Vol. 95, No. 1, 1980 BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS
July 16, 1980 Pages 97-102

# EFFECTS ON ADENYLATE CYCLASE ACTIVITIES OF UNSATURATED FATTY ACID INCORPORATION INTO RAT LIVER PLASMA MEMBRANE PHOSPHOLIPIDS. SPECIFIC MODULATION BY LINOLEATE.

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Received February 14, 1980

SUMMARY

The adenylate cyclase activities of the rat liver plasma membrane were measured simultaneously with the incorporation of acyl chains into the membrane phospholipids using oleyl CoA, linoleyl CoA or arachidonyl CoA thioester. The basal, fluoride - and glucagon - stimulated adenylate cyclase activities were increased by the incorporation of linoleate into the plasma membrane phospholipids. Oleyl CoA did not alter the adenylate cyclase activities whereas arachidonyl CoA, at high concentration, decreased the adenylate cyclase activities. These data indicate a specific effect of phospholipid molecular species containing linoleate.

## INTRODUCTION

Several experimental approaches have been employed to evaluate the importance of membrane phospholipids for adenylate cyclase activities. For example, membranes have been treated with phospholipases (1, 2, 3) organic solvants (4) non ionic detergents and lysophospholipids (5). These treatments resulted in changes in basal activity, hormone stimulation or both. Houslay and al (6) fused phospholipid vesicles with membranes and observed changes in both the temperature dependancy and the activity of adenylate cyclase ; Engelhard and al (7, 8) reported modifications of basal, fluoride- and $PGE_1$- stimulated activities in LM cell membranes as a result of the manipulation of the polar headgroup and fatty acid compositions ; recently Axelrod and al (9) in rat reticulocytes have correlated the activation of the isoproterenol-sensitive adenylate cyclase to the transmethylation of phosphatidylcholine into phosphatidylethanolamine and to changes in membrane fluidity.

Lysophosphatidylcholines have been demonstrated to inhibit basal (10) adenylate cyclase activity as well as fluoride (5, 11) or glucagon (11) - stimulated adenylate cyclase activities. Lysophospholipids are present in rat liver plasma membranes and can be produced by the phospholipase A acti-

0006-291X/80/130097-06$01.00/0

vities (12, 13, 14). It was reported that the plasma membranes could incorporate long chain fatty acyl-CoA thioesters in their phospholipids (15). Such transacylation processes could represent a mechanism allowing the decrease in lysophospholipid amount and/or the formation of specific molecular species which would modify the lipid environment of the adenylate cyclase.

To evaluate the effects of such modifications upon adenylate cyclase activity, we have developped a technique for the simultaneous measurement of fatty acyl chains incorporation into the phospholipids and of the adenylate cyclase activities. The present study compares the effect of oleate, linoleate and arachidonate incorporations on basal, fluoride - and glucagon - stimulated adenylate cyclase activities.

## MATERIAL AND METHODS

- Linoleic acid, oleyl-CoA, coenzyme A, ATP, dithiothreitol, albumin and EDTA were obtained from Sigma. Cyclic AMP, creatine phosphate and creatine kinase were from Boehringer-Mannheim.
[1-$^{14}$C] oleyl-CoA (50 mCi/mM), linoleic acid (51 mCi/mM), arachidonic acid (55,5 mCi/mM), cyclic [$^{3}$H] AMP (39.8 Ci/mM) and [$\alpha$-$^{32}$P] ATP (20 Ci/mM) were purchased from New England Nuclear.

- [1-$^{14}$C] linoleyl-CoA and [1-$^{14}$C]-arachidonyl-CoA thioesters were prepared using the mixed anhydride method described by Wieland and Rueff (16) and purified according to Seuberg (17). The specific activities used were 10 µCi/µmole. Their ultraviolet spectrum were typical of acyl-CoA derivatives and exhibited a maximal absorbance at 257 nm and a $A_{232}/A_{257}$ ratio ranging from 0.53 to 0.62 (theoretical ratio 0.55). The yields of synthesis were between 40 and 60 % when based on fatty acid incorporation in the thioester.

- Plasma membranes were prepared from male Wistar rats weighing 200 g according to the procedure devised by Neville up to step 12 (18). The fraction collected above the sucrose gradient layer of density 1.19 was resuspended in 1 mM $NaHCO_3$ and centrifuged at 25.000 x g for 15 min. The membrane fraction (0.6-0.9 mg protein/g liver) was stored in liquid nitrogen. Protein determinationswere done according to Lowry et al (19).

- Transacylation and adenylate cyclase simultaneous measurements. Transacylase activity was measured by the incorporation of[$^{14}$C] acyl chains from [$^{14}$C] acyl-CoA thioesters into the plasma membrane phosphatidylcholine and phosphatidylethanolamine. Adenylate cyclase activity was measured by the conversion of [$\alpha$-$^{32}$P] ATP into cyclic [$^{32}$P] AMP. The standard reaction mixture (0.1 ml) contained : 50 mM Tris-HCl pH 7.5; 5 mM $MgCl_2$; 1 mM dithiothreitol, 10 µM cyclic AMP ; 1 mM EDTA ; 0.5 mM ATP and [$\alpha$-$^{32}$P] ATP, about 1 µCi ; [$^{14}$C] acyl CoA thioester at varying concentration, 0.5 % (w/v) albumin and 0.4 mg/ml membrane protein. In addition, creatine kinase (50 units) and phosphocreatine (5 mM) were added as an ATP regenerating system. The reaction was initiated by the addition of membranes and carried out at 30°C for 10 min. Reaction was stopped by cooling in ice and by diluting with 1 ml $H_2SO_4$, pH 4-5. Samples were then centrifuged at 1 600 x g for 15 min. cAMP was purified from the supernatant according to Salomon et al. (20) using a sequential chromatography on Dowex and alumina columns, cyclic [$^{3}$H] AMP being added at the begining of the purification step to monitore cyclic [$^{32}$P] AMP recovery.

The membrane pellet was extracted with 1 ml chloroform-methanol (1/1 ; v/v). Organic phase was then dried under $N_2$ and lipids spotted on silicagel G plates. Chromatography was carried out in the following system ; chloroform-methanol-acetic acid-water (25/15/4/2)(21). Spots visualized by exposure to iodine vapor or by autoradiography were scrapped and 0.5 ml of methanol was added to each counting vial before adding 2.5 ml of scintillation fluid.

## RESULTS AND DISCUSSION

In our membrane preparations, the linoleate incorporation into the endogenous phosphatidylcholine and phosphatidylethanolamine was about ten fold higher when using linoleyl-CoA than with linoleic acid and CoA (Table I). The increases in adenylate cyclase activities measured under the same incubation conditions were also ten fold higher in the presence of linoleyl CoA than with linoleic acid. Thus, these increases in adenylate cyclase activities appeared to be related to the acyl incorporation.

The membrane phosphatidylcholine and phosphatidylethanolamine acylation levels were measured increasing oleyl CoA, linoleyl CoA and arachidonyl CoA concentrations (fig. 1). At low concentration, arachidonyl CoA was the best acyl donor in phosphatidylcholine as well as in phosphatidylethanolamine. Saturation of the lysophospholipid-acyltransferase occured at about 25 µM arachidonyl CoA while the linoleate incorporation was linear up to at least 100 µM linoleyl CoA. The transacylation from oleyl CoA was also linear but remained low when compared to the transacylation rates measured using either arachidonyl CoA or linoleyl CoA thioesters.

The adenylate cyclase activity measured simultaneously depended on the acyl thioester tested (fig. 2). There was a good correlation between the incorporation of linoleate into the membrane phospholipids and the increase in basal, fluoride - and glucagon - stimulated adenylate cyclase activities. Incorporation of arachidonate, up to 3 nmoles/ mg protein, did not alter adenylate cyclase activity while the incorporation of 3 nmoles/mg protein of linoleate increased adenylate cyclase activity by 50 %. For the highest concentrations of arachidonyl CoA tested, the arachidonate incorporation plateaued and the adenylate cyclase activities decreased. Thus this inhibitory effect appears to be related to the arachidonyl CoA itself and not to its incorporation into the phospholipids. It can be noted that the same concentration in oleyl-CoA did not produce any inhibition.

The different effects of linoleate and arachidonate incorporation into the phospholipids clearly demonstrated that there is no relation between the variations in adenylate cyclase activities and 1) the unsaturation degree of the transacylated fatty acids at variance to what had been described for

TABLE I. Membrane protein (40 µg) were incubated in the standard reaction mixture with or without either 0.1 mM [1-$^{14}$C] linoleyl CoA or 0.1 mM [1-$^{14}$C] linoleic acid plus 0.1 mM CoA. Values are the mean of two determinations. Numbers between brackets represent the fold increase in adenylate cyclase activity upon linoleic acid or linoleyl CoA additions. [NaF] was 10 mM and [glucagon] was 1 µM.

| Exp. | Addition | nmoles/10 min/mg protein Linoleate incorporated into | | Adenylate cyclase activity (pmoles/10 min/ mg protein) | | |
|---|---|---|---|---|---|---|
| | | Phosphatidyl-choline | Phosphatidyl-ethanolamine | basal | +NaF | +glucagon |
| I | none | | | 189±5 | 2795±11 | 1308±5 |
| | 0.1 mM linoleic acid + 0.1 mM CoA | 0.74±0.04 | 0.35±0.04 | 228±2 (x1.2) | 3089±19 (x1.1) | 1640±54 (x1.25) |
| II | none | | | 127±13 | 1584±44 | 992±4 |
| | 0.1 mM linoleyl CoA | 6.70±0.32 | 4.70±0.26 | 322±24 (x2.5) | 4000±43 (x2.5) | 2084±15 (x2.1) |

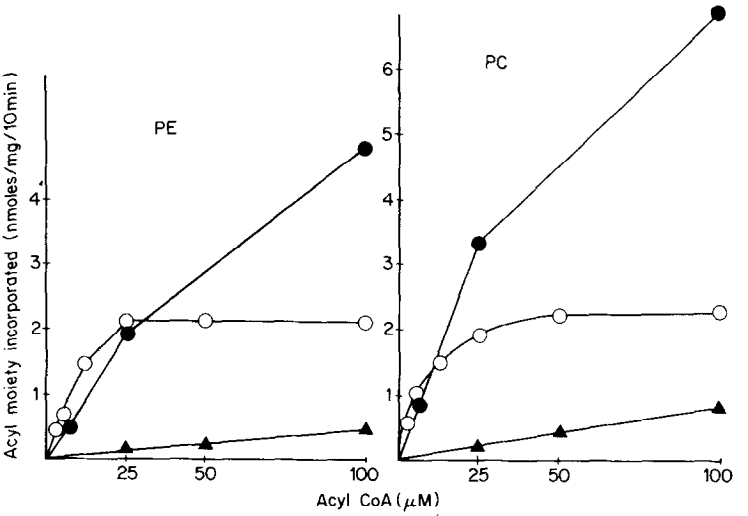


FIGURE 1. Fatty acyls incorporation into plasma membrane phosphatidylcholine (PC) and phosphatidylethanolamine (PE) from various acyl-CoA thioesters. Transacylase activities were determined in the standard reaction mixture. All the values are the mean of two determinations. ▲—▲ oleyl CoA, ●—● linoleyl CoA, ○—○ arachidonyl CoA.

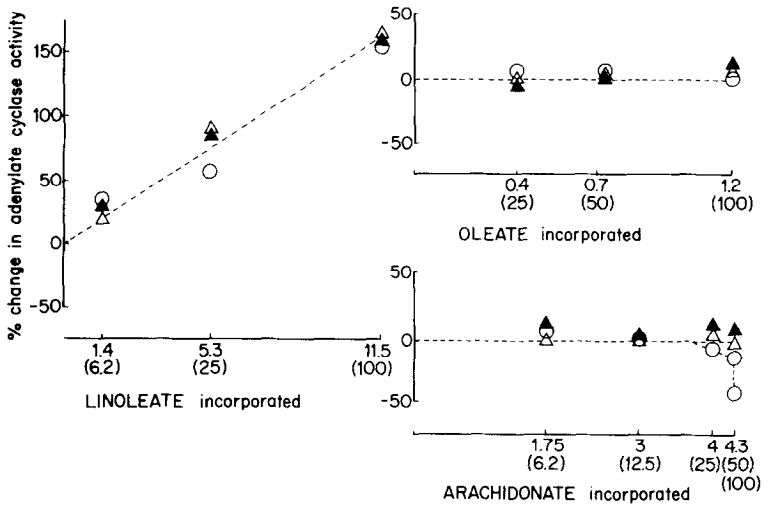


FIGURE 2. Relation between the incorporation of the various acyl groups into the phospholipids and the adenylate cyclase activity. The acyl group incorporations are those of PC + PE reported in figure 1 and are expressed in nmoles/10 min/mg protein. The numbers in parentheses refer to the μM concentration values for which these incorporations were obtained. Transacylation and adenylate cyclase measurements were made simultaneously as described under material and methods. The percent change in adenylate cyclase activity was plotted as a function of the amount of acyl incorporated into the membrane phospholipids. ○—○ basal, ▲—▲ NaF-stimulated and △—△ glucagon-stimulated adenylate cyclase activity.

the $PGE_1$-stimulated adenylate cyclase in LM cells (8), 2) the decrease in the lysophospholipid level in the membrane fraction, lysophosphatidylcholine being known to inhibit adenylate cyclase (5, 11). Thus the adenylate cyclase activities enhancement when linoleate was incorporated into the plasma membrane phospholipids cannot be explained by a modification as a whole of the membrane fluidity. However the specificity of the reacylation process might occur at two different levels : it may be dependent of the acyl CoA thioester used and/or it may be restricted to some specific areas of the plasma membrane. In the latter hypothesis the formation of 2-linoleyl phospholipid molecular species could carry local changes in the plasma membranes physico chemical properties. Anyhow the adenylate cyclase stimulation was specifically correlated to the synthesis of phospholipid molecular species containing linoleate.

ACKNOWLEDGEMENTS

This work was supported by D.R.E.T. 78 34 020 00 480 75 01

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