THE ROLE OF CYCLIC NUCLEOTIDES IN THE DEVELOPMENT AND FUNCTION OF RAT MAMMARY TISSUE

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1. Introduction

It has been previously reported that the tissue content of cyclic AMP in rat mammary gland rises continuously to the end of pregnancy and then falls progessively to its lowest value by the 16th day of lactation [1], suggesting that the growth and development of the gland are related to the issue content of cyclic AMP, while the initiation, and scale, of lactation may be related to the decreasing content of the nucleotide. In studies with rat mammary gland explants in culture [2] cyclic AMP inhibited the increase of enzyme activities normally associated with lactogenesis, in particular the activities of those enzymes involved in lipogenesis, and also markedly depressed the synthesis of DNA, RNA and fatty acids. This analogy with the pleiotypic response observed for other cells grown in culture [3], and the reversal by cyclic GMP of the pleiotypic inhibition of 3T3 cells treated with dibutyryl cyclic AMP [4], led us to investigate the simultaneous fluctuations of both cyclic nucleotides throughout the lactation cycle.

The results reported here show a coordinated change in the levels of cyclic AMP and cyclic GMP during the different stages of the cycle, with the cyclic AMP being high during pregnancy and low in lactation and cyclic GMP showing the reverse pattern, which supports the hypothesis [5] that cyclic GMP is involved im promoting cellular events that are antagonistic to those mediated by an elevation of cellular cyclic AMP. The process of differentiation of the mammary gland during pregnancy, its response to hormones and the promotion of specific cell programmes for the synthesis of the characteristic components of milk may all be mediated by modification of the ratio between the two nucleotides.

2. Methods

Mated female rats were taken either on the 10th, 15th or 20th days of pregnancy or on the 1st, 10th or 15th days of lactation and their abdominal mammary glands removed under nembutal anaesthesia preparatory to freezeclamping the tissue. The nucleotides were extracted as previously described [6] and then freeze-dried. They were dissolved, immediately before use, in 10 mM acetate buffer, pH 4.0, to give an extract equivalent to 1 g tissue/ml.

2.1. *Measurement of cyclic AMP and cyclic GMP*

Cyclic AMP was estimated by a radioisotope dilution method with cyclic AMP-binding protein [1,6]. Cyclic GMP was assayed by a modification of a described method [7] using 600 μg of a cyclic GMP-binding protein purified from lobster-tail muscle through the ammonium sulphate (55%) stage [8]. No further purification of the dialysed ammonium sulphate fraction was attempted as this results in severe losses of cyclic GMP-binding activity [9]. 40 μl of tissue or milk extract were used in a final incubation volume of 100 μl. The millipore filters with the protein-bound cyclic GMP were dried and placed in counting vials containing 15 ml of

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scintillant (4 g POP; 0.45 g dimethyl POPOP/litre toluene) and counted (40% efficiency).

For each tissue sample, the milk content was determined and a correction applied for the cyclic AMP and cyclic GMP contents of the milk in the extract.

3. Results and discussion

The content of cyclic AMP and cyclic GMP in the rat mammary gland at different stages of the lactation cycle are shown in Fig. 1, where the results are given as pmoles $\times g^{-1}$. (although it may be noted that a very similar pattern is obtained when the results are expressed on the total tissue basis).

The results show an opposite, and coordinated, change in the levels of the two nucleotides throughout the lactation cycle with the cyclic AMP being high during pregnancy and low in lactation while cyclic GMP shows the reverse pattern. The values for

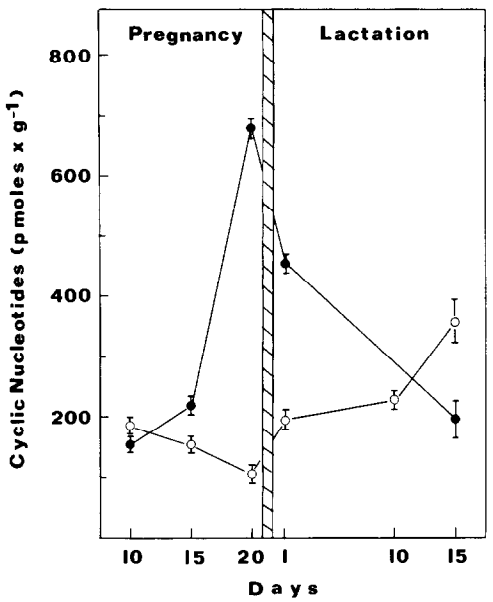

Fig. 1. Tissue contents of cyclic AMP 2nd cyclic GMP in the rat mammary gland at different stages of the lactation cycle. Results are given as the mean values ± SEM and are expressed as pmoles $\times g^{-1}$ milk-free tissue. (Rat milk contained 25000 ± 140 pmoles of cyclic AMP and 264 ± 27 pmoles cyclic GMP per ml). Each value represents the mean of not less than 5 animals. (•) cyclic AMP (○) Cyclic GMP.

cyclic AMP shown here conform closely to those previously reported [1]. The ratio cyclic AMP/cyclic GMP shows a diphasic pattern, with a rapid, and continuous rise throughout pregnancy and a profound decline during lactation. The transition, at parturition, coincides in time with the considerable increase in the metabolic activity of the gland consequent on the initiation of lactation. The inverse relationship between the tissue cyclic AMP content and a number of metabolic parameters, suggesting that the development of the gland is related to its content of cyclic AMP and the initiation and scale of lactation to the removal of the nucleotide, has been discussed elsewhere [1,2,6].

The tissue content of cyclic GMP, which is about 2×10^{-7}M at the middle of pregnancy, declines slowly, but continuously, during this stage to reach only about 50% of this value near parturition, and then starts increasing again in lactation finally to reach its peak on the 15th day of lactation (3.5×10^{-7}M). The minimum value of 10^{-7}M at the end of pregnancy and the maximum of 3.5×10^{-7}M at the peak of lactation are both within the range of concentrations found in many other tissues [5] and comparable, for example, to the changes reported for rat heart after perfusion with acelycholine ($1-3 \times 10^{-7}$M) [10]. At the middle of pregnancy, the concentrations of both cyclic nucleotides are very similar (cyclic AMP/cyclic GMP = 0.81) but, near parturition the level of cyclic AMP is about seven times that of cyclic GMP and at the end of lactation the cyclic GMP content has risen to nearly twice that of the cyclic AMP (ratio = 0.55), clearly showing that each nucleotide preponderates in one of the two main stages of the lactation cycle. Although it is generally considered that the cyclic GMP concentration in a given tissue is usually 1/10th to 1/100th of the level of cyclic AMP in the same tissue, there are many conditions in which they are more comparable, e.g. cerebellum, rat thymus gland and lung, sperm, guinea pig macrophages etc. [5,11]. The fact that the cyclic AMP content in milk (2.5×10^{-6}M) is 10-times that of cyclic GMP (2.6×10^{-7}M) at a stage at which the difference in the intracellular concentrations of the nucleotides is less manifest, suggests that the rate of extrusion of the cellular cyclic GMP from mammary tissue is lower than that for cyclic AMP and that this may serve to maintain a high level of

cyclic GMP in the tissue during lactation. The high extrusion rate of cyclic AMP into the milk may represent a mechanism for ensuring the maintenance of low levels of the nucleotide during lactation.

Further support for the reciprocal control of tissue concentrations of the two nucleotides may be adduced from regulatory influences acting at the level of their enzymatic hydrolysis by phosphodiesterase. Beavo et al. [12] reported that concentrations of cyclic GMP in the range 8×10^{-8}–2×10^{-6} M can stimulate (2–3 fold) the rate of the phosphodiesterase-catalyzed hydrolysis of cyclic AMP in a number of mammalian tissues. It has also been observed that a regulatory mechanism by which the cyclic AMP levels are modulated by increasing cyclic GMP exists in thymocytes and fibroblasts [13,14].

Although cyclic GMP may exert an independent, or mono-directional, role of considerable importance during lactogenesis by its known stimulatory effect on protein synthesis [15,16], the coordinated and opposite changes of the two cyclic nucleotides throughout the lactation cycle is suggestive and may indicate that the antagonism between cyclic AMP and cyclic GMP could be the main modulatory mechanism for the adaptation of the gland and for its responsiveness to hormonal stimulation. The present results support the hypothesis of Goldberg et al. [5] that there are bidirectional systems in which stimulation or inhibition of a process is achieved by a simultaneous drop in concentration of cyclic AMP as that of cyclic GMP is increased.

This 'dualistic' hypothesis is supported by many observations that cyclic GMP promotes events in the cell that are antagonistic to those mediated by cyclic AMP, e.g. the promotion of differentiation by high intracellular concentrations of cyclic AMP and of mitogenesis by high levels of cyclic GMP [17–19]; the effect of cyclic AMP to decrease the responsiveness of postganglionic neurones to impulses and of cyclic GMP to increase it [20]; the requirement of a certain ratio of the two nucleotides to mediate the induction of the immune response [21]; some hormone action, like that of insulin, which acts to lower the intracellular level of cyclic AMP, may also cause a marked increase of cyclic GMP [7] and the fact that cyclic GMP may be related to the F-prostaglandins, much as cyclic AMP relates to the E-prostaglandins, these being the two types of prostaglandins of opposite action [22].

Some of these activities may be highly relevant to the mammary gland a) the role of cyclic AMP as promoter of differentiation could be of special significance to the development of the gland during pregnancy, a stage at which the level of this nucleotide is high; b) the rise of cyclic GMP after the onset of lactation may explain the wave of mitosis which follows shortly after parturition in the rat [23], a process which is also significant (in addition to increasing the number of secretory cells present) in that it may, as suggested by Stockdale and Topper [24], make these cells specially susceptible to environmental factors capable of modifying the metabolic pattern; c) the complex endocrine control of the mammary gland and the tissue response to hormones may be expressed through the dualistic system of opposing cyclic AMP and cyclic GMP levels which could, as has already been shown for other cells grown in culture [3,4], also modulate the pleiotypic control of mammary cells [2].

It is possible that, at the time of parturition, changes of the various factors which oppose the production, or action, of cyclic AMP would be initiated by the generation of one, or more, 'active signals', as has been proposed by Goldberg et al. for other tissues [5], represented by an elevation of the cyclic GMP concentration and that the signals lowering the cyclic AMP level are complementary events permitting a greater response linked to cyclic GMP. The fact that cyclic GMP promotes the hydrolysis of cyclic AMP by the phosphodiesterase [12], an enzyme the activity of which is increased in the mammary gland with the onset of lactation [1,6], lends support to the idea that cyclic GMP contributes actively to the depletion of cyclic AMP which follows parturition.

In addition to this dualistic system of control, performed by the opposing action of cyclic AMP and cyclic GMP, it seems probable that each cyclic nucleotide may exert its own monodirectional modulatory control at different levels in the metabolic machinery of the cell although it may be that the effect of cyclic AMP in the mammary gland is different from the pattern obtaining in general in other tissues where the presence of cyclic AMP is primarily linked to the mobilization of energy reserves and the production of energy.

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