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FEED-BACK CONTROL OF MILK SECRETION IN THE GOAT BY A CHEMICAL IN MILK

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SUMMARY

1. In order to investigate the nature of the inhibition of milk secretion during a long milking interval, goats were treated in three possible ways: (i) milked twice daily at 08.00 h and 16.00 h or (ii) milked thrice daily at 00.00 h, 08.00 h and 16.00 h, or (iii) milked thrice daily at 00.00 h, 08.00 h and 16.00 h, but at 00.00 h the milk removed was replaced with an equal volume of isosmotic sucrose solution. The latter treatment was carried out in order to subject the gland to a degree of physical distension equivalent to that on treatment (i).

2. On either thrice-daily milking or thrice-daily milking with sucrose replacement, milk secretion rate over the 16.00–08.00 h period was significantly higher (by about 10% in both cases) than on twice-daily milking.

3. Secretion rates of lactose, milk protein, citrate and calcium during the 00.00–08.00 h period were similar on either thrice-daily milking or thrice-daily milking with sucrose replacement; the secretion rate of fat was significantly higher on thrice-daily milking with sucrose replacement.

4. Secretion rates of Na^+, K^+ and Cl^- were significantly higher on thrice-daily milking with sucrose replacement. In the case of Na^+, the increased Na^+ secretion rate was sufficient to create a normal Na^+ concentration in the milk/sucrose mixture removed at the next milking. In the cases of K^+ and Cl^-, their secretion rates were not sufficient to restore their concentrations to normal by the next milking.

5. It is concluded that physical distension does not cause the reduction in milk secretion which normally takes place in the latter part of a long milking interval. Instead it is concluded that there is in milk a locally-active chemical inhibitor, which reduces milk secretion by negative feed-back during this time.

INTRODUCTION

The control of milk secretion remains an enigma despite years of work on the physiology, endocrinology and biochemistry of lactation. However, one major influence on the rate of milk secretion is the frequency of suckling or milking. In ruminants, milk removal is required for this effect; the milking stimulus alone has no effect, since applying an extra milking to one udder half has no effect on milk yield of the other udder half (Morag, 1973; Henderson, Blatchford & Peaker, 1983). This shows that local intramammary mechanisms are important in the control of secretion

during lactation. Intramammary mechanisms also play important roles in controlling secretion at the onset and cessation of lactation (Fleet & Peaker, 1978; Maule Walker & Peaker, 1980; Peaker, 1980); for instance mammary distension by the accumulated milk is responsible for the arrest of secretion after the complete cessation of lactation in the goat (Peaker, 1980). The inhibition of milk secretion which takes place during lactation after several hours of milk accumulation has been generally assumed to be caused by the physical presence of milk accumulating in, and distending, the mammary gland (e.g. Petersen & Rigor, 1932; Schmidt, 1971). However, there is some evidence suggesting that a chemical in milk may inhibit milk secretion (see Linzell & Peaker, 1971*a*). In the present study, the nature of the inhibition of secretion with milk accumulation is investigated using a technique which distinguishes between the physical presence of the accumulated milk and the presence of chemical constituents of this milk. The findings of the experiment are also relevant to the study of the mechanism of milk secretion.

METHODS

Animals. The experiment was conducted on a group of six goats, which, at the start of the experiment, had been lactating for 170–176 days and were yielding 2·1–4·1 kg milk per day. Normally the goats are milked twice-daily at approximately 08.00 h and 16.00 h. At each milking the weight of milk removed and the time of milking was recorded; milk secretion rates were then calculated in ml/h.

Experimental protocol. In the first experimental period of 4 days, the animals were milked thrice-daily at approximately 00.00 h, 08.00 h and 16.00 h. Next the animals were returned to twice-daily milking for 2 days, in order to avoid any possible carry-over effects. In the second experimental period, again of 4 days, the animals were milked thrice-daily at approximately 00.00 h, 08.00 h and 16.00 h, but after each midnight milking a volume of sterile 300 mM-sucrose solution, equal to the volume of milk removed, was infused into the lumen of the mammary gland via the teat canal. Milk secretion rates during the two experimental periods are compared with those during the control periods on twice-daily milking. The control periods were: C1, the 3 days preceding the first experimental period; C2, the 2 days between the two experimental periods; C3, the 3 days succeeding the second experimental period.

All treatments were on one mammary gland only; the other mammary gland continued to be milked twice daily at 08.00 h and 16.00 h throughout. It has been shown that unilateral thrice-daily milking has no effect on the yield of the contralateral gland milked twice-daily (Henderson *et al.* 1983).

The replacement of the milk removed at 00.00 h with an equal volume of isosmotic sucrose was designed to mimic the physical presence of the removed milk, but without the presence of the chemical constituents of milk. It was considered desirable to check that the distribution of sucrose within the udder was similar to that of the milk prior to milking; for instance the infused sucrose solution might have remained only in the cistern and not reached the alveoli, whereas milk would have been present before milking in both the cistern and alveoli. An experiment was carried out to test this possibility.

At a milking, a certain amount of milk remains in the alveoli of the mammary gland. This amount is approximately 10–15% of the yield at the milking. In order to ascertain whether the infused sucrose solution reaches the alveoli, a volume of isosmotic sucrose solution equivalent to the volume of milk secreted in 8 h was infused into the cisterns of four goats, which had just been milked. The milk/sucrose mixture lying in the cistern was then sampled via an intracisternal catheter. If the sucrose solution remained in the cistern and did not enter the alveoli, then the concentration of milk-specific substances in the solution in the cistern should be negligible. Table 1 shows that, on the contrary, considerable mixing of the sucrose solution and the alveolar milk took place very quickly. The amount of lactose in the sucrose/milk mixture at 10 min post-infusion can only be accounted for by the mixing of all the alveolar milk with the sucrose solution. Therefore entry of

the infused solution into the alveoli was immediate. The results of the samplings at 30 min and 6 h post-infusion show that lactose continued to enter the sucrose solution, but at a rate much slower than that seen in the first 10 min post-infusion. This slower rate can be accounted for by the normal secretion rate of lactose by the gland; the fast rate over the first 10 min can only be accounted for by the mixing of a considerable quantity of lactose, which was already present in the gland. It is concluded therefore that the infused sucrose solution was distributed rapidly throughout the mammary gland.

TABLE 1. Lactose concentration in the milk/sucrose mixture following infusion of a volume† of isosmotic sucrose solution at 37 °C into a mammary gland of four goats.‡ (mean ± S.E. of mean)

Time post-infusion	10 min	30 min	6 h
Lactose concentration (mM)	11·9 ± 3·8	13·7 ± 3·3	58·0 ± 4·9

† The volume infused was equivalent to the volume of milk secreted over an 8 h period.
‡ The goats were milked just before the infusion.

Intramammary infusion. Sterile 300 mM-sucrose solution at approximately 37 °C was pumped into the cistern by a Watson Marlow 601 pump (Watson Marlow, Falmouth, Cornwall) via an 18 gauge needle cannula inserted into the teat canal. The infusion rate was approximately 400 ml/min. The solution had been sterilized by passage through a membrane filter of 0·2 μm pore size. Sucrose was of AnalaR grade. Linzell, Mepham & Peaker (1976) and Peaker (1977) have discussed the validity of such intramammary infusions in terms of their failure to induce osmotic water movements.

Chemical analyses. Milk from the experimental gland was sampled at each milking and analysed for Na^+, K^+, Cl^- and lactose (Neville & Peaker, 1981), protein (Mabon & Brechany, 1982), fat (Fleet & Linzell, 1964), citrate (White & Davies, 1963) and calcium (atomic absorption spectrophotometry). The presence of sucrose in some samples did not interfere with lactose measurement. Secretion rates of these components were calculated from milk secretion rates and their concentrations in the milk.

RESULTS

Thrice-daily milking increased milk secretion rate over the 16 h interval by approximately 10%, a figure similar to that seen in previous studies using goats in this laboratory (Henderson & Peaker, 1983; Henderson *et al.* 1983). There was no carry-over effect from the period of thrice-daily milking, since the C2 (control period between treatments) figure was similar to the other control figures (Table 2). Since by analysis of variance there was no significant difference between the three control periods, control figures were bulked before analysis. Thrice-daily milking with sucrose replacement increased milk secretion by as much as did thrice-daily milking (Table 2).

The rates of secretion of various components of milk were also investigated (Table 3). Secretion of the three major components of milk, lactose, fat and protein proceeded at similar rates (or indeed greater rates in the case of fat) whether the milk removed after 8 h was replaced with sucrose solution or not. The secretion of calcium and citrate was also not affected whether the milk removed after 8 h was replaced or not, but secretion of Na^+, K^+ and Cl^- was higher after sucrose infusion.

Table 4 shows the concentrations of the various components in milk 8 h after the sucrose replacement. If the secretion rate of any component were not affected by sucrose infusion, the concentration of that component in the milk/sucrose mixture would be expected to be approximately one-half of control values. This was the case for lactose, protein, citrate, fat and calcium. However, none of the monovalent ions

TABLE 2. Milk secretion rates (ml/h) over the 16 h interval during control periods and during periods of thrice-daily milking and thrice-daily milking with sucrose solution replacement† (mean ± S.E. of mean)

Control periods	Milk secretion rates	Experimental periods	Milk secretion rates
C1	64·3 ± 12·2	3 × milking	68·5 ± 11·5***
C2	61·3 ± 11·1	Sucrose replacement	68·0 ± 12·8*
C3	61·6 ± 10·6		
C_T	62·4 ± 11·3		

Protocol: twice-daily milking for 3 days (C1); thrice-daily milking for 4 days; twice-daily milking for 2 days (C2); thrice-daily milking with sucrose replacement for 4 days; twice-daily milking for 3 days (C3).

Analysis of variance showed no significant differences between the three control periods and therefore data have been bulked for analysis (C_T).

By comparison with C_T (paired t test) * $P < 0.05$, *** $P < 0.001$.

† For details, see text.

TABLE 3. Rate of secretion of milk and its components between 00.00 and 08.00 h during thrice-daily milking, or thrice-daily milking with sucrose replacement† (mean ± S.E. of mean)

	Thrice-daily milking	Sucrose replacement
Milk (ml/h)	70·7 ± 11·7	72·6 ± 13·3
Lactose (mmol/h)	9·0 ± 1·5	9·6 ± 1·7
Fat (g/h)	2·37 ± 0·47*	3·08 ± 0·49*
Protein (g/h)	1·77 ± 0·21	1·93 ± 0·47
Citrate (mg/h)	75·8 ± 24·6	69·1 ± 28·2
Calcium (mg/h)	60·5 ± 8·9	70·8 ± 12·2
Na^+ (mmol/h)	1·03 ± 0·13***	2·60 ± 0·27***
K^+ (mmol/h)	3·52 ± 0·64*	4·58 ± 0·84*
Cl^- (mmol/h)	3·33 ± 0·52**	5·44 ± 0·77**

Rates of secretion were calculated as mean figures for the 4 days of thrice-daily milking, or thrice-daily milking with sucrose replacement, respectively. Comparisons by paired t test: * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

† For details see text.

TABLE 4. Effect of sucrose replacement on composition of the milk/sucrose mixture at the next milking (mean ± S.E. of mean)

	Control	Sucrose replacement‡
[Na^+] (mM)	15·8 ± 0·7	19·9 ± 2·4
[K^+] (mM)	53·1 ± 1·8***	32·4 ± 1·0***
[Cl^-] (mM)	49·0 ± 1·9*	39·9 ± 2·3*
[Lactose] (mM)	140·3 ± 1·7***	68·9 ± 2·0***
[Protein] (g/100 ml)	2·9 ± 0·2***	1·6 ± 0·2***
[Citrate] (mg/100 ml)	105·2 ± 19·9**	43·8 ± 10·4**
[Fat] (g/100 ml)	3·8 ± 0·4**	2·2 ± 0·1**
[Calcium] (mg/100 ml)	97·6 ± 3·3***	50·7 ± 1·6***

† Control figures are for the 08.00 h milking, the morning before the first sucrose injection.

‡ Mean of all four 08.00 h milkings, following sucrose replacement.

* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$ (paired t test).

conformed to this scheme. Na^+ secretion increased sufficiently to restore the concentration of Na^+ in the milk/sucrose mixture to its normal concentration in milk. While the secretion rates of K^+ and Cl^- increased following sucrose infusion (Table 3), these increases were not sufficient to restore their concentrations to normal levels for milk (Table 4).

DISCUSSION

Linzell *et al.* (1976) first used a technique similar to that employed in the present study. They injected 100 ml 300 mM-lactose into the lumen of the goat mammary gland, 2 h after morning milking, and studied the secretion rate of various components in milk. They showed that there was little or no water movement into or out of the diluted milk over the 6 h period in which it lay in the gland. The present study, using a much greater dilution of milk, with consequently greater concentration gradients between the intracellular fluid and milk, confirms many of their conclusions. Both the present and the earlier study show that secretion of protein, citrate and calcium is unaffected by intramammary infusion of an isosmotic disaccharide solution while Na^+, K^+ and Cl^- entered milk at above the normal rates. The present study also shows that secretion rate of neither lactose nor fat was reduced by the procedure. The monovalent ions entered the stored milk, tending to restore milk concentrations of these substances to normal. In the case of Na^+, the concentration was fully restored while for K^+ and Cl^- this restoration was only partial.

The secretion of monovalent ions in milk has been reviewed by Peaker (1978), following studies by Linzell & Peaker (1971*b*, *c*) and it was concluded that Na^+ and K^+ are freely distributed between intracellular fluid and milk according to the potential difference across the apical cell membrane. In the present study Na^+ attained its normal concentration in the diluted milk, while K^+ did not. Under the influence of a high K^+ concentration gradient, particularly shortly after sucrose infusion, K^+ movements into milk increased but even after 8 h these movements proved insufficient to allow the establishment of the normal K^+ concentration in the milk/sucrose mixture. Clearly free distribution of K^+ did not take place during this experiment, suggesting that either the permeability of the apical membrane to K^+ is relatively low, or that during normal milk secretion, K^+ is not freely distributed across the apical cell membrane.

Peaker (1978) noted that there is uncertainty regarding Cl^- movements but in his model showed free movement of Cl^- into milk, aided by both concentration and electrical gradients and opposed by an active Cl^- pump which would keep the milk Cl^- concentration low. The present study suggests that the rate of movement of Cl^-, from intracellular fluid into milk is relatively slow.

Neville & Peaker (1981) have suggested that ionized calcium in milk is essential to preserve the integrity of the mammary epithelium. The present study shows that concentrations of calcium in milk considerably below normal were adequate for this function, since there was no evidence for disruption of the mammary epithelium.

During the sucrose infusion experiment, the milk removed at the midnight milking was replaced with an equal volume of isosmotic sucrose solution; there would therefore be an equal degree of physical distension within the gland during the subsequent 8 h whether the gland remained unmilked or whether it was milked and the milk replaced by sucrose. The rate of milk secretion over the 16 h (16.00–08.00) period was as high when milk was replaced by sucrose solution after 8 h as when the animals were simply milked at this time. This clearly demonstrates that physical distension does not cause the inhibition of milk secretion during the latter half of the 16 h

period. Instead it is the presence of milk itself which, on twice-daily milking, reduces the rate of milk secretion at this time.

It is clear that removal of milk after 8 h of accumulation removed a locally active inhibitory influence on milk secretion. It would appear that relief from negative feed-back inhibition followed removal of a chemical component(s) of milk. Relief from chemical inhibition can also account for the results of Linzell & Peaker (1971*a*) who showed that very frequent milking, with the aid of physiological doses of oxytocin, increases milk secretion.

It is not known whether the inhibitor is secreted continuously or whether its secretion accelerates as accumulation of milk takes place. The evidence that hourly milking with the aid of oxytocin can increase milk secretion rate (Linzell & Peaker, 1971*a*; Blatchford & Peaker, 1982) suggests continuous secretion of the inhibitor, since hourly milking is thought to act by milking out a chemical inhibitor. Certainly the inhibitor is already present in the milk by 8 h after last milking, so it is not just secreted towards the end of a long milking interval. If the inhibitor is secreted continuously, then how are its effects controlled? It is possible that as milk accumulates within the gand, the sensitivity of the secretory cells to the inhibitor increases. This presents a possible way in which both chemical and physical effects could contribute to the inhibition of secretion; while the present study shows the necessity of the chemical factor for the inhibition to take place, it does not preclude a subsidiary role for physical effects in modulating the response of the secretory cells to the chemical inhibitor. Alternatively the inhibitor may not be secreted at a constant rate, but its secretion rate might increase as accumulation of milk takes place. For a fuller discussion of the possible actions of the inhibitor see Henderson (1983).

The identity of the chemical inhibitor is unknown, but Maule Walker & Peaker (1980) found that prostaglandin $F_{2\alpha}$ plays a local inhibitory role in the mammary gland during late pregnancy in the goat, and they suggested that prostaglandin $F_{2\alpha}$ may play a role in inhibiting the rate of secretion at that time. Further work is required to determine whether prostaglandin $F_{2\alpha}$ can also reduce secretion rate during lactation.

The present study makes it clear that the increased milk yield on thrice-daily milking in goats may be accounted for by the milking out of a local chemical inhibitor. It is suggested that this mechanism may also explain the increased milk yield on three or four times milking in the cow. Indeed this control of milk secretion by a chemical in milk may be a general mechanism to match secretory rate to the demand of the young in many species.

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