Biochimica et Biophysica Acta, 628 (1980) 263—276
© Elsevier/North-Holland Biomedical Press

BBA 29188

EFFECT OF MEMBRANE PHOSPHOLIPID COMPOSITION CHANGES ON ADENYLATE CYCLASE ACTIVITY IN NORMAL AND ROUS-SARCOMA-TRANSFORMED CHICKEN EMBRYO FIBROBLASTS

SUSAN GIDWITZ [a], JEFFRY E. PESSIN [b], MICHAEL J. WEBER [b], MICHAEL GLASER [b] and DAN R. STORM [a]

[a] *Department of Pharmacology, University of Washington, Seattle, WA 98195 and* [b] *Departments of Microbiology and Biochemistry, University of Illinois, Urbana, IL 61801 (U.S.A.)*

(Received July 17th, 1979)

Key words: Phospholipid composition; Adenylate cyclase; (Chicken embryo fibroblast)

Summary

Adenylate cyclase specific activities in membranes isolated from chicken embryo fibroblasts transformed by Rous sarcoma virus are significantly lower than the specific activity of the enzyme in normal membranes. Since normal and transformed membranes have different phospholipid and fatty acid compositions, adenylate cyclase activities were examined in normal and transformed membranes which had been supplemented with polar head groups or fatty acids. Basal, fluoride, and prostaglandin E_1-stimulated activities changed systematically with phospholipid composition. Increases in the primary amino group of the phospholipid polar head groups or the average degree of fatty acid unsaturation both inhibited adenylate cyclase activity. In general, adenylate cyclase activities in normal membranes were more sensitive to phospholipid compositional changes compared to adenylate cyclase in transformed membranes. The data indicate that the lower adenylate cyclase activities in transformed membranes are not solely attributable to phospholipid changes but do suggest that increases in the percentage of phosphatidylethanolamine may contribute to the lower adenylate cyclase activities in transformed membranes.

Abbreviation: SDS, sodium dodecyl sulfate.

Introduction

The specific activity of adenylate cyclase in membranes isolated from Rous-sarcoma-transformed chicken embryo fibroblasts is significantly lower compared to normal membranes [1—3]. The mechanism for changes in adenylate cyclase activity accompanying Rous sarcoma transformation has not been elucidated. Anderson and Pastan have proposed that a viral product causes some modification of the plasma membrane which decreases adenylate cyclase activity [4]. This general hypothesis was strengthened by solubilization studies which demonstrated that the specific activities of adenylate cyclase from normal and Rous-sarcoma-transformed chicken embryo fibroblasts were identical when the enzyme was solubilized with a number of different nonionic detergents [3]. Detergent solubilization studies suggested that the differences between adenylate cyclase activities in normal and transformed membranes are due either to differential modulation of enzyme activity by an effector which requires intact membranes for its effects, or indirect effects due to altered membrane environment.

A number of studies have implicated the importance of membrane lipids for adenylate cyclase activity [5—15]. Technology has now been developed for the in vivo modification of the fatty acid or polar head group composition of chicken embryo fibroblasts in tissue culture [16,17]. In the present study, the effects of membrane phospholipid composition changes on adenylate cyclase activity in normal and transformed membranes are compared.

Materials and Methods

Materials. Calf serum and trypsin were purchased from GIBCO. Tryptose phosphate broth was from Difco. Fatty acid free bovine serum albumin was purchased from Pentex. Fatty acids were from Nucheck and they were more than 99% pure. Choline, *N,N*-dimethylethanolamine, *N*-methylethanolamine, and ethanolamine were from Eastman Kodak while 3-aminopropanol, and (—)-2-aminobutanol were purchased from Aldrich Chemicals. Prostaglandin E_1 was the kind gift of Dr. John Pike, Upjohn Co.

Primary cell culture. Chicken embryo fibroblasts were grown as previously described [18]. Primary cultures were prepared from 10-day-old chicken embryos from which the head and internal organs had been removed. The carcass was then incubated with 0.25% trypsin to dissociate the cells. The cells were centrifuged at 1200 rev./min for 10 min and resuspended in Dulbecco's modified Eagle medium high glucose formula containing penicillin and streptomycin and supplemented with 4% calf serum and 1% heat inactivated chicken sera. Cells were plated at $6 \cdot 10^6$ cells/100 mm dish and grown for 3 days at 41°C before replating as secondary cultures.

Secondary and tertiary cell cultures for lipid supplementation studies. Lipid supplementations of chicken embryo fibroblasts were carried out by the method of Hale et al. [16]. Cells were grown as described above, but were plated in either the standard medium, the standard medium plus biotin but without tryptose phosphate broth, or in delipidated medium (Dulbecco's medium without choline supplemented with biotin, delipidated calf and

chicken sera). Removal of lipid from calf and chicken sera was accomplished by multiple alcohol and ether extractions as previously described [19]. Phospholipid polar head group supplementations were performed on secondary or tertiary cultures plated in delipidated medium with a biotin concentration of 1 μg/ml. Choline or its analogues were added as supplements 3 to 6 h after plating at a concentration of 40 μg/ml. Normal cells were plated at $1.25 \cdot 10^6$ to $3.75 \cdot 10^6$ cells/100 mm dish and transformed cells at $1.88 \cdot 10^6$ to $6.00 \cdot 10^6$ cells/100 mm dish. These plating densities resulted in similar cell densities for all cell types when cells were harvested. Transformed cells were harvested approximately 28 h and normal cells approx. 40 h after supplementation.

Fatty acid supplementation was performed on secondary or tertiary cultures plated in delipidated medium with a biotin concentration of 10^{-4} μg/ml. Normal cells were plated at approximately $2.5 \cdot 10^6$ cells/100 mm dish and transformed cells at $3.1 \cdot 10^6$ cells/100 mm dish for all fatty acid supplements. Cells supplemented with different fatty acids were at comparable cell densities when harvested. Fatty acids were added to the medium at a final concentration of 20 μg/ml in the form of bovine serum albumin complexes. Bovine serum albumin fatty acid complexes were prepared by the method of Spector and Hoak [20]. Fatty acid supplements were added 20 h after plating and the cells were harvested 26 h later.

Viral infection was performed by adding the Schmidt-Ruppin strain of Rous sarcoma virus, subgroup A (RSV-SR-A), at a multiplicity of infection of approximately one.

Cells were harvested and membranes were prepared as previously described [3]. Membrane preparations were stored in 1 mM Tris-HCl, pH 7.5 at −60°C.

Lipid determinations. Lipids were extracted by the general method of Bligh and Dyer [21] as described by Ames [22]. For phospholipid determinations, the cells were grown for two passages in [^{32}P]phosphate to uniformly label all phospholipids. Phospholipids were separated by two-dimensional thin layer chromatography on Silica gel G [23]. Phospholipids were visualized by autoradiography on Kodak No-Screen film, eluted and counted for ^{32}P in scintillation cocktail. Fatty acid compositions were determined after extraction of the phospholipids by the method of Bligh and Dyer and separations of phospholipids from neutral lipids on a short unisil column [24]. Fatty acid methyl esters were prepared in sodium methoxide-methanol. The methyl esters were analyzed by gas-liquid chromatography on a 1.5 M column of 10% SP-2340 on 100/120 Chromasorb W AW. The *cis-trans* isomers were resolved in a 3 M column of 15% OV-275 on 100/120 Chromasorb PAW-DMCS. Cholesterol concentrations were determined by gas liquid chromatography using a 1.5 M column of 3% SE-30 on 80/100 Gas Chrom Q.

Adenylate cyclase assay. Adenylate cyclase was assayed at 37°C according to the general method of Salomon et al. [25] in 50 μl containing 1 mM α-[^{32}P]-ATP (400 cpm/pmol), 2 mM cyclic AMP, 5 mM $MgCl_2$, 1 mM EDTA, 1 mM β-mercaptoethanol, 5 mM theophylline, 1.1% bovine serum albumin, and 20 mM Tris-HCl, pH 7.5. 1 mg/ml creatine kinase and 20 mM phosphocreatine were present as an ATP regenerating system. ATP used in assays was purified by DEAE-Sephadex A-25 chromatography followed by Dowex AG-50 chromatog-

raphy. The concentrations of GTP, Gpp(NH)p, NaF, prostaglandin E_1 and epinephrine, when present, were 100 μM, 100 μM, 15 mM, 14 μM and 50 μM, respectively. Recovery of cyclic AMP was monitored using ^{3}H-labeled cyclic AMP. Each sample contained 40 to 100 μg of membrane protein. Data points are the mean of triplicate determinations with the standard deviation of less than 5%.

Sodium dodecyl sulfate-polyacrylamide gel electrophoresis. Protein samples were subjected to electrophoresis on 7.5% SDS polyacrylamide slab gels using the method of Laemmli [26].

Protein. Protein was determined by the method of Lowry et al. [27] using bovine serum albumin as standard.

Results

Modification of the phospholipid composition

In order to modify the phospholipid polar head group composition, the cells were plated in delipidated medium and allowed several hours to attach to the culture dish. Choline or choline analogues were added to the medium, and the cells were grown for 28 h (transformed) or 42 h (normal) and then harvested. It had been previously established [16] that incorporation of polar head group analogues was essentially complete 24 h after supplementation. Transformed cells were harvested earlier than normal cells because the transformed cells detach readily from the tissue culture dish at later periods of time. The growth of normal or transformed cells was unaffected by supplementation with either choline analogues or fatty acids.

The phospholipid compositions of normal and transformed cells grown in delipidated medium supplemented with choline (control) or choline analogues are reported in Table I. Incorporation of the supplements into membrane phospholipids was generally very good. For example, normal cells supplemented with *N,N*-dimethylethanolamine synthesized phosphatidyldimethylethanolamine as 58% of the total phospholipid content. With the exception of ethanolamine supplementation of normal cells, most choline analogues were incorporated to at least 40% of the total phospholipid content. Although ethanolamine supplementation of normal cells increased the phosphatidylethanolamine composition 10.7% over choline supplemented cells, phosphatidylcholine levels still exceeded phosphatidylethanolamine. Supplementation with choline analogues had little or no effect on the phosphatidylinositol plus phosphatidylserine, sphingomyelin or cardiolipin content. The supplements generally replaced phosphatidylcholine and phosphatidylethanolamine to varying degrees. In ethanolamine supplemented cells, the increase in phosphatidylethanolamine appeared to be a consequence of direct replacement of phosphatidylethanolamine for phosphatidylcholine.

Supplementation with choline or choline analogues also had some effects on the phospholipid fatty acid composition (Table II). Cells supplemented with ethanolamine or (—)-2-aminobutanol had a higher percentage of long chain fatty acids compared to other supplemented cells. The number of double bonds/100 fatty acids remained relatively constant, although normal cell membranes consistently contained more unsaturated fatty acids than transformed

TABLE I

PHOSPHOLIPID COMPOSITION OF CELLS GROWN IN DELIPIDATED MEDIUM + CHOLINE OR CHOLINE ANALOGUES

PC, phosphatidylcholine; PE, phosphatidylethanolamine; PI, phosphatidylinositol; PS, phosphatidylserine; Sp, Sphingomyelin; CL, cardiolipin; PMEA, phosphatidylmethylethanolamine; PDMEA, phosphatidyldimethylethanolamine; PAB, phosphatidylaminobutanol; PAP, phosphatidylaminopropanol.

Cells and supplement	Phospholipid composition (% phosphate)									
	PC	PE	PI + PS	Sp	CL	PMEA	PDMEA	PAB	PAP	Other *
Normal cells										
Choline	49.6	21.9	14.2	9.6	2.0	0.4	—	—	—	2.4
Dimethylethanolamine	8.4	8.4	16.3	5.7	1.5	—	58.2	—	—	1.5
Methylethanolamine	14.8	5.6	17.0	6.7	1.6	48.0	2.8	—	—	3.5
Ethanolamine	38.0	32.6	15.8	8.3	1.9	0.4	—	—	—	2.9
Aminobutanol	24.7	8.2	20.9	5.5	2.4	1.2	—	33.5	—	3.6
Aminopropanol	21.8	7.0	14.5	9.0	1.6	—	—	—	44.0	2.1
Transformed cells										
Choline	46.8	23.8	12.6	10.3	2.1	3.5	—	—	—	0.9
Dimethylethanolamine	11.4	13.3	13.1	5.8	1.6	0.5	52.8	—	—	1.5
Methylethanolamine	14.4	5.6	16.2	6.8	1.7	47.2	4.5	—	—	3.7
Ethanolamine	25.4	43.0	16.7	7.9	2.1	2.3	—	—	—	2.5
Aminobutanol	25.7	7.9	17.9	6.1	2.2	3.0	3.0	—	35.1	2.1
Aminopropanol	20.2	10.8	14.2	8.3	1.8	—	—	—	43.0	1.7

* Other phospholipids included phosphatidylglycerol, lysophosphatidylcholine (<0.05%), lysophosphatidylethanolamine and other unidentified compounds.

TABLE II

FATTY ACID COMPOSITION OF CELLS GROWN IN DELIPIDATED MEDIUM + CHOLINE OR CHOLINE ANALOGUES

Conditions of the experiment were the same as Table I.

Cells and supplement	Fatty acid composition (% by weight)										
	16:0	16:1	18:0	18:1	18:2	20:4	Other	>C18	% Saturated	% Poly-unsaturated	Double bonds per 100 fatty acids
Normal cells											
Choline	14.7	5.1	14.4	44.2	2.7	5.4	13.5	18.5	31.1	14.8	103
Dimethylethanolamine	12.0	7.0	14.5	48.7	3.2	5.0	9.6	14.1	28.6	12.7	100
Methylethanolamine	8.7	2.9	22.4	41.0	3.1	7.7	14.2	21.4	33.7	16.2	102
Ethanolamine	15.6	3.8	17.0	34.3	3.9	9.9	15.5	24.9	35.8	22.3	124
Aminobutanol	11.0	—	28.2	31.4	3.5	11.1	14.8	25.4	42.7	20.3	110
Aminopropanol	12.7	4.3	19.7	38.5	4.2	8.9	11.7	20.2	35.4	19.5	115
Transformed cells											
Choline	14.8	5.5	14.3	54.4	3.2	2.8	5.0	7.4	30.1	8.6	88
Dimethylethanolamine	12.9	7.1	13.4	55.4	3.7	2.9	4.6	7.1	27.6	8.7	91
Methylethanolamine	7.0	1.2	23.2	53.1	3.4	5.5	6.6	11.4	32.2	11.9	97
Ethanolamine	12.6	1.5	19.9	43.5	3.1	5.2	14.2	19.0	34.7	14.4	100
Aminobutanol	9.6	0.9	24.1	45.6	2.6	3.0	14.2	16.8	36.5	9.7	83
Aminopropanol	13.3	4.2	20.0	51.4	3.7	2.7	4.7	7.0	34.6	8.3	82

cell membranes. This compositional data indicates that the phospholipid polar head group composition cannot be varied without some minor changes in the fatty acid composition. However, the changes in polar head group composition affected by supplementation with choline analogues were generally quite large compared to fluctuations in fatty acid composition.

Modification of the fatty acid composition of chicken embryo fibroblasts

Fatty acid supplementation was carried out by addition of fatty acid-bovine serum albumin complexes to the delipidated medium 20 h after plating the cells and then allowing growth to continue for another 26 h. These conditions allowed for both maximal incorporation of the supplement and good growth of the cells. The fatty acid composition of membrane phospholipids isolated from cells supplemented with several unsaturated fatty acids is reported in Table III. Incorporation of all fatty acid supplements was excellent. Supplementation with 18:1(*t*) markedly reduced the percentage of saturated fatty acids, but did not alter the percentage of polyunsaturated nor long chain fatty acids compared to choline supplemented cells (Table IV). Growth of the cells in the presence of 18:2, 18:3 or 20:4 increased the percentage of polyunsaturated fatty acids approximately five-fold. Concommitant with the increases in polyunsaturated fatty acids were large increases in the number of double bonds/100 fatty acids. In each case, fatty acid supplements appeared to replace 16:1 and 18:1. The phospholipid composition of fatty acid supplemented cells was essentially invariant (Table V).

Since changes in membrane cholesterol can affect physical properties of the membrane and adenylate cyclase activity [10,28—30], the cholesterol contents of the lipid supplemented membranes were also determined. No major changes in cholesterol content of normal or transformed membranes occurred as a consequence of fatty acid or polar head group supplementations.

Protein composition of supplemented membranes

Membranes obtained from all lipid supplemented cells were subjected to SDS-polyacrylamide gel electrophoresis. No changes in SDS gel patterns as a consequence of either polar head group or fatty acid supplementation were evident.

Alteration of adenylate cyclase activity in polar head group supplemented membranes

Changes in the phospholipid polar head group composition of normal or transformed cells had significant effects on adenylate cyclase activity (Table VI). Supplementation with the series choline-dimethylethanolamine-methylethanolamine-ethanolamine illustrates an interesting correlation. This series progresses from a quaternary amine to a primary amine by successive removal of methyl groups from the nitrogen. With transformed and normal cells, successive removal of methyl groups lowered basal adenylate cyclase activity. However, adenylate cyclase in transformed membranes was much less sensitive to these phospholipid compositional changes than adenylate cyclase in normal membranes. Both normal and transformed adenylate cyclase activities exhibited a negative correlation with the primary amino character of the mem-

TABLE III

FATTY ACID COMPOSITION OF CELLS GROWN IN DELIPIDATED MEDIUM + CHOLINE + EXOGENOUS FATTY ACID

Cells and supplement	Fatty acid composition (% by weight)														
	16:0	16:1	18:0	18:1	18:1(*t*)	18:2	18:3	10:2	20:4	20:5	22:0	22:4	22:5	24:1	Other
Normal cells															
Choline	17.4	9.8	12.9	40.9	—	2.5	—	1.9	5.6	0.6	1.4	0.3	0.7	1.5	4.5
+ 18:1(*t*)	4.4	3.6	3.6	19.9	54.1	2.4	0.5	0.6	5.5	0.4	0.9	0.2	0.6	1.1	2.2
+ 18:2	10.8	1.7	8.9	8.8	—	45.7	—	3.8	9.2	1.1	2.8	0.2	0.7	2.9	3.4
+ 18:3	8.3	1.9	9.7	10.1	—	1.5	44.1	0.5	6.8	6.2	1.0	0.4	1.4	1.7	6.4
+ 20:4	15.7	1.9	11.7	11.0	—	1.0	—	0.4	32.7	0.6	0.7	18.2	0.7	—	5.4
Transformed cells															
Choline	16.6	8.7	13.3	46.8	—	2.2	0.6	0.7	3.5	0.7	1.1	0.7	0.5	1.1	3.5
+ 18:1(*t*)	5.3	4.5	4.4	26.2	47.9	2.3	0.6	0.3	3.7	0.4	0.8	0.1	0.4	0.7	2.4
+ 18:2	14.8	1.9	11.1	11.3	—	42.3	—	4.6	4.5	0.9	1.7	0.2	0.5	2.3	3.9
+ 18:3	10.0	2.1	10.5	13.2	—	1.7	43.7	0.4	6.0	2.2	1.1	0.8	1.3	1.7	5.3
+ 20:4	16.3	2.4	10.8	14.5	—	1.2	0.3	0.5	24.8	1.2	0.9	21.1	1.0	—	5.0

TABLE IV

FATTY ACID COMPOSITIONAL PARAMETERS FOR CELLS GROWN IN DELIPIDATED MEDIUM + CHOLINE + EXOGENOUS FATTY ACID *

The parameters calculated from data in Table III.

Cells and supplement	>C18	% Saturated	% Poly-unsaturated	Double bonds per 100 fatty acids *	18:1/20:4
Normal cells					
Choline	13.9	32.6	12.6	98	7.30
+ 18:1(*t*)	10.7	9.3	11.1	66 (120)	3.62 (13.5)
+ 18:2	23.0	24.5	61.4	160	0.96
+ 18:3	21.6	19.7	61.8	217	1.49
+ 20:4	54.0	29.0	54.0	209	0.34
Transformed cells					
Choline	10.1	32.3	9.2	91	13.4
+ 18:1(*t*)	7.4	11.2	8.3	61 (109)	7.08 (20.0)
+ 18:2	16.6	29.8	53.5	137	2.51
+ 18:3	16.1	22.8	56.8	200	2.20
+ 20:4	50.9	29.7	50.7	195	0.58

* Corrected for different weight of different fatty acids. Numbers in parentheses include 18:1(*trans*).

brane phospholipids. This parameter was calculated from the phospholipid compositions (Table I) by assigning phosphatidylethanolamine a value of 1; phosphatidylmethylethanolamine, 2/3; phosphatidyldimethylethanolamine, 1/3; and phosphatidylcholine, 0 (Fig. 1).

Stimulation by fluoride varied somewhat with polar head group supplementation; however, these changes were not pronounced. In contrast, stimula-

TABLE V

PHOSPHOLIPID COMPOSITION OF CELLS GROWN IN DELIPIDATED MEDIUM + CHOLINE + EXOGENOUS FATTY ACID

PC, phosphatidylcholine; PE, phosphatidylethanolamine; PI, phosphatidylinositol; PS, phosphatidylserine; Sp, sphingomyelin; CL, cardiolipin; PMEA, phosphatidylmethylethanolamine.

Cells and supplement	Phospholipid composition (% phosphate)						
	PC	PE	PI + PS	Sp	CL	PMEA	Other *
Normal cells							
Choline	51.5	20.0	13.3	8.9	1.7	2.4	2.2
+ 18:1(*t*)	46.8	24.2	13.5	8.3	1.7	3.0	2.4
+ 18:2	50.9	20.6	12.5	11.1	1.8	1.9	1.2
+ 18:3	51.4	21.1	11.0	11.1	1.5	2.6	1.3
+ 20:4	51.2	17.8	12.9	12.1	1.3	2.0	2.7
Transformed cells							
Choline	45.8	26.0	12.4	10.4	2.0	1.4	2.1
+ 18:1(*t*)	41.7	29.9	11.9	12.1	1.6	1.1	1.8
+ 18:2	43.2	28.6	11.0	11.1	1.5	2.6	1.3
+ 18:3	42.3	26.8	11.8	11.7	1.8	3.4	2.2
+ 20:4	43.9	25.6	13.2	12.3	1.2	1.8	2.0

* Other phospholipids included phosphatidylglycerol, lysophosphatidylcholine (<0.05%) lysophosphatidylethanolamine and other unidentified compounds.

TABLE VI

ADENYLATE CYCLASE ACTIVITIES OF CELLS GROWN IN DELIPIDATED MEDIUM + CHOLINE OR CHOLINE ANALOGUE

Numbers in parentheses indicate fold stimulation over basal activity.

Cells and supplement	Adenylate cyclase activities (pmol/mg/10 min)		
	Basal	Fluoride	Prostaglandin E_1
Normal cells			
Choline	110	1628 (15)	264 (2.4)
Dimethylethanolamine	95.7	1734 (17)	258 (2.7)
Methylethanolamine	66.0	1360 (21)	238 (3.6)
Ethanolamine	46.2	980 (21)	224 (5.3)
Aminobutanol	63.8	880 (14)	230 (3.6)
Aminopropanol	60.5	756 (13)	244 (3.7)
Transformed cells			
Choline	34.0	898 (26)	88.4 (2.6)
Dimethylethanolamine	27.0	826 (31)	78.3 (2.7)
Methylethanolamine	24.6	740 (30)	68.9 (2.7)
Ethanolamine	22.2	817 (37)	122 (5.5)
Aminobutanol	19.4	570 (29)	42.7 (2.2)
Aminopropanol	18.0	442 (25)	59.4 (3.3)

tion by prostaglandin E_1 was much more sensitive to phospholipid compositional changes. For example, supplementation with phosphatidylethanolamine increased the degree of prostaglandin E_1 stimulation by a factor of two in normal and transformed membranes. (Prostaglandin E_1 + GTP) and (prostaglandin E_1 + Gpp(NH)p) stimulated adenylate cyclase 2.4-fold and 10-fold, respectively, both in normal and transformed cells grown in the presence of choline.

Ethanolamine, 2-aminobutanol, and 3-aminopropanol constitute a set of related analogues which all contain a primary amino group but differ in the structure of the alkyl chain between the amino and alcohol functions. Basal adenylate cyclase activity in transformed membranes was relatively insensitive to these phospholipid structural changes, although fluoride and prostaglandin E_1 stimulated activities decreased considerably upon supplementation with 2-aminobutanol or 3-aminopropanol. In contrast, supplementation of normal cells with these two analogues increased basal adenylate cyclase activity with little or no effect on fluoride and prostaglandin E_1 stimulated activities.

Alteration of adenylate cyclase activity in fatty acid supplemented cells

Modification of the fatty acid composition had significant effects on adenylate cyclase activities in normal and transformed membranes (Table VII). Supplementation with 18:1(*t*) had little effect with either membrane system. However, supplementation with *cis* polyunsaturated fatty acids caused a decrease in basal, fluoride stimulated and prostaglandin E_1 stimulated adenylate cyclase activities. This effect was most pronounced in normal membranes where incorporation of 20:4 decreased basal activity by a factor of three.

Attempts were made to correlate adenylate cyclase activities with various fatty acid compositional parameters. No correlations could be drawn between

TABLE VII

ADENYLATE CYCLASE ACTIVITIES OF CELLS GROWN IN DELIPIDATED MEDIUM + CHOLINE + EXOGENOUS FATTY ACIDS

Numbers in parentheses indicate fold stimulation over basal activity.

Cells and supplement	Adenylate cyclase activities (pmol/mg/10 min)		
	Basal	Fluoride	Prostaglandin E_1
Normal cells			
Choline	132	1901 (14)	412 (3.1)
+ 18:1(*t*)	116	2204 (19)	510 (4.4)
+ 18:2	77.9	1169 (15)	223 (2.9)
+ 18:3	34.3	789 (23)	80.6 (2.3)
+ 20:4	43.6	937 (22)	113 (2.6)
Transformed cells			
Choline	31.8	851 (27)	110 (3.5)
+ 18:1(*t*)	29.9	783 (26)	112 (3.7)
+ 18:2	29.3	718 (25)	73.7 (2.5)
+ 18:3	17.2	533 (31)	36.1 (2.1)
+ 20:4	16.9	507 (30)	38.4 (2.3)

adenylate cyclase activity and the percentage of fatty acids longer than 18 carbons, the percent of saturated fatty acids, the percent of polyunsaturated fatty acids or the ratio of 18:1 to 20:4. Excellent correlations ($|r| > 0.98$) were found with both basal and prostaglandin E_1 activities and the number of *cis* double bonds/100 fatty acids. Increases in the number of double bonds/100 fatty acids were associated with a decline in both basal and prostaglandin E_1

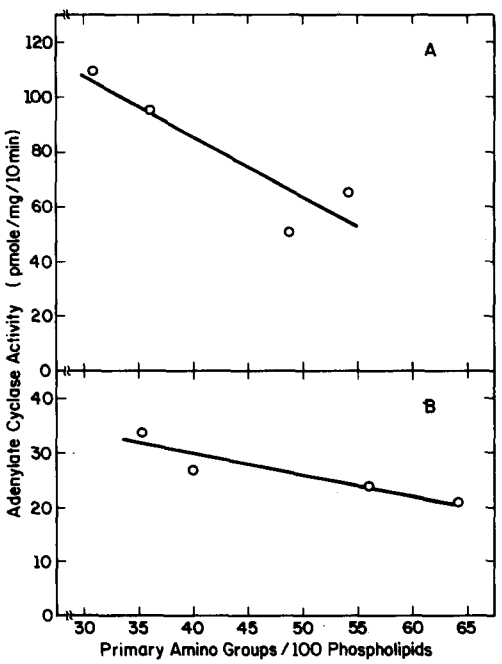
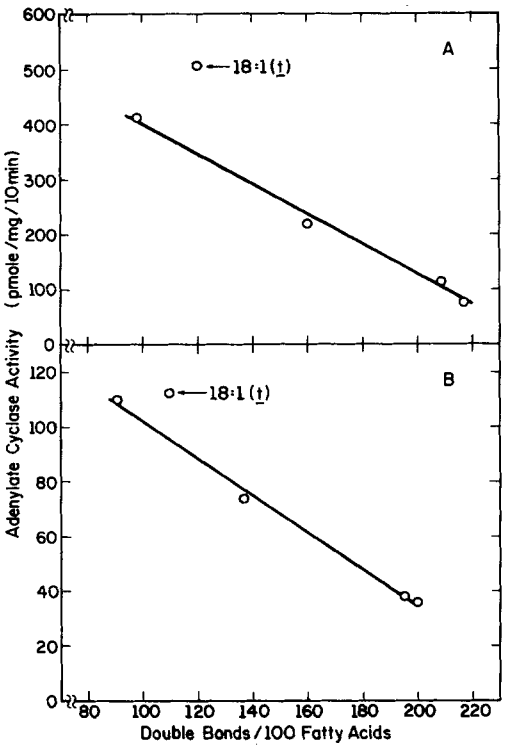


Fig. 1. Correlation between basal adenylate cyclase activity and primary amino group characters of mem brane phospholipids. Data are taken from Tables I and VI. A, normal membranes; B, transformed mem branes.



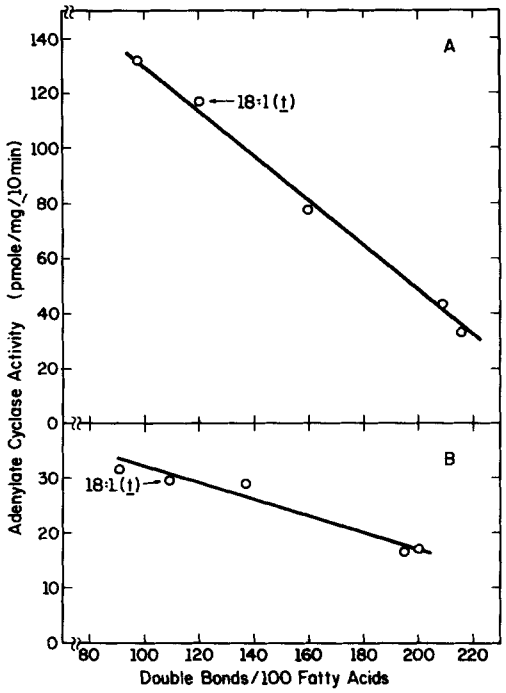


Fig. 2. Correlation between prostaglandin E_1-stimulated adenylate cyclase activity and number of double bonds/100 fatty acids of membrane phospholipids. Data are taken from Tables IV and VII. A, normal membranes; B, transformed membranes.

Fig. 3. Correlation between basal adenylate cyclase activity and number of double bonds/100 fatty acids of membrane phospholipids. Data are taken from Tables IV and VII. A, normal membranes; B, transformed membranes.

stimulated activities, and this effect was most pronounced with normal membranes (Figs. 2, 3).

Discussion

The membrane phospholipid compositions of normal and transformed chicken embryo fibroblasts are significantly different. Since adenylate cyclase is an integral membrane protein system, it might be postulated that the lower activity of the enzyme in transformed membranes is due to an altered phospholipid environment. Changes in the membrane phospholipid or fatty acid compositions can cause significant alterations of adenylate cyclase activities [14,15]. Therefore, the influence of membrane phospholipid and fatty acid compositional changes on adenylate cyclase in normal and transformed membranes was systematically examined.

Adenylate cyclase in chicken embryo fibroblasts is quite sensitive to the membrane phospholipid and fatty acid composition. Basal adenylate cyclase activity in normal membranes varied over a four-fold range, depending upon the lipid supplement. Prostaglandin E_1-stimulated activity was even more sensitive to the membrane lipid composition and varied over a six-fold range with

different lipid supplements. In general, adenylate cyclase activity in transformed membranes was much less sensitive to polar head group or fatty acid compositional changes.

There are a number of systematic correlations between adenylate cyclase activities and membrane phospholipid compositional parameters. For example, enzyme activity in normal and transformed membranes decreased regularly as the degree of fatty acid unsaturation increased. Increases in the primary amino group character of phospholipids also resulted in a decline in adenylate cyclase activity.

These correlations between chicken embryo fibroblasts adenylate cyclase activities and phospholipid compositions are strikingly different from those previously identified for adenylate cyclase in LM cell membranes [15]. With LM cells, increases in the either the primary amino group character or number of double bonds/fatty acid increased adenylate cyclase activity. These two studies suggest that other components of the membrane may moderate the sensitivity of the enzyme to its lipid environment. With current technology, it is impossible to quantitate the absolute amounts of adenylate cyclase present in membrane preparations. Some of the variations in adenylate cyclase activity accompanying lipid supplementation could conceivably be due to altered amounts of the protein in the membranes. This possibility cannot be rigorously excluded; however, SDS gels of the various supplemented membranes were indistinguishable.

The data presented in this study indicate that the changes in phospholipid or fatty acid composition are probably not solely responsible for the lower adenylate cyclase activities in transformed cells. When normal and transformed cells are grown in standard media supplemented with tryptose phosphate broth, the ratios of phosphatidylethanolamine to phosphatidylcholine are 0.58 and 0.81 for normal and transformed membranes respectively. In addition, the two membrane systems differ in the ratio of 18:1/20:4 and the number of double bonds/100 fatty acids. When all of the phospholipid compositional and adenylate cyclase data for the supplemented membranes is analyzed, it is difficult to unambiguously identify any single lipid compositional parameter which may account for the lower adenylate cyclase activity in transformed membranes. The data do suggest that the higher levels of phosphatidylethanolamine in transformed membranes may contribute to the lower adenylate cyclase activities. However, it was not possible to vary the phosphatidylethanolamin composition without some minor changes in fatty acid composition. Therefore, any conclusions concerning the relationship between changes in phosphatidylethanolamine and adenylate cyclase which occur during transformation of chicken embryo fibroblasts are tentative.

The adenylate cyclase system in animal cell membranes is a multisubunit complex comprised of several regulatory subunits and a catalytic subunit [31—35]. Although changes in the phospholipid composition may contribute to the lower adenylate cyclase activities in transformed membranes, there is evidence that Rous-sarcoma-transformed chicken embryo fibroblasts contain decreased amounts of a protein which activates adenylate cyclase [4]. Since the adenylate cyclase activities in normal and transformed membranes are normalized by detergent solubilization [3], this implies that the effects of the

regulatory factor may be indirectly dependent upon the membrane environment. The data presented in this study are consistent with this hypothesis and demonstrate that the enzyme in normal membranes is much more sensitive to phospholipid changes than adenylate cyclase in transformed membranes.

Acknowledgements

We wish to thank Diane Toscano and Barbara Larrain for skillful technical assistance. This work was supported by National Science Foundation Grant PCM 79-15390 (D.R.S.), National Institutes of Health Grants GM 21953 (M.G.), CA 12467 (M.J.W.), and Research Career Development Awards AI 00120 (D.R.S.), CA 00092 (M.J.W.) and GM 00193 (M.G.).

References

1 Anderson, W.B., Johnson, G.S. and Pastan, I. (1973) Proc. Natl. Acad. Sci. U.S.A. 70, 1055—1059
2 Anderson, W.R., Lovelace, E. and Pastan, I. (1973) Biochem. Biophys. Res. Commun. 52, 1293—1299
3 Gidwitz, S., Weber, M.J. and Storm, D.R. (1976) J. Biol. Chem. 251, 7950—7951
4 Anderson, W.B. and Pastan, I. (1975) Adv. Cyclic Nucleotide Res. 5, 681—698
5 Sutherland, E.W., Rall, T.W. and Menon, T. (1962) J. Biol. Chem. 237, 1220—1227
6 Pohl, S.L., Krans, H.M.J., Kozyreff, V., Birnbaunner, L. and Rodbell, M. (1971) J. Biol. Chem. 246, 4447—4454
7 Rethy, A., Tomasi, V. and Trevisani, A. (1971) Arch. Biochem. Biophys. 147, 36—40
8 Rethy, A., Tomasi, V. and Trevisani, A. and Barnabei, O. (1972) Biochim. Biophys. Acta 290, 58—69
9 Rubalcava, B. and Rodbell, M. (1973) J. Biol. Chem. 248, 3831—3837
10 Puchwein, G., Pfeuffer, T. and Helmreich, E.J.M. (1974) J. Biol. Chem. 249, 3232—3240
11 Orly, J. and Schramm, M. (1975) Proc. Natl. Acad. Sci. U.S.A. 72, 3433—3437
12 Houslay, M.D., Hesketh, T.R., Smith, G.A., Warren, G.B. and Metcalfe, J.C. (1976) Biochim. Biophys. Acta 436, 495—504
13 Brivio-Haugland, R.P., Louis, S.L., Musch, K., Waldeck, N. and Williams, M.A. (1976) Biochim. Biophys. Acta 433, 150—163
14 Engelhard, V.H., Esko, J.D., Storm, D.R. and Glaser, M. (1976) Proc. Natl. Acad. Sci. U.S.A. 73, 4482—4486
15 Engelhard, V.H., Glaser, M. and Storm, D.R. (1978) Biochemistry 17, 3191—3200
16 Hale, A., Pessin, J.F., Palmer, F., Weber, M.F. and Glaser, M. (1977) J. Biol. Chem. 252, 6190—6200
17 Pessin, J.E., Salter, D.W. and Glaser, M. (1978) Biochemistry 17, 1997—2004
18 Weber, M.J. (1973) J. Biol. Chem. 248, 2978—2983
19 Horwitz, A.F., Halten, M.E. and Burger, M.M. (1974) Proc. Natl. Acad. Sci. U.S.A. 71, 3115—3119
20 Spector, A.A. and Hoak, J.C. (1969) Anal. Biochem. 32, 277—302
21 Bligh, E.G. and Dyer, W.J. (1959) Can. J. Biochem. Physiol. 37, 911—917
22 Ames, G.F. (1968) J. Bacteriol. 95, 833—843
23 Glaser, M., Ferguson, K. and Vagelos, P.R. (1974) Proc. Natl. Acad Sci. U.S.A. 71, 4072—4076
24 Ferguson, K.A., Glaser, M., Bayer, W.H. and Vagelos, P.R. (1975) Biochemistry 13, 1969—1977
25 Salomon, Y., Landos, C. and Rodbell, M. (1974) Anal. Biochem. 58, 541—548
26 Laemmli, U.K. (1970) Nature 227, 680—685
27 Lowry, O.H., Rosebrough, N.A., Farr, A.L. and Randall, R.J. (1951) J. Biol. Chem. 193, 265—275
28 Papahadjoupoulos, D. (1974) J. Theor. Biol. 43, 329—337
29 Sinha, A.K., Shattil, S.J. and Colman, R.W. (1977) J. Biol. Chem. 252, 3310—3314
30 Klein, I., Moore, L. and Pastan, I. (1978) Biochim. Biophys. Acta 506, 42—53
31 Pfeuffer, T. and Helmreich, E.J.M. (1975) J. Biol. Chem. 250, 867—876
32 Orly, J. and Schramm, M. (1976) Proc. Natl. Acad. Sci. U.S.A. 73, 4410—4414
33 Limbird, L.E. and Lefkowitz, R.J. (1977) J. Biol. Chem. 252, 799—802
34 Ross, E.M. and Gilman, A.G. (1977) Proc. Natl. Acad. Sci. U.S.A. 74, 3715—3719
35 Naya-Vigne, J., Johnson, G.I., Bourne, H.R. and Coffine, P. (1978) Nature (London) 272, 720—722