REVERSIBLE SUPPRESSION OF MILK SECRETION BY CONCANAVALIN A

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Received 25 August 1976

1. Introduction

Our previous research [1,2] has shown that intramammary infusions of the plant alkaloids colchicine or vincristine suppress ongoing secretion of milk in the goat. These agents are known to inhibit the assembly of microtubules [3], organelles which are reputed to give structural integrity to the golgi apparatus [4,5] and to secretory processes of the cell [6,7]. Recent observations indicate an association of cytoplasmic microtubules with components of the plasma membrane in several cell types [6,8], including the lactating mammary cell [9], and that the plant lectin, concanavalin A (Con A), can cause a redistribution (gathering) of these microtubules as a result of its binding to cell-surface receptors [10]. Since Con A represents an entirely different approach than colchicine and the vinca alkaloids to perturbation of cytoplasmic microtubules, its capacity to inhibit milk secretion was evaluated. The lectin produced a suppression closely resembling those by the plant alkaloids. It has already been shown that apical plasma membrane of the lactating cell effectively binds Con A [11] and that the ectoenzyme, 5′-nucleotidase, of the membrane is thereby inactivated [12]. It is a reasonable working hypothesis that substances interacting with Con A in this membrane are involved directly in the milk secretion (exocytosis) mechanisms.

2. Materials and methods

The goats employed were in mid-lactation (3–5 months) and yielding 2–3 liters of milk per day. None of the animals had been subjected to previous intramammary infusions during their current lactations. Two sources of Con A were evaluated: one with the protein dissolved in sterile 5% glucose solution (Calbiochem, LaJolla, California, USA); the other a purified, dry, salt-free protein (Sigma Chemical Company, St. Louis, Missouri, USA). These and other materials studied were made to 5 ml with distilled water after which each solution was infused into one-half of a goat's udder. Consecutive complete milkings were taken at 12 h intervals (0900 and 2100) starting from 24–36 h before an infusion and for 60 h to 10 days following it. All infusions were made immediately following a complete milking. The infusion procedure and methods to quantitate the major milk constituents (fat, protein and lactose) have been described [1,2].

In order to check whether the response to Con A was of a general nature, such as to foreign proteins, the following infusates were evaluated: 25 mg of conalbumin (Schwarz/Mann, Orangeburg, New York, USA) dissolved in 5 ml of water, 0.5 ml of fresh human serum (approx. 100 mg of protein) made to 5 ml with water, and 25 mg of Con A (Sigma) dissolved in 5 ml of water which solution was then heated 5 min in a boiling water bath to denature the protein and cooled to 38°C.

3. Results and discussion

Con A produced substantial depressions in milk flow in the four animals tested. The results presented in fig.1 are representative. Both sources of Con A were effective, the Calbiochem preparation at a somewhat lower concentration range (about one-half) than that

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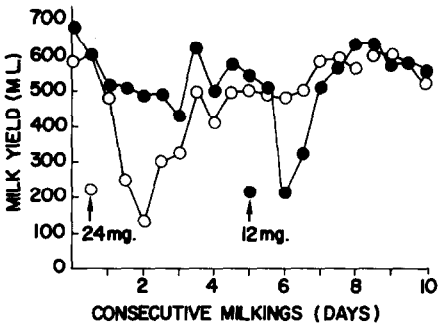

Fig.1. The effects of intramammary infusions of concanavalin A (Calbiochem) on yields of milk obtained at consecutive 12 h intervals from the left (o–o–o) and right (•–•–•) halves of a goat's udder. The first infusion contained 24 mg of the lectin administered into the left half of the udder immediately following the milking on day 0.5; the second infusion containing 12 mg was inserted into the right half of the udder after the milking on day 5.

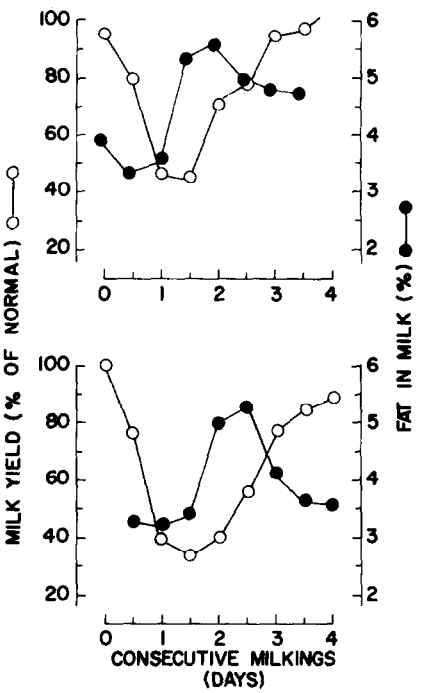

Fig.2. The relationship between the depression in milk yield (o–o–o) induced by an intramammary infusion of concanavalin A (upper) or of colchicine (lower) on the fat content (•–•–•) in consecutive 12 h milkings of two goats. The concanavalin A (18 mg) or colchicine (5 mg) both from Calbiochem, were infused into half the udder after the milking on day zero. The colchicine data are from ref. [2].

from Sigma. Infusions of conalbumin, human serum, heat-denatured Con A and water alone produced no significant depression in milk yield. An infusate was considered to have depressed the milk yield significantly when any one of the first five milkings from the infused side fell at least 100 ml below the average for the two milkings on that side immediately preceding the infusion. All eight infusions of native Con A produced significant depression by this criterion (223–454 ml below the pre-infusion averages) and it appears that the suppressing effect on milk flow is specifically dependent on configuration of the native protein. To our knowledge this is the first evidence that Con A can act as an antagonist of exocytosis.

The suppression of lactation obtained with Con A closely resembles that induced by colchicine. It occurs only on the treated side of the udder as shown in fig.1, and thus appears to be a localized effect directly on the mammary tissue. It reaches a maximum in 24–36 h and is fully reversed by about 96 h (see figures herein and ref. [1]). Milk obtained during the depressed yield period is generally within the range of normal composition for the major milk components (fat 2.5–7.0%, protein 2.5–5.0%, lactose 4.3–5.0%). All of these observations hold for both agents. A further parallel exists in the levels of fat and protein in the milks as recovery to normal flow begins. As shown in fig.2 and 3 both agents tend to effect a rise in the fat and protein of the milk at that time. The somewhat earlier response to the Con A may be due to its acting on the cell surface whereas the colchicine may have to achieve an effective concentration within the cell. Electron microscopy studies of the effects of colchicine on lactating mammary tissue (goat and rat) indicate that the agent inhibits release of fat droplets and the contents of secretory vesicles from the cell leading to a build up of these components within the cell (B. H. Stemberger, C. J. Knudson, and S. Patton, unpublished). Thus as the capacity to secrete is regained, these 'backed up' constituents elevate levels of fat and protein in the milk (figs.2 and 3).

While colchicine is a relatively small molecule, mol. wt 399.43, Con A is a protein with a mol. wt 110 000 [13]. On the evidence that 25 mg of Con A produces a suppression in milk flow equivalent to that from

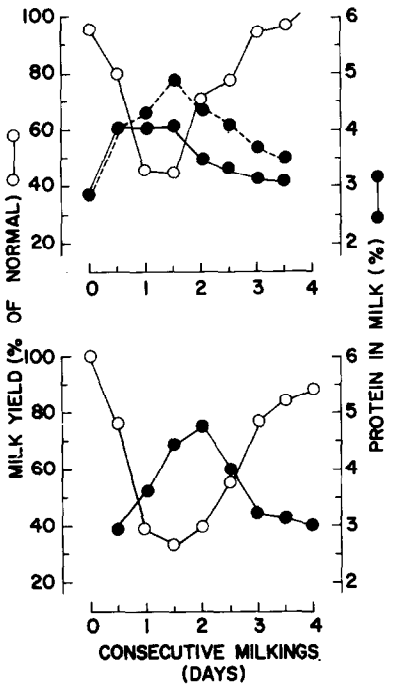

Fig.3. The relationship between the depression in milk yield (o–o–o) induced by an intramammary infusion of concanavalin A (upper) or of colchicine (lower) on the protein content (•–•–•) in consecutive 12 h milkings of goats. The same animals and infusates are involved as in fig.2 except that trends in milk protein data (only) for a third goat infused with 24 mg of concanavalin A is shown by the broken line-connected data.

about 5 mg of colchicine, the Con A is roughly 50 times as effective as the colchicine on a molecular basis. One way to rationalize the quite similar effects of the two agents on milk secretion is by the idea that both interfere with microtubule function, colchicine by inhibiting their formation within the cell and Con A by causing their aggregation (dysfunction) through the mediation of Con A receptors in the cell surface as described by Albertini and Clark [10]. If indeed Con A perturbs the milk secretion mechanism by altering surface components of the lactating cell, a highly useful probe of the secretion mechanism is at hand.

The fact that milk-fat globules are enveloped in plasma membrane at the time of their secretion makes secretory surface of the cell conveniently accessible for study [14]. However, demonstration that Con A acts directly on the lactating cell to inhibit its secretory activity will be required before the lectin can be seriously considered as a molecular probe of exocytosis.

Acknowledgements

Fat and protein analysis were performed at the University's Central Milk Testing Laboratory through the courtesy of Dr Herbert C. Gilmore. The research was supported in part by grant HL 03632 of the USA Public Health Service.

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