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MEMBRANE REGULATION OF LIVER AND LUNG MICROSOMES UNDER LOW OXYGEN TENSION

C.Casals, L.M.Herrero, Y.Soriano, P.García-Barreno and A.M.Municio

Department of Experimental Medicine, Hospital Provincial and Department of Biochemistry, Faculty of Chemistry, Universidad Complutense, Madrid, Spain

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A highly monitorized animal model has been developed for the study of the influence of low oxygen tension on lipid composition, microviscosity and regulation of enzyme activities involved in the phospholipid synthesis of hepatic and pulmonary microsomes.

Microviscosity decreased in liver microsomes whereas no difference was shown in that of microsomal membrane core of hypoxemic lung. Nevertheless, phospholipid and cholesterol content of both liver and lung membranes changed significantly. Microsomal membranes of hypoxemic liver increased the unsaturation degree of fatty acids, whereas hypoxemic lung membranes become more saturated, mainly due to the increase of palmitic acid. The adaptive response of lung was confirmed by the high increase of the deacylation-reacylation mechanism.

Oxygen and oxidants produce pathological changes in the lung (1-6) affecting both endothelial and alveolar cells. The toxic effect of long-term O_2 therapy is manifested by progressive respiratory distress and thickenning of the alveolar walls, producing finally massive atelectasis and acute pulmonary œdema. There is evidence that oxygen alters lung surfactant and reduces the synthesis of its major component, dipalmitoylglycerophosphocholine (7,8) and it may responsible for all or part of the pulmonary effects of oxygen (3,7-10).

Concerning the regulatory properties of oxygen on enzyme activities, Gilder and McSherry (11) suggested the existence of a non specific mechanism of inhibition of phosphatidylcholine synthesis by oxygen at hyperbaric pressure using rabbit lung slices. On the contrary, synthesis of HbF can be stimulated by exposing animals to hypoxic stress (12). Patients with chronic obstructive pulmonary disease constitute the largest and homogeneous group of patients with chronic hypoxemia. Chronic arterial hypoxemia affects also to persons living at high altitudes. Exposure to hypoxia for prolonged

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periods results in a number of compensations which minimize the effects of reduced PO_2: ventilatory, circulatory and hematological. Lowered oxygen tension elicits specific effects on some enzyme activities (13) although the influence of oxygen on enzyme adaptation has not been widely studied.

Also, the influence of endogenous membrane enzymes on membrane phospholipids is well-documented. Such reactions can alter the composition and fluidity of the lipid environment, thus altering the activity of physiologically important membrane enzymes and transport processes. The fatty acid composition of particular microsomal phospholipids is altered by ethanol feeding (14). The ethanol consumption induces a more fluid environment within the membrane core of liver plasma membranes, lower cholesterol being responsible, at least partially, for the observed variations in the microviscosity (15). It has been suggested that an adaptive adjustment of the phospholipid composition of membranes causes increased membrane rigidity (16). Adaptive responses to bacterial membrane fluidity concomitant with changes in the fatty acid composition have been described (17).

The present paper reports on initial studies of the effect of experimental low arterial PO_2 on both microsomal membrane fluidity and mechanisms of phospholipid synthesis from rabbit lung and liver.

Materials and Methods

Experimental animal model. The experiments were performed on adult male New Zealand white rabbits. They were kept in individual cages under identical conditions of temperature (23±2°C) and humidity (65%) and fed a standard diet (Panlab Lab.ref.102). The 4-animal control group were kept for 30 min at normal arterial PO_2 (77 mmHg) and arterial blood pH 7.4±0.1. The 4-animal low arterial PO_2 group were kept at low arterial PO_2 (43 mmHg) and arterial blood pH 7.3 ± 0.1, for 30 min.

Rabbits were premedicated with intramuscular injection of ketamine (10 mg/Kg). Anesthesia was induced by slow intravenous injection of ketamine (10 mg/kg) at intermittent doses via ear vein. A tracheostomy was performed to all animals and afterwards a cannula was tied into the trachea attached to a IMV Neonatal Ventilator. Flows of O_2 and NO_2 and respiratory rate were controled to keep either normal or low arterial PO_2 as well as an arterial blood pH of 7.4 for 30 min. Arterial blood gases, pH and bicarbonate were continously monitorized (Gas Check 938 AVL) in blood from catheterized right iliac artery. Sodium, potassium, calcium and glucose were also determined using an Automated Stat Routine Analyzer (Astra-4, Beckman) and creatinine with a creatinine analyzer 2 (Beckman). At the end of the experiments, lungs and liver were removed, frozen immediately with liquid N_2 and maintained at -80°C until use.

Subcellular fractionation. Organs were minced and carefully washed with ice-cold 50 mM potassium phosphate buffer (pH 6.8 for lung and 7.4 for liver), containing 150 mM KCl, 1 mM EDTA, 1 mM DTE and 10% glycerol. Organs were homogenized with 5 ml/g of the same buffer in a Potter-Elvehjem homogenizer provided with a Teflon pestle (four

30-s strokes). Tissue homogenates were first centrifuged for 5 min at 2 445 g and afterwards twice for 15 min at 17 300 g in a Sorvall RC2B centrifuge. Postmitochondrial supernatants were centrifuged at 105 000 g for 60 min in a Beckman L-5 ultracentrifuge. Microsomes were resuspended carefully in ice-cold 50 mM tris/HCl buffer, pH 7.4, containing 150 mM KCl and 0.5 mM DTE and resedimented by centrifugation at 150 000 g for 30 min. Microsomal pellets were resuspended in 4 ml of the same buffer. All procedures were carried out at 0-4°C. Microsomal preparations were immediately used for enzyme assays, protein determination and fluorescence polarization determination.

Enzyme assays. *De novo* glycerolipid biosynthesis was assayed by using 3 mM L-(U-^{14}C)glycerol 3-phosphate (sp.rad. 0.1 Ci/mol), 0.4 mM (9,10-^{3}H)palmitate bound to bovine serum albumin (BSA) (sp.rad. 1.5 Ci/mol), 0.2 mM CoA, 4 mM ATP, 0.3 mM CDP-choline, 0.3 mM CDP-ethanolamine, 3 mM $MgCl_2$, 150 mM KCl, 0.5 mM DTE and 0.4 mg of microsomal proteins in a final volume of 1 ml with 50 mM tris/HCl buffer, pH 7.4. Incubations were carried out at 37°C for 30 min.
Competition between palmitic and either linoleic or arachidonic acid for the glycerolipid biosynthesis was performed by using 3 µmol of glycerol 3-phosphate, 0.2 µmol of (9,10-^{3}H)palmitate bound BSA (sp.rad. 1.5 Ci/mol and either 0.2 µmol of (1-^{14}C)linoleate bound BSA (sp.rad. 0.5 Ci/mol) or 0.2 µmol of (1-^{14}C)arachidonic acid bound BSA (sp.rad. 0.05 Ci/mol). Other components of the mixture and incubation conditions as described above. Labeled and unlabeled fatty acid sodium salts were sonicated at 6-8 microns for 15-30 min at 0-4°C in a MSE sonicator in 50 mM tris/HCl buffer, pH 7.4, containing 0.5 mM DTE and 150 mM KCl. When the solution was completely clear, BSA (fraction V, fatty acid-free) from a concentrates stock (20%) in the same buffer, was added with gentle mixing to obtain 1 mg albumin/0.1 µmol fatty acid. The stock was frozen at -20°C until use.
Both, acyl-CoA:lysophosphatidylcholine acyltransferase and acyl-CoA hydrolase activities were simultaneously determined by incubation of 30 nmol of (1-^{14}C)palmitoyl-CoA (sp.rad. 1 Ci/mol), 0.2 µmol of 1-palmitoyl-*sn*-glycero-3-phosphocholine and 0.2 mg of microsomal protein in a final volume of 1 ml with 90 mM tris/HCl buffer, pH 7.4, containing 2.4 mM $MgCl_2$ and 30 mM KCl. The incubation was carried out for 30 min at 37°C.

Reaction product analysis. All assay reactions were stopped by adding 5 ml of chloroform-methanol (2:1, by vol.). The total lipid-soluble products were obtained by a modification of the method of Bligh and Dyer (18) and the lipid classes were isolated by t.l.c. on silica-gel G plates with chloroform-methanol-water (65:25:4, by vol.) as the developing solvent. Lipid areas of the chromatogram were visually detected by exposure to I_2 vapour and scraped off into counting vials containing 15 ml of scintillation cocktail (toluene scintillation mixture with 0.5% PPO and 0.03% POPOP-triton X100-water, 10:5:1 by vol.). Samples were counted in a Packard 3255 scintillation spectrometer with external-standard correction for quenched samples.

Analytical methods. Protein concentration was determined by the method of Lowry *et al.* (19), with bovine serum albumin as standard. Lipid phosphorous was measured according to Rouser *et al.* (20). Cholesterol was estimated by enzymatic spectrophotometric method (21). Fatty acid analysis in phospholipid fractions was carried out as described (22).

Fluorescence polarization. 1,6-Diphenylhexa-1,3,5-triene was incorporated into membranes (0.1 mg protein/ml) using a stable dispersion of the probe in 50 mM tris/HCl buffer, pH 7.4, containing 0.5 mM DTE and 150 mM KCl (final concentration 9 μM), for 30 min at 37°C. Fluorescence polarization was measured at 25°C in a Perkin-Elmer spectrofluorimeter equipped with a polarization attachment. Measurement conditions and corrections as described (23). Microviscosity ($\bar{\eta}$) was calculated from the P values through the relation $\bar{\eta} = 2P/(0.46-P)$ (24).

Results and discussion

One 4-animal group has been used as control and other 4-animal group was submitted to hypoxemic conditions according to the conditions reported above.

Table 1 shows the values of fluorescence polarization (P) and microviscosity ($\bar{\eta}$) of microsomal membranes of lung and liver from hypoxemic and control rabbits.

Polarization values indicate clearly a more fluid environment within the membrane core of liver microsomes of the group of hypoxemic animals than that of the control group; however, there was no significant difference between the microviscosity of the microsomal membranes from the control and hypoxemic lungs.

An insight to the lipids for these microsomes indicates that phospholipid and cholesterol content of both lung and liver microsomal membranes decreases significantly under the hypoxemic conditions (Table 2). It is known that the molar ratio of cholesterol/phospholipid is inversely correlated with the fluidity of a variety of natural membranes (25,26) and that the enrichment or depletion, respectively, of human erythrocyte membrane cholesterol decreases or enhances the fluidity (27,28). Thus, the changes of phospholipid and cholesterol content observed in the hypoxemic microsomal membranes of liver could account, at least partially, for the enhancement of their fluidity. On the other side, DPH is assumed to be aligned with the phospholipid acyl chains (29) and the degree of saturation of the fatty acyl side chains of the phospholipids are

Table 1. Effect of hypoxia on the microviscosity ($\bar{\eta}$) of microsomal membranes

Animal	Organ	P	$\bar{\eta}$ (Pa.s)
Control	Lung	0.276±0.007	0.30±0.02
	Liver	0.248±0.005	0.23±0.01
Hypoxia	Lung	0.276±0.006	0.30±0.01*
	Liver	0.222±0.005	0.18±0.01**

(*) n.s. (**) $P<0.01$

Table 2. Effect of hypoxia on phospholipid and cholesterol content of microsomal membranes

Organ	µmol PL/ mg total lipids	µmol Ch/ mg total lipids	µmol Ch/ µmol PL	mg Ch/ mg proteins
Control lung	1.24	0.61	0.49	0.11
liver	1.40	0.27	0.19	0.03
Hypoxia lung	0.62	0.24	0.38	0.07
liver	0.75	0.07	0.09	0.01

determinants of fluidity; *cis* double bounds introduce kinks which prevent close packing and thus enhance fluidity (30,31).

The fatty acid composition of total phospholipids from dif - ferent types of microsomal membranes is given in Table 3.

The average degree of unsaturation exhibits in both cases a significant variation although in the opposite sense. Thus, hypo- xemic lung membranes become more saturated whereas those of hypo- xemic liver increase the unsaturation degree.

Studies on the physical properties of mitochondrial membranes show that their changes are related with variations in the phos -

Table 3. Fatty acid composition of microsomes from control and hypoxemic lung and liver#

Fatty acid	LUNG		LIVER	
	CONTROL	HYPOXEMIC	CONTROL	HYPOXEMIC
14:0	11.1(1.5)	5.8(0.8)	-	3.0(0.5)
14:1	17.0(2.3)	3.1(0.4)	-	4.6(0.6)
15:0	4.9(0.7)	2.0(0.3)	-	-
16:0	209.4(28.8)	250.5(34.5)*	140.0(19.5)	123.7(17.0)
16:1	19.7(2.7)	15.0(2.0)	11.4(1.6)	8.4(1.5)
18:0	103.6(14.3)	121.5(16.8)*	154.0(21.5)	158.0(21.8)
18:1	127.9(17.6)	133.4(18.4)	155.5(21.7)	126.1(17.4)
18:2	108.0(14.8)	106.7(14.7)	181.7(25.4)	236.2(32.6)*
18:3	3.0(0.5)	4.8(0.7)	7.2(1.0)	13.0(1.8)
20:2	4.0(0.6)	13.1(1.8)	4.3(0.6)	3.0(0.5)
20:4	101.0(14.0)	54.3(7.4)*	48.1(6.7)	37.5(5.2)
Unsaturation degree	1.12	0.87	1.05	1.11

(#) Values are given as µg fatty acids/mg phospholipids. Per - centages are given in brackets.

(*) $P<0.01$

Table 4. Synthesis of phosphatidylcholine from labelled palmitate and glycerol 3-P

ORGAN	(^{3}H)palmitate (nmol)	(^{14}C)glycerol 3-P (nmol)	(^{3}H)/(^{14}C)
Control lung	2.50±0.18	3.14±0.25	0.8
liver	3.47±0.22	7.07±0.42	0.5
Hypoxia lung	3.77±0.29	1.34±0.09	2.8
liver	3.20±0.31	0.91±0.09	3.5

pholipid composition (32). Also adaptive responses to bacterial growth in the presence of ethanol induces a more fluid membrane concomitant with changes in the fatty acid composition (17). A similar behaviour takes place in microsomal membranes of hypoxemic liver in which the microviscosity is inversely correlated with the unsaturation degree. Nevertheless, lung exhibits a quite different response and the decrease of the unsaturation degree in the hypoxemic lung membranes is mainly due to the increase of the levels of palmitic acid and the simultaneous diminution of arachidonic acid (Table 3). Taking into account the presence of palmitic acid acylating the main component of pulmonary surfactant, its high increase under the hypoxemic conditions could reflect a compensatory change in lipid composition to counteract the low access of oxygen to the most fundamental tissue. Table 4 shows a high participation of *de novo* pathway for the phosphatidylcholine synthesis in both control lung and liver. However, hypoxia induces in both organs a shift towards the deacylation-reacylation mechanism. Table 5 confirms the net increase of acyl transferase activity in hypoxemic lung using palmitoyl-CoA as fatty acid donor. It agrees with the higher ex -

Table 5. Synthesis of phosphatidylcholine by acylating the lysoderivative with (^{14}C) palmitoyl-CoA

ORGAN	acyl transferase activity (nmol of PC)	acyl hydrolase activity (nmol of FA)
Control lung	7.60±0.88	5.25±0.45
liver	8.32±0.89	6.00±0.78
Hypoxia lung	10.38±0.75	5.00±0.50
liver	7.30±0.64	7.69±0.65

tent of palmitic acid in microsomal phospholipids in hypoxemic lung as an adaptive response to the limiting oxygen availability. Under these conditions, other enzymes as succinate dehydrogenase semms also to be modulated for compensation of the lack of oxygen as this forms part of electron transport activities which account for the bulk use of oxygen, yielding anergy to the cell (13).

Incorporation experiments of labeled glycerol 3-P, without exogenous palmitate, show the existence of endogenous fatty acids incorporating into phospholipids. It is the reason of the low $(^{3}H)/(^{14}C)$ ratios obtained in the double labeled experiments for the phosphatidylcholine synthesis in control organs. Competition experiments using palmitate and either linoleate or arachidonate show the utilization of endogenous unsaturated fatty acids in both control and hypoxemic animals.

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