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LINOLEATE INCORPORATION INTO RAT LIVER MEMBRANES PHOSPHOLIPIDS : EFFECT ON PLASMA MEMBRANE ATPASE ACTIVITIES AND PHYSICAL PROPERTIES

Michelyne Breton, Claude Wolf and Odile Colard

Laboratoire de Biochimie, C.H.U. Saint-Antoine
27, rue Chaligny, 75571 Paris Cédex 12
France

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SUMMARY : Plasma membrane phospholipids were modified by incubation in the presence of linoleyl-CoA with or without added lysolecithin (LPC) for various lenghth of time. In the absence of LPC, a maximum of 10 nmoles linoleyl-phosphatidylcholine (PC) were synthesized and the ATPase specific activities were not affected whereas in the presence of LPC ,when linoleyl-PC synthesis rose from 10 to 80 nmoles, the ATPase activities were decreased. The decrease was similar in the Na,K- or in the Mg-dependant-ATPase and reached maximally 30-40 %. LPC by itself did not modify the ATPases. A concomitant decrease in DPH polarization was observed when linoleate was incorporated into phospholipids. We concluded that the decreased ATPase specific activities may be due to an increased fluidity of membranes produced by linoleyl- PC synthesis. We compare this modulation of ATPases by the membrane fluidity with the specific effect of linoleyl- PC species on adenylate cyclase.

In a previous work on isolated hepatocyte plasma membranes, we described a method using the membrane-associated acyltransferase activities (1) to modify enzymatically the fatty acid composition of phospholipids and to measure simultaneously the activity of an intrinsic membrane enzyme system, the adenylate cyclase. We observed an enhancement of basal and glucagon-stimulated adenylate cyclase correlated with the incorporation of linoleate into membrane-associated phospholipids whereas oleate and arachidonate incorporations were without effect (2, 3). ATPase, another membrane enzyme system has been shown to be modulated by phospholipid fatty acid

Abbreviations:
PC, phosphatidylcholine; LPC, lysophosphatidylcholine; DPH, 1,6-diphenyl 1-3-5-hexatriene.

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modifications (4, 5, 6). So the question arises : is the effect of linoleate in hepatocyte plasma membranes specific for adenylate cyclase activity or also effective on ATPase activities. Here we report that the NaK-ATPase and Mg-ATPase plasma membrane-enzymes are not modulated by linoleate incorporation into membrane-associated phospholipids at the levels which were able to provide the adenylate cyclase enhancement previously described. However decreasing effects in ATPase activities were observed with higher linoleate incorporation which provided a decrease in microviscosity as measured by fluorescence polarization.

MATERIALS AND METHODS. ($1-^{14}C$) linoleic acid was from New England Nuclear. Pyruvate kinase and lactate deshydrogenase mix was from Boehringer Mannheim. All other chemicals were from Sigma Chemical Co.
(14-C) linoleyl-CoA was prepared using the mixed anhydride method described by Wieland and Rueff (7) and purified according to Seuberg (8). The specific activity used was 10 Ci/mole. Plasma membranes were prepared from Wistar rats according to the procedure of Neville (9) as previously described (1). Protein determinations were done according to Lowry (10). Phospholipid phosphorus content was measured using the method of Bartlett (11).

Incorporation of linoleic acid into membrane phospholipids. 0.4 mg/ml of plasma membranes in 50 mM Tris-HCl pH 7.4 containing 5 mM $MgCl_2$, 1 mM DTT, 0.5 % fatty acid free albumin with 50 or 100 µM (14-C)linoleyl CoA and without or with 64 µM egg LPC were incubated at 37° C for various lenghts of time. In all experiments the incorporation of linoleate into PC was monitored by the appearance of labeled PC from (14-C) linoleyl CoA. After incubation, the lipids of a fraction corresponding to 40 µg membrane protein were extracted and separated by thin layer chromatography on silica gel plates (Schleicher & Schüll) with chloroform, methanol, acetic acid, water (25, 15, 4, 2, by vol.) as solvent system. The PC fraction was visualized by exposure to iodine vapor and counted in a liquid scintillation counter (Intertechnique SL 3000).

ATPases assay. ATPases were measured spectrophotometrically at 340 nm by coupling the production of ADP to the oxydation of NADH via the pyruvate kinase and lactate dehydrogenase reactions essentially as described by Catterall and Pederson (12) and validated using liver plasma membranes (13). The ouabain sensitive enzyme was designated as Na,K-ATPase and the ouabain resistant as Mg-ATPase. The incubation mixture in a final volume of 1 ml Tris-HCl (25 mM) pH 7.5 contained : 5 mM ATP, 5 mM $MgCl_2$, 120 mM NaCl, 12.5 mM KCl, 0.6 mM phosphoenolpyruvate, 0.4 mM NADH, 1 unit pyruvate kinase, 1 unit lactate dehydrogenase and 5-8 µg membrane protein with or without 10 mM

ouabain. The reaction was started by addition of the membrane fraction after the enzymes, pyruvate kinase and lactate dehydrogenase had been added to the incubation mixture and preincubated for 5 min. in order to eliminate the ADP contained in the reaction medium. When used, the ouabain was added with the membrane fraction.

Fluorescence measurement : Control or modified membranes (150-200 µg protein) were suspended in 2 ml 0.9 p.1000 NaCl solution and labeled for 20 min at 37° C with 1,6-diphenyl-1-3-5-hexatriène (DPH) with the ratio DPH to lipid, 1/200. Fluorescence polarization was measured with constant stirring in a Jobin Yvon JYE spectrofluorometer. The sample was excited by a vertically polarized light at 357 nm and the fluorescence of the sample was analized at 430 nm. The averaged fluorescence life-time of the DPH has been evaluated previously by the single photon technique at 9.5 nsec. The apparent microviscosity is estimated according to Shinitzky and Barenholz (14).

RESULTS

We have previously observed (1) that in rat liver plasma membranes linoleyl-CoA from about 20 µM to 100 µM was the best incorporated acyl-CoA into the endogenous phospholipids. When LPC was added, the incorporation of linoleate into the PC fraction increased 4 to 6 fold thus obtaining 25-40 nmoles/mg protein incorporated during a 10 min-incubation time. In the present work, extending incubation times and using optimum amounts of linoleyl-CoA and LPC, we incorporated into PC up to 80-100 nmoles linoleate per mg protein. We used this LPC linoleyl transferase activity to modify the plasma membranes in order to study the phospholipid-ATPase interactions. Various linoleate incorporations were obtained by adding or not LPC and by varying the linoleyl-CoA concentration and the incubation times. A typical linoleate incorporation is shown in fig.1. When the membranes were incubated without LPC, the incorporation into the native phospholipids did not exceed 10 nmoles linoleate/mg protein.

The total ATPase activity was not significantly changed when linoleyl-CoA was incubated with the membrane without added LPC (fig.2). This showed that the acyl-CoA by itself and its

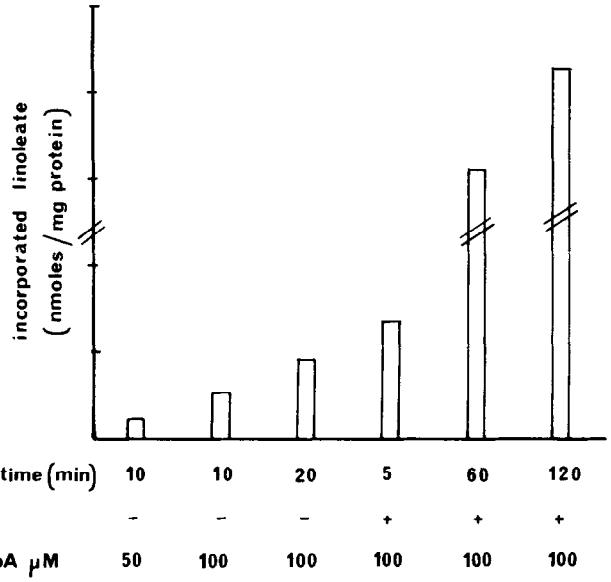

Fig.1 Effect of time, of LPC and linoleyl CoA concentration on linoleate incorporation into PC. Plasma membrane incubation and lipid extraction are described under Materials and Methods.

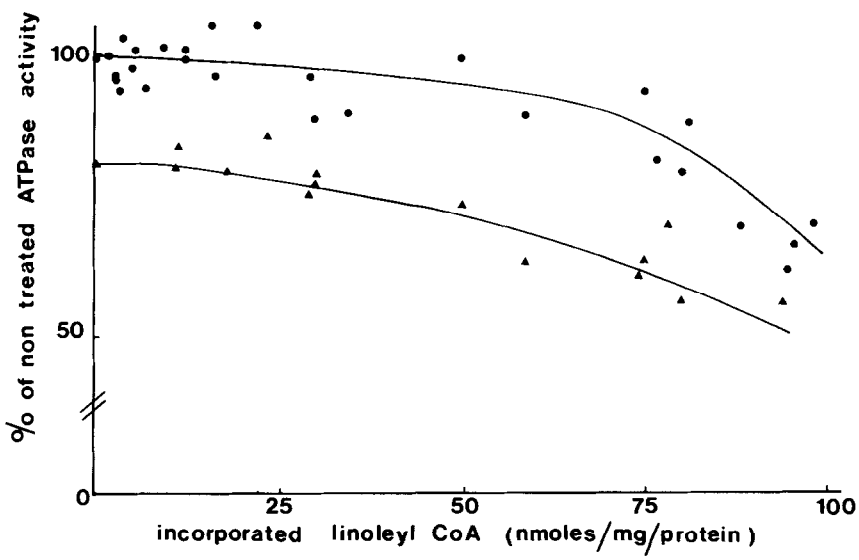

Fig.2 Effect of linoleate incorporation into plasma membrane phospholipids on ATPase activities. Linoleate incorporation into plasma membrane PC and ATPase assays are described under Materials and Methods. ATPase activities are expressed as percentage of total ATPase in non treated membranes . The total ATPase was 97 ± 18.0 μmoles/mg protein/h (based on 30 determinations).
●—● total-ATPase ▲—▲ Mg-ATPase. Control values for ATPase activities were determined for each linoleate incorporation time.

incorporation into the native endogenous phospholipids (up to 10 nmoles linoleate incorporated) were without effect on ATPase activities. 64 µM LPC added alone to the membranes did not affect specific activities of the ATPases. However when the membranes were incubated with LPC and linoleyl CoA, the ATPases activities decreased slowly until about 60 nmoles linoleate incorporated/mg protein into the PC. This decrease became faster above 60 nmoles linoleyl- PC synthesized per mg protein. The activity measured in the presence of ouabain (the Mg-ATPase) was always about 20 % lower than the total ATPase. Thus Na,K-ATPase and Mg-ATPase varyied in the same manner and presented a 30-40 % decrease of activity when linoleyl-PC synthesis was 80-100 nmoles/mg protein.

The fluidity of the plasma membrane lipid phase was determined using the fluorescence polarization and fluorescence lifetime of DPH (fig.3) ; the fluorescence polarization of treated membranes in which linoleate incorporation did not exceed 10 nmoles/mg protein was not significantly different from non-treated membranes. When LPC was added, the higher incorporations of linoleate into PC provided a gradual decrease in the apparent microviscosity (respectively 4.18, 3.90, 3.56 and 3.25 Poise at 20 °C for the control, 16, 46, 65 nmoles linoleate incorporated/mg protein). The Arrhenius plots allow a comparison of flow activation energies which are shown here to decrease throughout the incorporation of linoleate (insert fig. 3). In addition the absence of a critical temperature in these membranes is noticed.

DISCUSSION

In a previous work (2) we observed in rat liver plasma membranes a stimulation of basal, fluoride and glucagon-stimulated adenylate cyclase activities correlated to the amount of

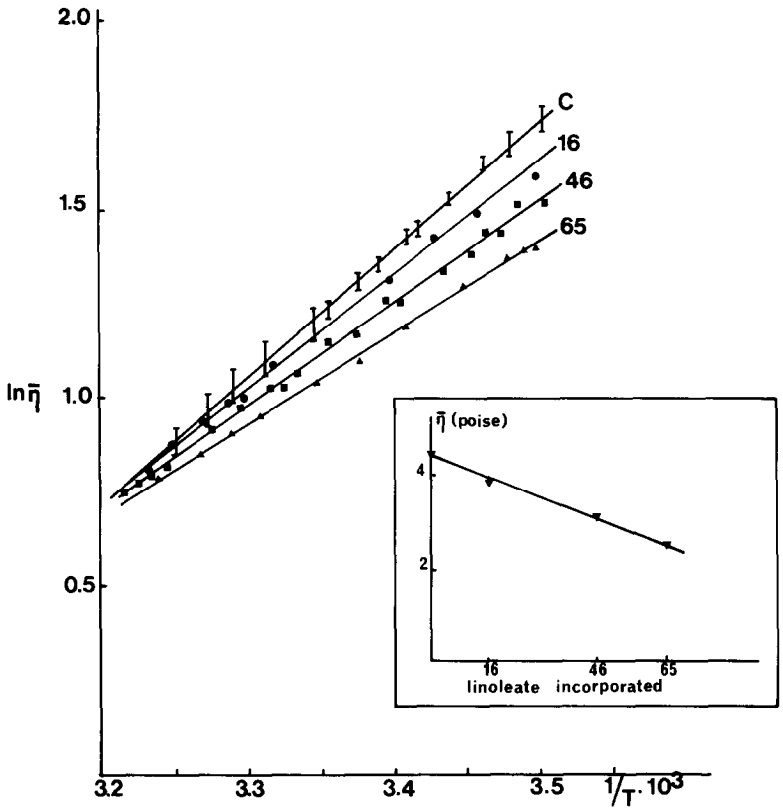

Fig.3 Effect of linoleate incorporation on the microviscosity of plasma membrane. Purified plasma membranes (150 µg) were incubated to obtain various linoleate incorporations, then centrifuged at 1200 x g for 10 min. The plasma membrane pellet was resuspended in 2ml NaCl 0.09 % and 1 nmole of DPH was added (ratio DPH to lipid 1/200) Arrhenius plots of the microviscosity scanned through the temperature ranges 10-35°C are displayed for non-treated membranes (C) and for various incorporation of linoleate : 16, 46, 65 nmoles of linoleate incorporated per mg protein, C(n=3). In the insert, microviscosity at 20 °C is plotted as a function of the incorporation of linoleate into PC.

linoleate incorporated into endogenous phospholipids. The cyclase activities increased twofold when about 5 nmoles of linoleic residues/mg protein were incorporated into PC. The present experiments showed that the incorporation of linoleate into endogenous phospholipids up to 10 nmoles/mg protein incorporated into PC did not affect the ATPase activities.Thus the modulation of adenylate cyclase by phospholipid molecular species containing linoleate is specific for the adenylate cyclase system. Moreover the low amounts of incorporated

linoleate which produced the adenylate cyclase enhancement did not modify the bulk fluidity of the membrane as the absence of variation in the polarization of the lipidic probe DPH indicated. However local changes in the physical properties of the plasma membranes which are not evidenced by DPH could occur. In any case the ATPase system is not sensitive to this specific or localized effect of linoleyl species.

Recent data (4, 5) showed that considerable amounts of polyunsaturated fatty acids incorporated into PC provided a twofold enhancement of the Na,K-ATPase activity and a 40 % decrease in the Mg-ATPase activity of the lymphocyte plasma membranes. It is the reason why we investigated the modulation of the ATPases by incorporating high amounts of linoleate into rat liver plasma membranes PC in the presence of LPC. LPC by itself did not affect the ATPase activities. Our results showed that when adding LPC, the linoleyl-PC synthesis provided an identical decrease in the Na,K-ATPase and in the Mg-ATPase activities with a concomitant decrease in the DPH polarization. The highest linoleate incorporation that we could reach in the liver plasma membrane PC was about 80 nmole/mg protein. In lymphocytes the large modifications of ATPase activities observed were around 150-200 nmoles unsaturated fatty acid incorporated/mg protein.

The modulation of ATPase activities by incorporating fatty acids appeared to be different in these two systems : in lymphocytes arachidonate incorporation increased two fold the Na,K-ATPase when oleate incorporation had nearly no effect. However the incorporation of both oleate and arachidonate resulted in an almost identical decrease in microviscosity (4). The Na,K-ATPase activation could thus be due to a specific phenomenom induced by the incorporated arachidonate (5). In hepatocyte plasma

membranes, the ATPase modulation seems related to an overall fluidity change, as demonstrated by the variations of DPH polarization and flow activation energies, induced by synthesis of membrane-associated linoleyl-PC. However such modifications in hepatocyte plasma membranes where 80 nmoles of newly synthesized PC would result in a 15 % increase in the content of phospholipids, are somewhat unlikely under physiological conditions. It is reason to think that structural features of particular molecular species could be more significant than the bulk lipid order to modulate membrane-associated enzyme as demonstrated by the specific effect of linoleyl-PC species on adenylate cyclase.

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