CHANGES OF THE ACTIVITIES OF ADENYL CYCLASE AND cAMP-PHOSPHODIESTERASE AND OF THE LEVEL OF 3'5' CYCLIC ADENOSINE MONOPHOSPHATE IN RAT MAMMARY GLAND DURING PREGNANCY AND LACTATION

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SUMMARY: The activities of adenyl cyclase, the low- and high-K_m cAMP phosphodiesterases and the tissue level of cAMP have been measured in mammary glands taken from pregnant and lactating rats. Adenyl cyclase reaches its maximum activity late in pregnancy and thereafter falls continuously throughout lactation. The high-K_m phosphodiesterase rises slowly in pregnancy but reaches a maximum late in lactation. The tissue cAMP content is maximal at the end of pregnancy and then falls progressively to its lowest level by the sixteenth day of lactation. These changes are discussed in relation to the metabolic adaptation of the gland and its responsiveness to hormonal stimulation.

INTRODUCTION

Growth and differentiation of the mammary gland depend on the action of a number of hormones acting in a sequential manner. Numerous studies have implicated cAMP as the second messenger in hormonal stimulation [1]. cAMP may also lead to the modification of nuclear proteins resulting in changes in the rate of formation of multiple classes of RNA [2] and can control the activities and rates of synthesis of some enzymes. Both of these actions can be related to the presence of phosphorylating protein kinases which are activated by cAMP.

In rat mammary gland there is a *post-partum* increase in the activities of enzymes associated with galactopoiesis, particularly those associated with lipogenesis [3]. Turkington & Riddle [4] noted that the phosphorylation of nuclear protein is associated with the hormonal activation of transcription and Majumder & Turkington [5] reported that mouse mammary epithelial cells contain two protein kinases, one of which is specifically activated by cAMP. During pregnancy the specific activity of these kinases increases some 7—9 fold.

The present results show a coordinated change in the activities of adenyl cyclase and the specific cAMP-phosphodiesterase (PDE) at different stages of the lactation cycle with the cyclase being high in pregnancy and low in lactation and PDE showing the reverse pattern. The tissue content of cAMP at the different stages of the lactation cycle is closely related to the relative activities of the two enzymes. The findings provide some evidence for the

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involvement of the nucleotide in the growth and metabolic activities of the gland.

MATERIALS AND METHODS

Animals. Mated female rats were taken either on the 10th, 15th or 20th days of pregnancy or on the 1st or 16th days of lactation. Two further groups were taken on the 16th day of lactation and were either weaned for 16 hr or were weaned for 16 hr and then resuckled for 2 hr. The rats were killed either by cervical dislocation for the preparation of extracts for enzyme assay or were anaesthetized prior to the removal of glands before preparation of extracts for cAMP assay.

Preparation of extracts for enzyme assay. The abdominal glands were removed, minced and then homogenized in a loose-fitting, all-glass Potter-Elvehjem homogenizer with 4 volumes of medium (40 mM Tris, pH 7.5; 1 mM $MgCl_2$; 1 mM dithiothreitol) at 0°. After straining through gauze the homogenate was centrifuged at 4000 *g* for 20 min. The supernatant from this was used for the estimation of PDE activity. The pellet was washed twice and finally resuspended in 1 vol. of the medium. This preparation of washed membranes was used for the assay of adenyl cyclase.

Preparation of extracts for cAMP determination. The abdominal glands were removed, freeze-clamped and then homogenized in 4 vol. ice-cold 0.5*N* $HClO_4$. After removal of the protein and perchloric acid and neutralization, the extract was lyophilized and finally dissolved in 0.2M acetate buffer, pH 4.0. Milk, taken from rats by hand-milking, was added to 0.5*N* $HClO_4$ and treated as above.

Enzyme assay procedures.

Adenyl cyclase activity. This was measured essentially by the method of Davis & Lazarus [6] i.e. 40 mM Tris, pH 7.5; 5 mM KCl; 15 mM $MgCl_2$; 0.4 mM [U-^{14}C]ATP (0.5μCi); 8 mM theophylline; 2 mM cAMP; 20 mM phosphoenolpyruvate; 2.5 units pyruvate kinase and 200 μg of bovine serum albumin. The incubation, at 37°, was for 15 min. and the reaction stopped by heating to 100°. cAMP was separated chromatographically using the solvent system A of Woods & Waitzman [7] and the cAMP cut out and counted.

cAMP-phosphodiesterase activity. Both the high- and low-K_m enzymes occur in mammary gland and were found to have K_ms, 70 and 6 μM respectively, in good agreement with those found in other tissues [8,9]. The activities of the two isoenzymes were measured essentially as described by Loten & Sneyd [10] and by Thompson & Appleman [11]. Assay conditions were: 40 mM Tris, pH 7.5; 5 mM $MgCl_2$; 1 mM dithiothreitol; 200 μg of bovine serum albumin and 10 μM (for the low-K_m enzyme) or 100 μM (for the high-K_m enzyme) cyclic [^{3}H]AMP, equivalent to 0.5 μCi. Incubation, at 37°, was for 10 min. and the reaction stopped by heating to 100°.

Measurement of cAMP. cAMP was estimated by the method of Gilman [12] using a commercial test-kit (Boehringer Corporation, London, W5 2TZ). For each tissue sample the milk content was measured and a correction applied for the cAMP content of the milk in the extract.

RESULTS AND DISCUSSION

The activities of adenyl cyclase and of the low- and high-K_m PDE during the lactation cycle are shown in Table 1 and Fig.1. By the 10th day of

Table 1. Activities of adenyl cyclase and phosphodiesterases in rat mammary gland at different stages of pregnancy and lactation.

	Adenyl cyclase	Phosphodiesterases	
		High K_m	Low K_m
Pregnant 10 days	0.73 ± 0.10	30.39 ± 4.27	12.70 ± 1.70
Pregnant 15 days	1.53 ± 0.16	45.55 ± 11.56	19.32 ± 1.30
Pregnant 20 days	4.34 ± 0.22	56.61 ± 3.91	20.22 ± 2.88
Lactating 1 day	1.52 ± 0.11	71.70 ± 6.84	20.31 ± 1.92
Lactating 16 days	0.32 ± 0.02	86.56 ± 11.56	23.37 ± 2.41
Weaned	0.24 ± 0.05	89.23 ± 26.55	24.17 ± 3.46
Weaned and resuckled	0.49 ± 0.07	62.12 ± 11.13	25.01 ± 3.26

Results are given as mean ± SEM (5 rats). Activities are expressed in mU/g milk-free tissue [3]; a unit is defined as the amount of enzyme producing 1 μmol of product/min at 37°C.

pregnancy, the adenyl cyclase activity is already relatively high and it increases continuously and sharply until the 20th day of pregnancy, to a value of 4.3 mμmoles/g/min. Following parturition, the activity falls sharply and by the first day after parturition it has reached a level only 35% of the maximal value. Thereafter, the activity falls further, to a minimum (7%) at 16 days lactation. Neither short-term weaning nor short-term weaning followed by resuckling sensibly changes this low value. The low-K_m PDE shows no appreciable change during pregnancy and only a 15% increase during lactation. On the other hand, the high-K_m PDE begins to increase during pregnancy, but its major increase is achieved during lactation, with a peak of activity on the 16th day. This pattern of enzyme changes, reflecting the relative rates of synthesis and degradation of cAMP, is consistent with the level of the nucleotide found in the tissue (Fig.1). cAMP reaches its highest value on the 20th day of pregnancy (ca. 7×10^{-7}M), when the adenyl cyclase activity is also at its peak value, and then drops to its lowest level by the 16th day of lactation, when adenyl cyclase is at a minimum and the PDE is at a maximum. These results suggest that those enzymes which depend for their action either on the release of cAMP, or on its presence as an activity modulator, would be more effective during pregnancy and less so during lactation. They would also suggest that the modification of the pattern of enzyme activities which occurs in the gland following parturition may, in part, be the response to a repression or derepression of gene expression by the prevailing level of cAMP in the cell.

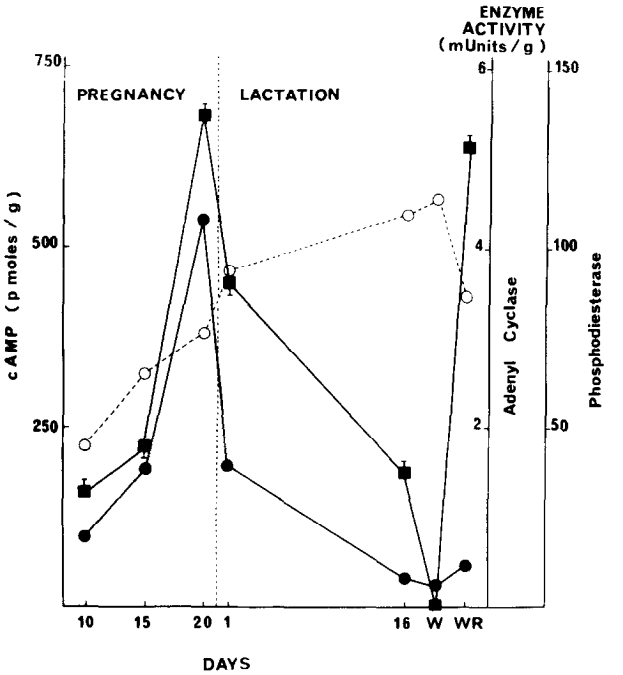

Fig.1. cAMP content and the activity of adenyl cyclase and cAMP phosphodiesterase in mammary gland at different stages of the lactation cycle.

The tissue content of cAMP was measured according to Gilman [12] and the values obtained were corrected for the cAMP content of the retained milk. Milk contained 2.5 ± 0.14 mμmoles cAMP/ml.

■, cAMP; ●, adenyl cyclase; ○, cAMP phosphodiesterase (low- + high-K_m activities). W, weaned rats; WR, weaned and resuckled rats.

The effects of the above changes on mammary gland metabolism may be examined from two viewpoints: firstly, the consequences of the changeover from a high cAMP level in pregnancy to one which is relatively low in lactation and secondly, the effects of the enzyme changes on the responsiveness of the tissue to hormonal stimulation.

It is now well established that the onset of lactation is accompanied by a sharp increase in the rate of lipogenesis [13]. The flux of glucose through the glycolytic and pentose phosphate pathways also increases at this time [13]. The higher rate is also due, in part, to a rise in the activity of the lipogenic enzymes, in particular acetyl CoA carboxylase and fatty acid synthetase, both of which increase more rapidly after parturition than do the enzymes of glycolysis or the pentose phosphate pathway [3]. These findings may be interpreted in the light of observations by Bricker & Levey [14] and of Tepperman & Tepperman [15] that high levels of cAMP are associated with a block in the pathway of lipogenesis. Rudack *et al.* [16] have also reported that high levels of cAMP in liver tend to repress the synthesis of lipogenic enzymes. If a

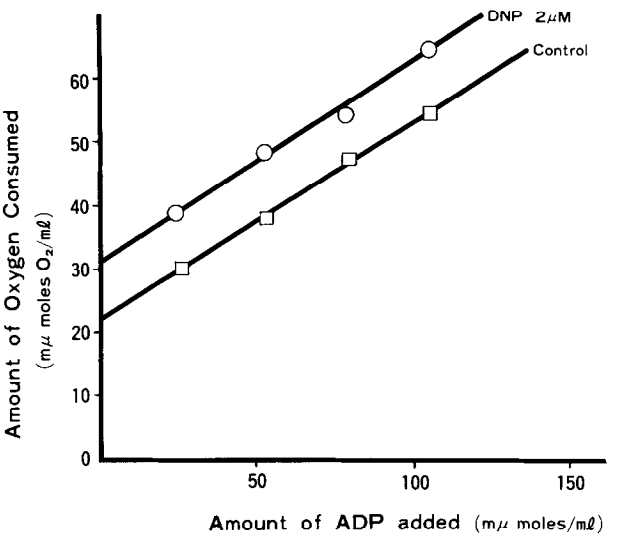

Fig. 4. Effect of small concentration of DNP on the stoichiometric relationship between oxygen and ADP consumed during state 3 respiration.
Experiments were performed as in Fig. 2, except that the medium contained 5 mM succinate and 5 mM glutamate and that in one set of experiments DNP was included to 2 μM. Mitochondria: 1.1 mg protein per ml.

is enlarged. This appears to have some bearing on so-called loosely coupled phosphorylation.

From the above findings it is concluded that at least in the present in vitro system the mitochondrial transitions are an energy requiring phenomenon. The results of Eisenhardt and Rosenthal clearly showed that certain amount of utilizable energy is actually stored in mitochondria of state 4, which is consumed on addition of ADP for the synthesis of ATP ("ATP jump", Ref. 2). It should be stressed here that according to this concept the ATP synthesis linked with electron transport occurs mostly after the depletion of the stored energy. After the completion of phosphorylation of added ADP, an extraneous electron transport should take place in order to supplement the energy stored in state 4 mitochondria. If the efficiency is comparable among the electron-transport dependent ATP synthesis, the electron-transport dependent energization to state 4 and the ATP synthesis elicitable at the expense of the stored energy, no "extraneous oxygen consumption" should occur. The fact, this was demonstrated, indicates that the efficiency is not comparable, and some extent of energy loss occurs which can be compensated for by "extraneous" election transport. The data of Fig. 4 suggest that the energy loss may occur in a manner similar to that occurring under the influence of a low concentration of classic uncouplers. Therefore the possibility can not be excluded that it reflects uncoupling occurring as an artifact in the in

by the presence of placental lactogenic hormone [23]. Thus, during pregnancy, with the placentae still present *in utero*, insulin action is depressed and hormone action, mediated via cAMP, can proceed since the cyclase is high and active, while PDE is low and not activated.

At parturition, the placentae are ejected and the postulated insulin effects on adenyl cyclase and PDE become more apparent, manifesting themselves as a depressed response to cAMP-mediated hormones.

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