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EPINEPHRINE- AND PROSTAGLANDIN-SENSITIVE ADENYL CYCLASE IN MAMMARY GLAND

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SUMMARY

Adenyl cyclase was measured in 1000 × *g* particulate fractions obtained from homogenates of lactating mammary tissues, or of cells derived thereof by collagenase treatment.

The enzyme from rat mammary cells was found to be stimulated by epinephrine (average 1.7- to 1.8-fold) and fluoride (about 7-fold), activities and stimulation by hormones and NaF being very variable.

Rabbit mammary cells yielded the bulk of adenyl cyclase in fractions of low centrifugal force (600 × *g*). The enzyme showed a maximum in activity around pH 8, and it was stimulated by various catecholamines (up to 3-fold) and prostaglandins (3- to 4-fold), K_a values for epinephrine and prostaglandin E_1 being 0.1 to 0.3 μM and 1 μM, respectively. Caffeine and aminophylline reduced apparent activity in the presence of 1 mM 3′,5′-AMP in assays. Storage of enzyme at 0 °C showed only small losses in activity or sensitivity to stimulation by epinephrine or fluoride.

Propranolol inhibited stimulation by epinephrine, as expected for a competitive inhibitor. Alpha-blocking drugs like phentolamine and phenoxybenzamine also inhibited stimulation by epinephrine, although not in a competitive fashion. Maximally effective doses of epinephrine and prostaglandin E_1 did not lead to additive stimulation.

Adenyl cyclase from mouse mammary cells was not studied systematically; however, it was also shown to be stimulated by epinephrine and prostaglandins.

INTRODUCTION

Adenyl cyclase has been recognized as a ubiquitous enzyme in mammalian tissues[1]. The cellular levels of 3′,5′-AMP and enzymes involved in its formation and destruction have been found to respond to certain tissue-specific hormones, explaining in part molecular aspects of hormone specificity or function. Surprisingly, no systematic studies on adenyl cyclase in mammary gland tissue have appeared to date. This organ, however, poses several interesting functional problems involving hormonal

regulation of development and intermediary metabolism[2]. Since 3′,5′-AMP has been implicated as a mediator of hormonal control in metabolic and developmental reactions, it seemed of interest to investigate enzymes, and their functional aspects, related to the 3′,5′-AMP pathway of cellular regulation in the mammary gland. In this communication some properties of epinephrine- and prostaglandin-sensitive adenyl cyclase from rabbit mammary gland are described; observations on enzymes from rat and mouse mammary gland are also included.

MATERIALS AND METHODS

Tissue and enzyme preparation

Virgin Wistar rats or Swiss white mice were bred under controlled conditions to allow timing of delivery dates. Pregnant white New Zealand rabbits were obtained directly from the supplier. They were usually used 1 to 2 days after delivery. Mammary lobes were excised from bled animals, and superficial connective tissue sheaths were thoroughly removed. Tissues were then cut and minced with razor blades and rinsed extensively in several changes of cold isotonic NaCl with good agitation to remove milk. For preparation of enzyme directly from minced tissue, each gram was homogenized in 5 ml of 20 mM Tris–HCl (pH 7.5) and 1 mM $MgCl_2$, using a ground glass homogenizer tube (Pyrex) with a motor-driven ground glass pestle. The use of Teflon pestles did not allow efficient rupture and homogenization of the mechanically quite resistant tissues, even if finely minced. After filtration of homogenates through glass wool, they were centrifuged at 1000 × *g* for 10 min. Adenyl cyclase activity was usually assayed in both the supernatant and the washed, resuspended pellet, with the bulk of activity occurring in the pellet. For the purpose of enzymatic digestion and preparation of isolated mammary cells, the procedure described by Rodbell[3] for adipose tissue was adopted as follows. The digestion mixture consisted of 140 mM NaCl, 9.1 mM sodium phosphate (pH 7.2), 2.5% bovine serum albumin, purified according to Chen[4], 5 mg/ml collagenase (EC 3.4.4.19) and 1 mg/ml glucose. The pH was readjusted to 7.2–7.4 with 1 M NaOH after addition of albumin and collagenase. About 1 g of minced mammary tissue was incubated with 3 ml of digestion buffer in 25 ml polypropylene vials. After gassing briefly with O_2, the vials were capped and incubated for 2 h at 37 °C under shaking at 160 strokes/min. Between 8 and 10 g of tissue were processed for any given experiment. Unlike in adipose tissue, full disintegration of mammary tissue could not be achieved. Incubations were terminated by filtering digests through fine nylon netting to retain undigested clumps of tissue. The nettings were rinsed with cold NaCl–phosphate buffer from which collagenase and glucose had been omitted and albumin had been reduced from 2.5 to 0.5%. The filtrate was centrifuged in a table centrifuge at low speed for about 1 to 2 min. Small amounts of floating fat cells and fat were withdrawn together with the supernatant fluid by use of Pasteur pipets. The combined sediments were rinsed 5 times with cold NaCl–phosphate buffer (0.5% albumin). The final pellet thus obtained contained small, barely visible, clumps of tissues or cellular aggregates. Microscopic examination indicated that rather uniform cell types were present, partly held together by residual collagen fibers. Lesser amounts of single cells were also present. No fat cells could be seen. In spite of incomplete digestion and

liberation of cells from mammary tissues of all three species studied, this preparation is referred to as "cells" in the subsequent text.

Compared to undigested tissue, the cells could easily be homogenized using Teflon pestles. Routinely, homogenization was performed by using 5 ml of 20 mM Tris–HCl (pH 7.5) and 1 mM $MgCl_2$ per ml of packed cells. Homogenates were likewise centrifuged at 1000 × *g*, and washed pellets were resuspended to yield about 5 to 10 mg/ml protein. Unless assayed immediately following preparation, small aliquots of 0.1–0.2 ml were stored under liquid N_2.

Enzyme assay

Adenyl cyclase was assayed with [α-^{32}P]ATP as substrate and radioactive 3′,5′-AMP was separated from other nucleotides by thin-layer chromatography on polethyleneimine-impregnated cellulose as described by Bär and Hechter[5] with time and cost saving improvements[6]. Incubations were carried out in siliconized 5 mm × 60 mm Kimax test tubes and contained in a total of 0.05 ml the following: 40 mM Tris–HCl (pH 8), 5 mM $MgCl_2$, 10 mM sodium creatinephosphate, 0.1 mg/ml creatine phosphokinase (EC 2.7.3.2), 0.5 mM sodium 3′,5′-AMP, 1% bovine serum albumin, 1 mM sodium [ethylenebis(oxyethylenenitrilo)]tetraacetate, 0.6 mM $CaCl_2$, 0.1 mM [α-^{32}P]ATP (approx. 600 000 cpm) and other additions as indicated later. Enzyme (0.1 to 0.4 mg/ml) was added to start reactions, which were carried out at 37 °C for 15–25 min. Incubations were terminated by addition of 5 μl of a cold solution of 20 mM ATP, 20 mM AMP, 20 mM 3′,5′-AMP and 200 mM EDTA (pH 7, sodium salts) and placing the tubes on ice. Aliquots of 3–5 μl were directly spotted on thin-layer plates, and after development in 0.25 M LiCl (10 cm total length) ultraviolet-marked spots of 3′,5′-AMP and ATP *plus* AMP were cut out and counted by liquid scintillation in a toluene-based fluor. Triplicate assays were performed, standard errors of means were below 5–10%.

Reagents

Bioreagents were purchased from Sigma Chemical Co., St. Louis, or Boehringer–Mannheim, New York. [α-^{32}P]ATP was obtained from International Chemical and Nuclear Corporation. D-Epinephrine was obtained from Winthrop Laboratories, New York, and synthetic arginine vasopressin from Mann Research. Sheep luteotrophic hormone (prolactin) was purchased from Mann Research, New York. Propranolol (Inderal) was a gift from Ayerst Laboratories, Montreal, and Rogitine (phentolamine) from Ciba Co. Ltd, Dorvall, Quebec. Phenoxybenzamine was synthesized and kindly supplied by Dr D. Cook. The following hormones were kindly donated: synthetic oxytocin by Dr R. Walter, Mt. Sinai School of Medicine, New York; synthetic adrenocorticotrophin (β^{1-24}-corticotrophin) by Dr W. Rittel, Ciba Co., Basel; synthetic (porcine) glucagon by Dr L. Jaeger, Max-Planck-Institute, München; prostaglandins E_1, $F_{1\alpha}$ and $F_{1\beta}$ by Dr J. Pike, Upjohn Co., Kalamazoo. Thyrotrophic hormone was a National Institute of Health standard, 1.44 I.U./mg.

Stock solutions of peptide hormones were prepared in distilled water (1 mg/ml) or in 0.01 M HCl (glucagon) and kept frozen at −20 °C. Prostaglandins were dissolved in 1 vol. 95% ethanol and diluted with 9 vol. of 0.2 mg/ml Na_2CO_3 solution (1 mg/ml prostaglandin). These stock solutions were kept frozen, and appropriate dilutions were made in distilled H_2O.

RESULTS

Rat mammary gland

Initial studies concentrated on rat mammary gland using lactating gland tissues from breeding stock animals, at the time of weaning of litters. Results were variable but indicated that specific activity of adenyl cyclase obtained after direct homogenization of minced tissues was low, *i.e.* below 2–3 pmoles·mg^{-1}·min^{-1} (unstimulated). Activity could be stimulated 2- to 4-fold by 10 mM NaF, however, hormonal stimulation (epinephrine, 0.1 mM) could not be observed. When adenyl cyclase was prepared from cells, *i.e.* following collagenase digestion of tissues from lactating animals, basal and fluoride-stimulated activities were higher, though still variable, and stimulation by epinephrine appeared to exist in some preparations. The mean basal activity ($\pm$ S.E.) in 13 preparations was 3.3 $\pm$ 0.3 pmoles·mg^{-1}·min^{-1}, fluoride-stimulated activity 29 $\pm$ 6 pmoles·mg^{-1}·min^{-1} and epinephrine-stimulated activity 5.8 $\pm$ 0.6 pmoles·mg^{-1}·min^{-1}. The average degree of stimulation by hormone was thus 70–80%, the difference between basal and stimulated enzyme being highly significant ($P < 0.01$, paired *t*-test). Neither oxytocin (10 μg/ml), arginine vasopressin (10 μg/ml), glucagon (20 μg/ml), adrenocorticotrophin (10 μg/ml), thyrotrophic hormone (100 μg/ml), prostaglandin E_1 (0.1 mM) showed any effect on the enzyme activity. Variable results and low hormonal sensitivity discouraged more intensive studies with adenyl cyclase from rat mammary tissue.

Rabbit mammary gland

Centrifugal fractionation. A homogenate of packed cells after collagenase treatment of rabbit tissue was subjected to differential centrifugation steps. Adenyl cyclase activity was measured in 600 $\times$ *g* and 20 000 $\times$ *g* subcellular particulate fractions and in the 20 000 $\times$ *g* supernatant. A typical experiment is reported in Table I. Two similar experiments showed 75 and 85% of basal activity in the 600 $\times$ *g* pellet. "Purification" of cyclase activity was negligible in either the 600 $\times$ *g* or 20 000 $\times$ *g* pellets, but slightly improved hormone sensitivity was evident in these fractions, particularly with epinephrine as the stimulant.

General properties. The pH dependence of stimulated and unstimulated activity was determined. Using different buffers to cover the range between pH 7 and 9.5, it was demonstrated that basal and fluoride- or epinephrine-stimulated adenyl cyclase

TABLE I

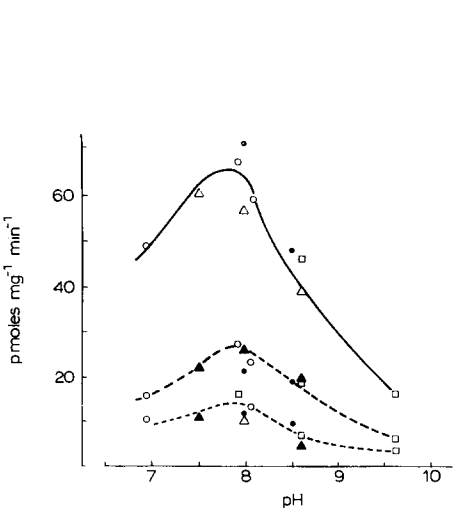
CENTRIFUGAL FRACTIONATION OF ADENYL CYCLASE FROM RABBIT MAMMARY CELLS

Centrifugations were carried out for 10 min at 600 $\times$ *g* and 30 min at 20 000 $\times$ *g*. Per cent activity refers to total activity (pmoles·min^{-1}) in subfractions. Epinephrine was 0.1 mM, NaF, 10 mM and prostaglandin E_1, 0.01 mM.

Fraction	*pmoles·mg^{-1}·min^{-1}*				*Per cent activity (basal)*
	Basal	*NaF*	*Epinephrine*	*Prostaglandin E_1*	
Homogenate	11.8	41.4	13.3	19.5	—
600 $\times$ *g* pellet	14.4	68.3	22.9	31.5	90
20 000 $\times$ *g* pellet	12.3	59.4	15.9	25.4	7
20 000 $\times$ *g* supernatant	1.7	7.3	1.7	3.0	3

exhibits a pH maximum between 7.8 and 8 (Fig. 1). To further assure adequacy of assay conditions, the effects of 3′,5′-AMP and caffeine or aminophylline on the enzyme were studied. Fig. 2 indicates that unlabeled 3′,5′-AMP added to the assay medium elevates apparent activity of adenyl cyclase with increasing dose both in the absence and (more markedly) in the presence of NaF. Caffeine and aminophylline reduce apparent activity, in the absence of NaF, over the entire range of concentrations of unlabeled 3′,5′-AMP. In the presence of NaF, however, the methylxanthines stimulated apparent activity above levels seen in the presence of 0.01 or 0.1 mM 3′,5′-AMP alone, while inhibition was seen only at 1 mM 3′,5′-AMP. The effects of methylxanthines probably indicate a dual action, *i.e.* inhibition of phosphodiesterase and partial inhibition of adenyl cyclase. Relative concentrations of total 3′,5′-AMP, and degrees of inhibition of cyclase and diesterase, respectively, may determine the complex results documented in Fig. 2. However, the presence of phosphodiesterase in the enzyme preparation has not yet been demonstrated directly in the present investigation.

When enzyme preparations (stored under liquid N_2) were thawed and kept on an ice bath, basal and epinephrine-stimulated activities remained relatively stable over a 4.5-h period, while fluoride-stimulated activity was reduced about 25%. Immediately after thawing (10-min period) slight transient elevations of activity by about 10–15% were repeatedly seen. This phenomenon may account partially for the variability of activities observed from experiment to experiment. Storage under liquid N_2 for days or a few weeks seemed to have no effect on the enzyme or its hormone sensitivity. However, storage for a period of 3 months under liquid N_2 led to



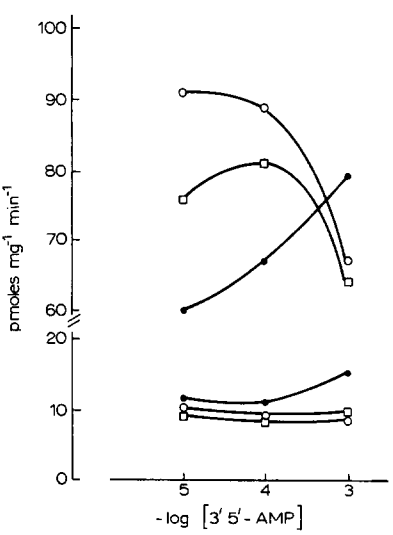


Fig. 1. pH dependence of adenyl cyclase from rabbit mammary cells in the absence of any additions (lower broken line), and in the presence of 0.1 mM epinephrine (upper broken broken line) or 10 mM NaF (solid line). Buffers were Tris–HCl (●), glycylglycine–HCl (▲), sodium *N*-2-hydroxyethylpiperazine-*N*′-2-ethanesulfonate (○) and glycine–HCl (□); all were 40 mM.

Fig. 2. Rabbit mammary cell adenyl cyclase and effects of 3′,5′-AMP and methylxanthines. Upper curves in the presence, and lower curves in the absence of 10 mM NaF. Caffeine (□) and aminophylline (○) were present at 1 mM.

considerable reduction of basal and fluoride-stimulated activity and to loss of epinephrine sensitivity (prostaglandin E_1 sensitivity was not assayed in these tests).

Addition of 1 mM EDTA, [ethylenebis(oxyethylenenitrilo)]tetraacetate or dithioerythritol to assays had no significant effect on either basal or fluoride-, prostaglandin E_1- or epinephrine-stimulated activities. Ca^{2+} at 1 mM reduced basal and fluoride-stimulated activity by about 80%.

Hormone effects. Initial experiments indicated that adenyl cyclase from rabbit mammary gland was sensitive to epinephrine and prostaglandin E_1. Other hormones including adrenocorticotrophin, glucagon, thyrotropic hormone, oxytoxin, arginine vasopressin, parathyroid hormone and prolactin showed no effect. Dose dependence of stimulation by epinephrine and related drugs is shown in Fig. 3. Both L-epinephrine and DL-isopropylnorepinephrine stimulated activity similarly in respect to degree and dose dependence, while L-norepinephrine required about 100 times higher doses for maximal stimulation. This and similar experiments indicated that concentrations for half maximal stimulation (K_a) were about 0.1 to 0.3 μM for L-epinephrine and DL-isopropylnorepinephrine and 5 to 10 μM for L-norepinephrine. D-Epinephrine also stimulated activity fully (K_a about 2 μM); phenylephrine acted as a weak agonist leading only to 20% of full activation (compared to epinephrine) at 0.1 mM.

Prostaglandins E_1, $F_{1\alpha}$ and $F_{1\beta}$ also stimulated the activity of adenyl cyclase from rabbit mammary gland, and dose–response effects are shown in Fig. 4. From this figure, the K_a values were estimated to be 1 μM for prostaglandin E_1 and 50 μM for prostaglandin $F_{1\beta}$. The value for prostaglandin E_1 was confirmed in further experiments; the effect of prostaglandin $F_{1\alpha}$, which was the least potent of the three prostaglandins available, was not tested at concentrations above 0.3 mM. Maximal stimulation achieved with prostaglandin E_1 was consistently higher than that obtained with epinephrine. Prostaglandin E_1 and epinephrine did not lead to additive

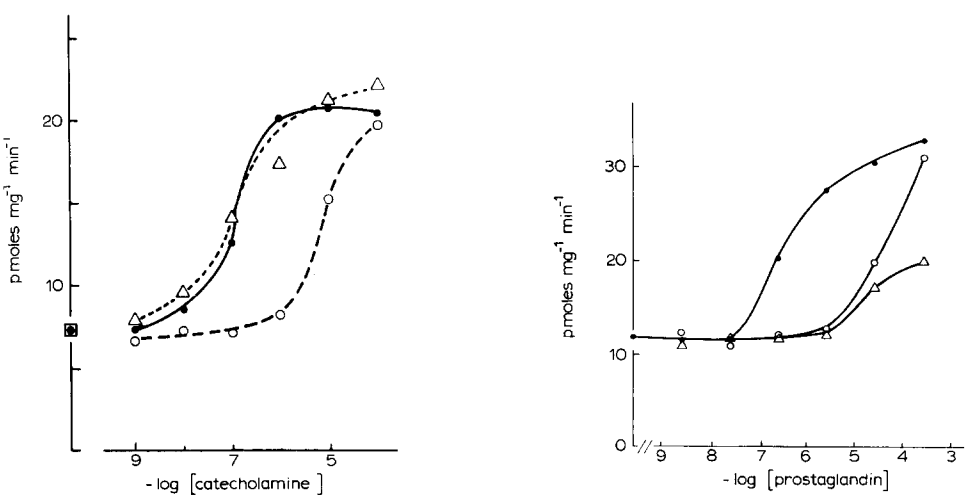


Fig. 3. Effect of catecholamines upon adenyl cyclase from rabbit mammary cells. L-Epinephrine (●), DL-isopropylnorepinephrine (△--△) and L-norepinephrine (○--○).

Fig. 4. Effect of prostaglandin E_1 (●), prostaglandin $F_{1\alpha}$ (△) and prostaglandin $F_{1\beta}$ (○) upon adenyl cyclase from rabbit mammary cells.

TABLE II

COMBINED ASSAY OF EPINEPHRINE AND PROSTAGLANDIN E_1 WITH ADENYL CYCLASE FROM RABBIT MAMMARY CELLS

Assay condition	*pmoles·mg^{-1}·min^{-1}*
Basal	13.3 ± 0.2
0.1 mM L-epinephrine	28.2 ± 0.6
0.01 mM prostaglandin E_1	38.2 ± 1.4
L-Epinephrine + prostaglandin E_1	39.0 ± 2.9

stimulation when each was present at its maximal effective dose, as evident from data in Table II.

Blocking drugs. The activation of adenyl cyclase by catecholamines could be completely abolished by the beta-adrenergic blocking drug propranolol. Alpha-blockers also appeared to block stimulation, and this unusual phenomenon was more closely examined. Dose–response curves of epinephrine activation were measured in the presence and absence of 1 mM phentolamine or phenoxybenzamine (Fig. 5). A shift of the dose-response curves to higher concentrations was noted, however, full activation was not reached in the presence of these drugs. The slight reduction of activity, evident above 0.1 mM epinephrine, in the presence of these drugs was confirmed in additional experiments. In a further experiment, propranolol shifted the epinephrine dose–response curve in a manner characteristic of a competitive antagonist.

Mouse mammary gland

Only a single experiment was carried out, testing the applicability of the collagenase-predigestion procedure to this tissue. Glandular tissue was obtained from three animals 2–3 days after delivery and treated as in the case of rat or rabbit. Enzyme activity was assayed in the standard preparation thus obtained, and results

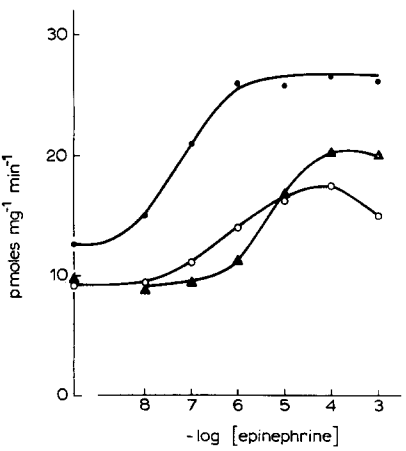


Fig. 5. Effect of α-blocking drugs on the dose–response curve for epinephrine stimulation of rabbit mammary cell adenyl cyclase (●). Both phentolamine (○) and phenoxybenzamine (▲) were present at 1 mM.

TABLE III

ADENYL CYCLASE FROM MOUSE MAMMARY GLAND

Stimulant	*pmoles·min*$^{-1}$*·mg*$^{-1}$
None	4.2 ± 0.2
0.1 mM epinephrine	5.8 ± 0.2
0.01 mM prostaglandin E_1	6.6 ± 0.2
0.01 mM prostaglandin $F_{1\alpha}$	5.1 ± 0.2
0.01 mM prostaglandin $F_{1\beta}$	6.1 ± 0.3

are listed in Table III. It appears that both epinephrine and prostaglandins stimulate activity. Arginine vasopressin, glucagon and parathyroid hormone showed no effect.

DISCUSSION

Mammary gland tissue is composed of several cell types. Disregarding elements of innervation and blood supply, secretory epithelial and myoepithelial cells can be considered the major functional cells involved in production, secretion and ejection of milk. Furthermore, fat cells and collagen fibers are present, the latter contributing as much as 80% of total mammary protein in the virgin rat[7]. Secretory cells, however, have been estimated to represent 70% of total cell number in the lactating gland[8]. The cellular source of enzyme activities prepared from intact tissue is thus not certain, and it appeared desirable, therefore, to attempt tissue digestion and cell separation. Other groups have also applied the collagenase digestion procedure of Rodbell[3] to mammary gland of the mouse[9] and rat[10]. In both cases, parenchymal cells have been obtained and characterized extensively in respect to morphological and biosynthetic properties. For the procedure used in this study, no fully isolated cells were obtained. However, the absence of fat cells in the final preparation has been confirmed by light microscopy. It appears that under the experimental conditions used, fat cells were extensively destroyed since only minor quantities of floating cells were observed, besides floating fat, during washing steps of tissue digests. Myoepithelial structures were never observed by light microscopy in cell preparations used in this study. One must assume that the procedure of tissue digestion used yielded mainly secretory cells, partly linked together by residual collagen fibers. The bulk of supportive collagen fibers was degraded efficiently by collagenase since the rigidity encountered with intact tissue, which complicated homogenization, had fully disappeared.

Low adenyl cyclase activities found in homogenates and centrifugal subfractions of whole tissues, both in the cases of rat and rabbit, indicate that the mechanically very disruptive procedure, involving ground glass homogenizers, may have destroyed part of this enzyme or its hormone sensitivity. After collagenase treatment, specific activities obtained were much higher. Hormone sensitivity of the rat enzyme, however, could not be clearly and consistently observed. The reason for this variability is not known. When preparing cells from mouse mammary tissue, Pitrelka *et al.*[9] found that certain morphological features of parenchymal cells, related mainly to their secretory functions, were damaged or altered. These effects could partly be avoided when glucose was included during digestion. No investi-

gation of ultrastructural changes of rat mammary cells was carried out in the present study to test whether correlations between cell integrity and cyclase activity exist. However, this is considered only as a remote possibility since methodology was applied rather evenly from batch to batch. In spite of irregular results obtained with rat mammary cells, it is evident that they contain an epinephrine-sensitive adenyl cyclase.

In contrast to the rat, studies with rabbit tissue gave very reproducible results from one preparation to the other. Both good specific activities and hormonal effects by epinephrine and prostaglandins were obtained. Again, intact tissue produced lower activities than freed cells. Since maximal stimulatory effects by epinephrine and prostaglandin E_1 were not additive when these hormones were incubated simultaneously, it appears that only one adenyl cyclase system responsive to both hormones was present. This offers also further support for the idea that the enzyme activity originated from a single cell type.

The order of potency of catecholamines and the blocking effect of propranolol indicate that rabbit mammary cells contain a β-adrenergic receptor. Usually, α-blocking drugs do not affect basal or epinephrine-stimulated adenyl cyclases, or they lead to increased stimulation, presumably due to the block of a cyclase-inhibiting α-adrenergic effect. The inhibitory actions of phentolamine and phenoxybenzamine observed in this study, however, were only seen in the presence of epinephrine. A similar observation has been made in the case of adenyl cyclase from rat erythrocytes[11]. Further studies seem necessary to elucidate the mechanistic basis and specificity of this effect.

It is interesting to note that adenyl cyclase from Ehrlich ascites cells (which originate from human mammary tissue) is also responsive to epinephrine and prostaglandin E_1 (ref. 12). In contrast to the enzymes from rabbit and mouse mammary cells, high doses of prostaglandin E_1 were necessary to stimulate Ehrlich cell adenyl cyclase, while prostaglandin $F_{1\alpha}$ and prostaglandin $F_{1\beta}$ were inactive. Thus, species differences with respect to hormone and structure–activity effects become apparent. Further studies are planned to show whether normal and malignant mammary tissues from other species also contain adenyl cyclases responsive to epinephrine or prostaglandins.

Two brief reports on adenyl cyclase in mammary tissue have appeared. Brown *et al.*[13] measured adenyl cyclase from lactating rats as well as from chemically induced breast adenocarcinoma. However, activity was measured in high speed particulate fractions after extensive (14 h) dialysis and was found to be 400 000 pmoles·mg^{-1}·min^{-1} (6 μmoles·mg^{-1} per 15 min, measured at 15 mg/4 ml Na_2HATP). This suggests the methodology used was at fault and adenyl cyclase has, in fact, not been measured in this study. Dousa and Rychlik[14] studied distribution of adenyl cyclase and phosphodiesterase in receptor tissues of neurohypophysial hormones, including mammary gland from lactating mice. They found highest specific adenyl cyclase activity in a microsomal fraction (measured only in the presence of NaF). In present studies on adenyl cyclase from rabbit tissue (Table I) and, preliminarily, from rat tissue, only negligible activity was seen in high speed centrifugal fractions. Neither group of workers reported on hormone sensitivity of their adenyl cyclase preparations from normal mammary tissue.

It is not known to what extent epinephrine or prostaglandin affects and con-

trols the function of the mammary gland in different species. Studies of metabolic and functional parameters, parallel with 3′,5′-AMP analysis and hormonal effects thereon, in intact glands seem necessary to answer this question. 3′,5′-AMP has been demonstrated in milk[15], and one may speculate that its secretion (which may or may not have any functional significance) could be influenced by hormones stimulating adenyl cyclase. Many intracellular effects of 3′,5′-AMP have been linked to its activating effect upon protein kinases. Such a 3′,5′-AMP-dependent protein kinase has also been found in rat mammary tissue[16], but specific control functions in the organ have not been demonstrated or postulated. It is possible that histone phosphorylation represents a mechanism of selective gene expression, as proposed for the 3′,5′-AMP effect on liver enzyme induction[17].

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