the mammary cell population. Secretory cell differentiation was monitored by measuring key enzyme activities and synthetic rates in freshly-prepared explants, and was shown to be enhanced by each long term manipulation. An increase from twice to thrice daily milking stimulated goat mammary cell differentiation within ten days (Wilde *et al.*, 1987b), and four times daily milking of dairy cows produced a similar effect after four weeks (Hillerton *et al.*, 1990). Conversely, unilateral once daily milking of goats for four weeks decreased secretory cell differentiation compared with contralateral glands milked twice daily (Wilde and Knight, 1990). Cell differentiation was also modulated by even the small perturbation of milk removal produced by incomplete milking, when this treatment was sustained for 24 weeks (Wilde *et al.*, 1989b). Inefficient milking of bovine mammary glands had a similar effect on gland morphology. Histological examination of tissue subjected to long periods of incomplete milking showed a proportion of glands in an advanced stage of involution, and also a higher proportion of smaller alveoli (Michel *et al.*, 1986, Ziesack *et al.*, 1986).

The effect of milking frequency or efficiency on cell differentiation may be exerted by the same autocrine inhibitor, as a sequel to its acute effect on milk secretion. A whey fraction containing this factor inhibited epithelial cell differentiation in primary cultures of mouse mammary cells. Induction of fatty acid synthetase activity (a key enzymic marker of differentiation) and casein synthesis was suppressed when the inhibitor fraction was added to differentiating cells early in culture, or was reduced when it was added to differentiated cells (Wilde *et al.*, 1991). The same fraction also reduced secretory rate and key enzyme activities in lactating rabbit mammary glands when injected via the teat duct (Wilde *et al.*, 1988b). Moreover, this fraction stimulated intracellular degradation of newly-synthesised casein in goat mammary explants, a process whose activity appears to be inversely related to the degree of secretory cell differentiation (Wilde *et al.*, 1989a).

The ultimate response to more frequent milking (thrice instead of twice daily), when this treatment was applied for virtually an entire lactation, was an increase in secretory cell number compared with the twice daily-milked contralateral gland. This was attributable, at least in part, to a stimulation of cell proliferation, as indicated by a higher rate of DNA synthesis in explants freshly-prepared from the thrice-milked gland (Wilde *et al.*, 1987b). However, it is possible that thrice daily milking also increased cell longevity in the thrice milked gland. In cows, a higher rate of DNA synthesis in quarters milked four times daily (and by inference a higher rate of cell proliferation) was observed after only four weeks (Hillerton *et al.*, 1990). This was supported by quantitative histology, which suggested that four times-milked glands of both heifers and cows had more epithelial cells per secretory alveolus. The reason for this more acute response is not clear; it may reflect the greater effect imposed by four times rather than three times daily milking, or may indicate a difference in sensitivity of cows and goats to manipulation of milking frequency. Clearly, in each species the response elicited depended on both the degree and duration of the manipulation of milk removal. It also varied with stage of lactation: four weeks thrice daily milking of goats stimulated cell differentiation in early lactation (Wilde *et al.*, 1987b) but not when introduced in week 17 (Knight *et al.*, 1990).

The effects on secretory cell differentiation and number would suggest that an effect of milking frequency or efficiency on milk yield might persist after treatment stopped. There was evidence of a small but significant carry-over effect in cow mammary glands after cessation of four times daily milking, perhaps as a result of a slight improvement in persistency during the treatment i.e. a reduction in the rate of decline in milk yield (Hillerton *et al.*, 1990). Increased persistency of milk secretion was also noted in cows milked three times daily for various periods up to five months (Pearson *et al.*, 1979; Poole, 1982), and this too appeared to have a carry-over effect when milking frequency reverted to twice daily. However, in neither study was the effect significant, and others have failed to observe any carry-over effect of frequent milking (Gisi *et al.*, 1986).

A persistent effect of frequent milking may occur only when the treatment is of sufficient duration and magnitude to elicit an effect on secretory cell number, as appeared to be the case in four times-milked quarters of cows (Hillerton *et al.*, 1990). If this were the case, it would be useful to be able to predict reliably the conditions required to stimulate cell proliferation, so that a long term improvement in productivity could be obtained by a limited period of frequent milking. The local mechanism mediating this growth response is, however, unclear at present. Evidence from mammary cell culture experiments indicates that it is not a further effect of the autocrine inhibitor of milk secretion (Wilde *et al.*, 1991). Instead, the response could occur through a change in the level of a local inhibitor of cell proliferation. An inhibitor of mammary cell proliferation *in vitro* has been isolated from bovine mammary tissue, and is secreted in milk associated with the milk-fat globule membrane (Bohmer *et al.*, 1987; Brandt *et al.*, 1988).

There are circumstances in which it would be advantageous for the effects of milking frequency to be reversible rather than persistent. For instance, milk yield could be reduced temporarily by a short period of once daily milking, without any long term effect on productivity. In goats, the rate of milk secretion recovered rapidly from a period of once-daily milking, even when milk yield had decreased by 26% and some loss of secretory cell differentiation had occurred ((Wilde and Knight, 1990). However, this treatment may have impaired a subsequent frequent milking response. When the same glands were switched from twice to thrice daily milking, not all goats responded, and the effect was small compared with previous studies (Henderson *et al.*, 1983; Wilde *et al.*, 1987b). Therefore, in spite of the absence in most cases of a carry-over effect on milk secretion, changes in milking frequency may nevertheless have a persistent influence on the subsequent response of the tissue to autocrine modulation. If so, this would be an important consideration when using frequent or infrequent milking as a tool for manipulating milk production, and also in the applicability of techniques for direct manipulation of autocrine inhibition *in vivo*.

MANIPULATION OF AUTOCRINE CONTROL

Characterization of the autocrine inhibitor and its mode of action may allow milk production to be controlled without resorting to a change in milking frequency. Milking three or more times a day obviously requires an increase in labour input, which often renders the operation uneconomic; more time spent milking also leaves less time for the other tasks of animal management. If feedback inhibition could be neutralized, perhaps by immunization against the inhibitor, these disadvantages of frequent milking would be avoided. A direct method for manipulating autocrine inhibition could also be applied in combination with once a day milking, when the objective is not to reduce output (short term once daily milking is sometimes used by European farmers to keep production within quota limits) but to minimise labour costs. Yield should be maintained better when animals are switched from twice to once a day milking if the predicted increase in feedback inhibition can be minimized or prevented altogether. Neutralization of autocrine inhibition could also reduce unwelcome variability in each cow's capacity to tolerate once daily milking (Carruthers *et al.*, 1989): this variable response is not explicable in other terms, and may itself be due to differing susceptibility to autocrine inhibition.

The potential use of autocrine control to manipulate milk secretion cannot be considered in isolation. Milk yield can be regulated by a number of means independently, notably by bovine growth hormone (bGH) treatment, which stimulates milk yield in dairy cows by 10-30%, principally through homeorhetic partitioning of nutrients towards the mammary gland (Bauman *et al.*, 1985; Davis *et al.*, 1988). The relative merits of these treatments, or of a combination of treatments, are worthy of evaluation. For example, bGH treatment and three times daily milking of goats had an additive effect on milk yield, with stimulation of up to 55% recorded for the combined treatment (Knight *et al.*, 1990b). Significantly, this hormone treatment and thrice daily milking together produced a synergistic increase in lactation persistency and mammary secretory cell differentiation. If increased persistency were indeed associated with a carry-over effect on cessation of such manipulations (as discussed above), then neutralization of autocrine control combined with bGH treatment could be used to achieve long term benefit from a limited period of treatment.

ROBOTIC MILKING

Within a few years, farmers may have the option of fully- automated milking by robotic milking stations, thereby dispensing with the need for labour in the milking parlour and the imposition of a milking regimen. It is likely that this would result in a greater increase in milking frequency than can presently be accomodated, since cows given free choice elect to be milked five or six times a day (Rossing, 1985). The benefits for milk production are obvious, particularly since evidence suggests that the yield limit is not reached by three times daily milking. It could also increase the persistency of lactation, for the reasons discussed above. Robotic milking would include the computerized monitoring of indices of animal health, such as milk conductivity and body temperature, so that as well as providing more time for husbandry, it could also bring about an improvement in animal welfare.

Robotic milking would effectively remove the need for direct manipulation of autocrine control, except perhaps when a temporary reduction in yield was required. However, it might nevertheless be advantageous to monitor the degree of autocrine inhibitor in animals milked by robot, in order to determine the optimum frequency of milking for each animal. Cows electing to be milked more frequently than was required to minimise autocrine inhibition would then be allowed only restricted access to the milking parlour.

CONCLUSION

Autocrine regulation by the feedback inhibitor modulates endocrine control of the mammary gland in a manner which acts to match supply of milk with the demand of the milking regimen. The details of this tactical control of mammary function remain to be uncovered. However, manipulation of autocrine control offers the prospect of controlling milk production in a novel and more specific way than has hitherto been possible.

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