A role for adrenaline and calmodulin in modulating cyclic AMP levels during the lactogenic cycle

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The effect on lactose production of several external modulators of intracellular cyclic AMP was studied in rat mammary gland tissue slices and explants. Adrenaline, a β-adrenergic receptor effector, forskolin, a direct adenylate cyclase activator and fluphenazine, a calmodulin inhibitor, all produced an increase in the intracellular level of cyclic AMP and a concomitant inhibition of lactose production. These results suggest a role for adrenaline and calmodulin in modulating cyclic AMP levels in mammary tissue during the lactogenic cycle.

Mammary gland *Cyclic AMP* *Lactose* *Calmodulin* *β-Adrenergic receptor*

1. INTRODUCTION

It is well known that cyclic nucleotides and Ca^{2+} are key regulatory signals in the normal progress of the lactogenic cycle in the rat and other mammals [1–3]. Cyclic AMP presents a sustained increase during pregnancy reaching a maximum near parturition and a minimum at the beginning of lactation. Levels of cyclic GMP, on the other hand, show opposite changes. This pattern suggests that cyclic AMP is a negative controlling factor for lactogenesis through phosphorylation of key proteins and/or repression mechanisms of those systems involved in the synthesis and liberation of milk constituents. The most dramatic and rapid effect of cyclic AMP in mammary tissue is the inhibition of lactose production, for which several explanations have been offered [4]. A unifying element for all of them has been the involvement of calmodulin, a low-M_r protein which binds Ca^{2+} and modulates several enzyme activities, particularly cyclic nucleotide phosphodiesterase which on activation lowers the intracellular cyclic AMP concentration. Both the activation of the high-K_m cyclic AMP phosphodiesterase by calmodulin and the increase in calmodulin during lactation are in accordance with the cyclic AMP decrease mentioned for this period [2,3].

Here, we describe experiments in which we have analyzed the effect of adrenaline (a β-adrenergic receptor effector), forskolin (an adenylate cyclase effector) and fluphenazine (a calmodulin inhibitor) on lactose production in rat mammary gland tissue. The results suggest that both adrenaline and calmodulin may play an important role in modulating cyclic AMP levels during the lactogenic cycle.

2. MATERIALS AND METHODS

Sprague-Dawley rats at middle pregnancy and lactation were used. The animals were killed by decapitation and the abdominal mammary glands were prepared directly for determination of enzymes and metabolites or for explant cultures.

Cyclic [8-^{3}H]AMP and L-[^{35}S]methionine were obtained from New England Nuclear; Dulbecco's modified Eagle's medium (DMEM) from Flow Laboratories; (−)-adrenaline, β-galactosidase and galactose dehydrogenase from Sigma; and for-

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skolin from Calbiochem. 2-Chloro-10-(3-aminopropyl)phenothiazine (CAPP), conjugated to Sepharose 4B (CAPP-Sepharose) was a gift from Dr E. Jedlicky (Dept of Biochemistry, Faculty of Medicine, University of Chile).

2.1. *Calmodulin assay*

The stimulation of activator-deficient cyclic AMP phosphodiesterase was determined as described in [2].

2.2. *Cyclic AMP and lactose determinations*

Cyclic AMP was determined by the competitive protein binding assay of Tovey et al. [5], and lactose by the double enzyme method (β-galactosidase/galactose dehydrogenase) [6].

2.3. *Adrenaline effect on cyclic AMP and lactose production*

This effect was studied in mammary gland explants from middle lactation, freed of pre-existent lactose [7], and cultured in DMEM. The incubation was performed in the presence of increasing concentration of (−)-adrenaline (10^{-7}–10^{-4} M) in 0.5 M ascorbic acid. Cyclic AMP levels in explants were determined after 10 min incubation; lactose determination in explants and media was carried out after 120 min incubation.

2.4. *Fluphenazine and forskolin effects in lactating mammary gland slices*

Rat mammary gland slices were prepared according to Loizzi et al. [4]. The slices were incubated with shaking in Krebs-Ringer bicarbonate solution in the presence and absence (control) of 200 μM fluphenazine or 100 μM forskolin. Tissue lactose and cyclic AMP were determined at the indicated times.

2.5. *Calmodulin characterization*

Mammary gland explants were cultured in DMEM containing bovine insulin (5 μg/ml), ovine prolactin (10 μg/ml) and corticosterone (1 μg/ml) [8]. [^{35}S]Methionine (100 μCi) was added to the media for 48 h, then the purification steps described by Charbonneau and Cormier [9] were used. A CAPP-Sepharose 4B column, the last step of purification, equilibrated with 10 mM Hepes, 0.5 M $CaCl_2$, pH 7.0, was washed with the same buffer containing 0.5 M NaCl. Calmodulin was eluted with 10 mM Tris-HCl, 5 mM EGTA, pH 8.0. Calmodulin activity and radioactivity were determined in the elution volume.

3. RESULTS AND DISCUSSION

Changes in cyclic AMP and lactose concentration in response to adrenaline (see section 2) are shown in fig.1. A sizeable increase in cyclic AMP in response to increasing concentrations of adrenaline was observed together with a concomitant decrease in lactose, in both the tissue explant and incubation medium.

To determine the effect of calmodulin on lactose production we incubated mammary gland slices in Krebs-Ringer bicarbonate in the presence and absence of 200 μM fluphenazine. As shown in fig.2, there is a sustained decrease with time in lactose concentration in the medium which reached 35% of the control level 90 min after addition of the inhibitor. The decrease in lactose was accompanied by an increase in cyclic AMP concentration which reached a maximum during the first minute of incubation (fig.2). The presence of calmodulin

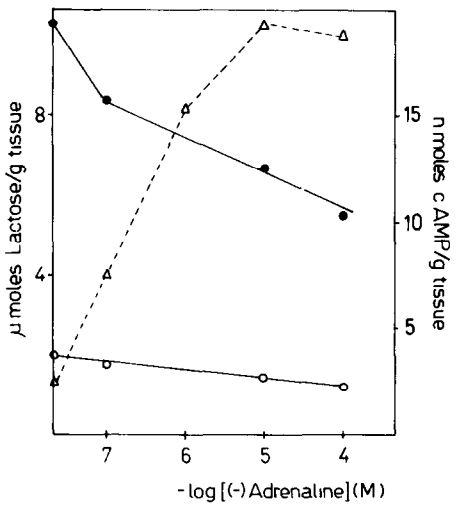

Fig.1. Effect of (−)-adrenaline on cyclic AMP production in explants of lactating mammary gland. (△) Tissue explant cyclic AMP; (●) tissue explant lactose; (○) lactose in the incubation media. Each value represents the mean of 3 determinations with SE not exceeding 5% for lactose and 10% for cyclic AMP values.

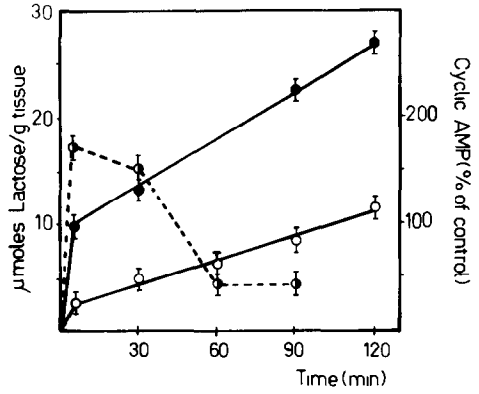

Fig.2. Fluphenazine (200 μM) effect on lactose secretion (○) and tissue cyclic AMP (◑); (●) lactose secretion without fluphenazine. Initial cyclic AMP value 7.0 ± 0.5 nmol/g. Each value represents the mean of 3 different determinations ± SE.

was verified by the reversible calcium-dependent binding property of this protein to immobilized phenothiazines (CAPP-Sepharose column). As shown in fig.3, there is a good correlation between the maximal radioactive profile and the maximal activation of phosphodiesterase after elution with EGTA. Incubation of mammary gland slices with forskolin at 100 μM resulted in an increase of 90% of tissue cyclic AMP and a decrease of 50% of lactose in the medium after 60 min incubation (not shown).

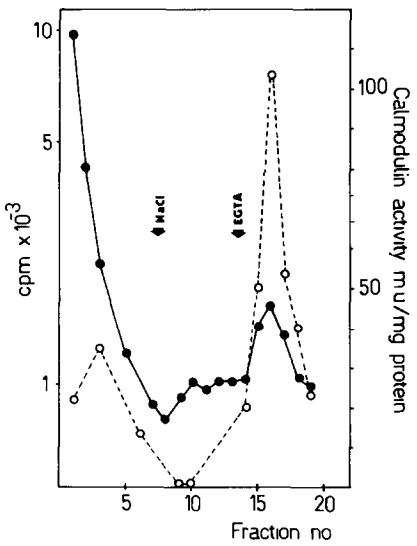

Fig.3. CAPP-Sepharose 4B chromatography of mammary gland calmodulin. Fractions were assayed for radioactivity (●) and calmodulin activity (○) (for details see section 2).

The inhibitory action of cyclic AMP on lactose production is a rapid process in which several factors would act as a whole, the inhibition of glucose uptake [10] and the decrease in α-lactalbumin production being the most direct effects [11]. On the other hand, the biochemical mechanisms which account for the decrease in cyclic AMP level, allowing the onset of lactogenesis, are also multiple; amongst these are the hydrolysis by cyclic AMP phosphodiesterase and the excretion of cyclic AMP through milk as lactation proceeds [4,12]. Our results suggest that both adrenaline and calmodulin may play an important role in modulating cyclic AMP levels during the lactogenic cycle. Adrenaline most likely exerts its effect by triggering β-adrenergic receptors [13] and together with other hormones acting through adenylate cyclase may be responsible for the rise in cyclic AMP. A role for calmodulin is not surprising as we recently presented evidence for the existence in mammary gland of a protein with similar characteristics [14]. Furthermore, we have shown that anticalmodulin in drugs such as phenothiazines are efficient inhibitors of the stimulatory action of prolactin on RNA and casein synthesis in mammary gland explants from virgin rats [14]. Taken together, our present results confirm the existence and functionality of calmodulin in the gland and support its role in association with Ca^{2+}, in differentiation as well as in prolactin action [14,15].

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