Lipogenesis in liver, lung and adipose tissue of rats fed with oleoylanilide

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(Received 8 November 1982/Accepted 18 January 1983)

1. Oleoylanilide was administered orally to groups of rats according to different patterns. 2. Subcellular fractionation of liver, lung and adipose tissue was then carried out in order to study the main enzyme activities involved in the lipogenesis. 3. The observed findings indicate that adipose tissue and lung are the main target organs for the anilide, adipose tissue being involved in a general decrease of the enzyme activities, whereas transacylation reaction exhibits the most marked depletion of all the enzyme activities in the lung. 4. The enzyme activities in liver were not markedly affected by this oral administration, although some data support the existence of a latent liver toxicity. 5. These data suggest that oleoylanilide has the capacity to alter lipid metabolism of lung and adipose tissue to a considerable extent, whereas no major effect was produced in the liver. 6. This different organ response could be related to the lymphatic gland via absorption of the substance.

Anilides, such as acetanilide, paracetamol and phenacetin, have been used extensively for many years as antipyretic analgesics. The addition of substituents to the ring of acetanilide has more effect on toxicity and analgesic potency than addition of methylene groups to the acyl moiety (Starmer *et al.*, 1971). However, no outstanding differences were found among the analgesic activities of the homologous series of anilides, from acetanilide to valer-anilide; the higher homologues were only weakly active and LD₅₀ values could not be obtained (Starmer *et al.*, 1971). Recently, Murphy & Vodyanoy (1982) have suggested that the surface properties of substituted stearanilides are different from those of the non-esterified fatty acids. This and other reports (Kemper *et al.*, 1982; Tena, 1982) are related to the aetiology of an epidemic of poisoning in Spain from denaturated rape-seed oil. This toxic syndrome is a new disease of multisystemic character (Vicario *et al.*, 1982; Noriega, 1982). In the acute stage the disease resembled pneumonia, whereas in the chronic stage illness has been associated with weight loss, weakness, muscular atrophy, neuromuscular and scleroderma-like symptoms and respiratory disturbances (Noriega, 1982). The histological characteristics are widespread damage of the endothelial lining of arterioles and venules, without damage to other vessel layers (Martinez-Tello *et al.*, 1982).

Taking into account that long-chain-fatty acid anilides have been suggested to be the cause of the symptoms observed and that a severe adipose-tissue

weight depletion is one of the main metabolic disorders exhibited by these patients, we attempted to study the metabolic influence of oleoylanilide on the main enzyme activities of lipid synthesis. The present paper details the influence of oleoylanilide on the synthesis *de novo* of phospholipids and triacylglycerols, fatty acid synthesis, as well as the transacylation and re-acylation reactions of phospholipids in lung, liver and adipose tissue from rats.

Materials and methods

Materials

All chemicals and solvents used in the present study were reagent and/or analytical grade. [1-¹⁴C]Palmitoyl-CoA, [U-¹⁴C]glycerol 3-phosphate, [1-¹⁴C]acetyl-CoA and 1-[1-¹⁴C]palmitoyl-*sn*-glycero-3-phosphocholine were obtained from The Radiochemical Centre (Amersham, Bucks., U.K.). NADPH, ATP, palmitoyl-CoA, glycerol 3-phosphate, oleic acid, EDTA, carbodi-imide derivatives, mercaptoethanol and scintillation reagents were purchased from Sigma Chemical Co. (St. Louis, MO, U.S.A.). Commercial pure olive oil was from Carbonell (Spain).

Synthesis of oleoylanilide

Equimolecular amounts of oleic acid, aniline and dimethylaminopropylethyl carbodi-imide dissolved in ethanol were at room temperature for 2–3 days. The anilide was obtained after concentrating the solution. The crude product was recrystallized from

ethanol and dried under vacuum. The chemical purity of this compound was assessed by elemental analysis and n.m.r. and i.r. spectroscopy.

Animals and treatment schemes

Three groups of male Wistar rats (150–180 g; three rats in each group) were fed standard rat chow and water *ad lib*. Each rat received daily 5 mg of oleoylanilide dissolved in 0.5 ml of pure olive oil, delivered by an oesophageal tube, for 10–15 days and various times without administration of the anilide allowed before the animals were killed. The same number of control animals received 0.5 ml of pure olive oil alone. All animals were killed at approximately the same time each morning; after decapitation, animals were exsanguinated and liver, lungs and abdominal adipose tissue were excised and carefully washed with 50 mM-Tris/HCl buffer, pH 7.4, containing 1 mM-EDTA and 0.25 M-sucrose at 4°C. Organs from each group were pooled and immediately chopped and homogenized in a Potter–Elvehjem homogenizer provided with a Teflon pestle with the same buffer (four strokes of 30 s each stroke; 6–10 ml/g of tissue).

Subcellular fractionation

Tissue homogenates were first centrifuged for 5 min at 2450 g and, afterwards, twice for 15 min at 27000 g in a Sorvall RC2B centrifuge. The post-mitochondrial supernatant was centrifuged at 105000 g for 60 min in a Beckman L-5 ultracentrifuge. The microsomal fraction was suspended in 4–5 ml of 50 mM-Tris/HCl buffer, pH 7.4, using a small glass Potter–Elvehjem homogenizer. All the fractionation procedures were performed at 0–4°C. Portions of the postmitochondrial, soluble and microsomal preparations were used for protein determinations. These preparations were used for various enzyme assays.

Enzyme assays

The synthesis *de novo* was assayed by using either [1-¹⁴C]palmitoyl-CoA or [U-¹⁴C]glycerol 3-phosphate as precursors. The standard assay mixture for the incorporation of palmitoyl-CoA consisted of 32.8 nmol of [1-¹⁴C]palmitoyl-CoA (sp. radioactivity 3 Ci/mol), 0.4 μmol of ATP, 1 μmol of glycerol 3-phosphate, 4 μmol of MgCl₂ and 0.5 mg of post-mitochondrial protein to a final volume of 1 ml with 75 mM-Tris/HCl buffer, pH 7.4, containing 0.5 mM-EDTA, 0.125 M-sucrose and 5% glycerol. The mixture was incubated at 37°C for 1 h.

The standard assay mixture for the incorporation of glycerol 3-phosphate consisted of 1 μmol of [U-¹⁴C]glycerol 3-phosphate (sp. radioactivity 0.5 Ci/mol), 64 nmol of palmitoyl-CoA, 0.4 μmol of ATP, 4 μmol of MgCl₂ and 0.5 mg of postmito-

chondrial protein to a final volume of 1 ml with the same buffer as before. The mixture was incubated at 37°C for 1 h.

The fatty acid synthesis was assayed by using 10 μM-[1-¹⁴C]acetyl-CoA (sp. radioactivity 4.96 Ci/mol), 100 μM-malonyl-CoA, 300 μM-NADPH and 0.25–0.50 mg of proteins from the soluble fraction to a final volume of 1 ml with 50 mM-Tris/HCl, pH 6.8, 0.125 M-sucrose, 1.8 mM-EDTA, 3.6 mM-mercaptoethanol and 6% glycerol. The mixture was incubated at 37°C for 30 min.

The lysolecithin reacylation mechanism and the acyl-CoA hydrolysis were simultaneously determined by incubation of 0.2 μmol of 1-palmitoyl-*sn*-glycerol-3-phosphocholine, 27 nmol of [1-¹⁴C]-palmitoyl-CoA (sp. radioactivity 0.7 Ci/mol) and 0.1 mg of microsomal proteins to a final volume of 1 ml with 95 mM-Tris/HCl buffer, pH 7.4, containing 75 mM-sucrose, 0.3 mM-EDTA and 3.3% glycerol. The incubation was carried out for 30 min at 37°C.

The lysolecithin transacylation mechanism was assayed with 0.4 μmol of 1-[1-¹⁴C]palmitoyl-*sn*-glycerol-3-phosphocholine (sp. radioactivity 0.05 Ci/mol) and 0.25–0.50 mg of proteins from the soluble fraction to a final volume of 1 ml with 50 mM-Tris/HCl buffer, pH 6.8, containing 0.125 M-sucrose, 2 mM-EDTA, 4.5 mM-mercaptoethanol and 6% glycerol. The mixture was incubated at 37°C for 30 min (Casals *et al.*, 1982).

Analysis of reaction products

All assay reactions were stopped by adding 5 ml of chloroform/methanol (2:1, by vol.). The total lipid-soluble products were obtained by the method of Bligh & Dyer (1959) 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 for separation of polar lipids. After chromatography, lipid areas of the chromatogram were visually detected by exposure to I₂ vapour and scraped off into counting vials containing 15 ml of scintillation cocktail {toluene scintillation mixture [0.5% 2,5-diphenyloxazole and 0.03% 1,4-bis-(5-phenyloxazol-2-yl)benzene in scintillation-grade toluene]}, Triton X-100 and water (10:5:1, by vol.). Samples were counted in a Packard 3255 scintillation spectrometer with external-standard correction for quenched samples.

Fatty acid analysis

Lipid fraction was dissolved in 0.5 ml of spectrograde hexane and 2.0 ml of boron trifluoride in methanol was then added. The mixture, in an atmosphere of N₂, was heated in a boiling water bath for 30 min. After cooling, 20 ml of distilled water was added, and the sample was extracted three times with hexane (2.5 ml). The upper layers were pooled

and evaporated to dryness under N₂ and the residue was redissolved in hexane. The fatty acid methyl esters in this solution were separated by g.l.c. on a Hewlett–Packard model 5830A gas chromatograph and columns of 10% Sp-2330 on 100/200 Chromosorb W (Supelco Inc., Bellefonte, PA, U.S.A.) at 180°C. Peak areas were automatically integrated.

Other methods

Protein concentration was measured by the method of Lowry *et al.* (1951), with bovine serum albumin as the reference standard.

Analysis of data

The values presented are the means $\pm$ s.d. obtained from the three groups of animals. Each one-group value for the enzyme assays was also the mean of triplicate determinations, with errors within 5% of the mean. A *P* value of <0.05 was used as the criterion for statistical significance.

Results

Synthesis of glycerolipids *de novo*

Results in Table 1 show the incorporation of [¹⁴C]palmitoyl-CoA into triacylglycerols and total phospholipids by a microsomal preparation from liver, lung and adipose tissue. In this first series of incorporation experiments, there appears to be a strong decrease in the fatty acid incorporation in both triacylglycerols and phospholipids from the adipose tissue of the oleoylanilide-treated animals. However, the major feature is the absence of

palmitate incorporation into adipose triacylglycerols. Lung also exhibited a decreased fatty acid incorporation into esterified lipids, mainly into triacylglycerols. In contrast, there is no difference in the uptake of labelled palmitate for either liver phospholipids or triacylglycerols.

The synthesis of triacylglycerols and of the most important classes of phospholipids of liver, lung and adipose tissue from rats treated according to different experimental schemes was assayed by using [¹⁴C]glycerol 3-phosphate as precursor. The response observed (Table 2) confirms the results obtained in the palmitate incorporation. Again in contrast, the incorporation of [¹⁴C]glycerol 3-phosphate into each lipid class of adipose tissue is practically absent, whereas pulmonary lipids show a clear and strong diminution in the synthesis of glycerolipids *de novo*. However, the recovery of the biosynthetic capacity of triacylglycerols and phosphatidic acid after a period free of oleoylanilide administration is clearly established in lung, whereas this is not observed by the adipose tissue. So it is apparent that adipose tissue does not incorporate either non-esterified fatty acids or glycerol 3-phosphate. On the other hand, the lipid-synthesis capacity of liver microsomes shows only a small but significant distortion of the control patterns with a clear recovery and even an increase of triacylglycerols and phosphatidic acid during the oleoylanilide-free period. The greatly elevated levels of triacylglycerols and phosphatidic acid seen after 45 days free of oleoylanilide administration could be the result of a latent liver toxicity; the marked inhibition of phosphatidylcholine and phosphatidylethanolamine synthesis during the recovery period and not during the treatment phase could be also the result of a slow accumulation of the anilide in the liver.

Furthermore, the fatty acid composition of total lipids from hepatic microsomes and mitochondria of oleoylanilide-treated animals does not exhibit any significant variation (Table 3).

Synthesis of fatty acids

To determine the relative importance of fatty acid incorporation from fatty acids formed endogenously from acetate, the total incorporation of [¹⁴C]acetyl-CoA into fatty acids was measured by using a 105 000g supernatant of liver, lung or adipose-tissue homogenate from both control and treated rats. It is seen in Table 4 that only adipose tissue exhibits a clear diminution of the fatty acid synthesis.

Re-acylation and transacylation reactions

Microsomes of liver, lung and adipose tissue from both control and oleoylanilide-treated rats were assayed for the acyl-CoA:lysophosphatidylcholine

Table 1. Incorporation *in vitro* of [¹⁻¹⁴C]palmitoyl-CoA into non-esterified fatty acids, triacylglycerols and total phospholipids by a microsomal preparation of liver, lung and adipose tissue from control rats and rats fed with oleoylanilide

Values are expressed as nmol of palmitoyl-CoA incorporated/0.5 mg of protein (means $\pm$ s.d.).

Organ	Lipid class	Control	Oleoylanilide treatment*
Liver	Non-esterified fatty acids	10.1 $\pm$ 0.4	10.3 $\pm$ 0.5
	Triacylglycerols	1.4 $\pm$ 0.1	1.3 $\pm$ 0.2
	Phospholipids	5.5 $\pm$ 0.2	5.3 $\pm$ 0.2
Lung	Non-esterified fatty acids	13.4 $\pm$ 0.7	16.0 $\pm$ 0.8
	Triacylglycerols	1.8 $\pm$ 0.1	0.1 $\pm$ 0.02
	Phospholipids	2.0 $\pm$ 0.1	0.8 $\pm$ 0.03
Adipose tissue	Non-esterified fatty acids	10.4 $\pm$ 0.6	16.9 $\pm$ 1.1
	Triacylglycerols	5.0 $\pm$ 0.3	0.06 $\pm$ 0.01
	Phospholipids	1.6 $\pm$ 0.2	0.12 $\pm$ 0.03

* The animals received 5 mg of oleoylanilide daily for 13 days and 4 days without administration elapsed before the rats were killed.

Table 2. *Incorporation in vitro of [U - 14 C]glycerol 3-phosphate into triacylglycerols and phospholipid classes by a microsomal preparation of liver, lung and adipose tissue from control and oleoylanilide-fed rats*

Values are expressed as nmol of glycerol 3-phosphate incorporated/0.5 mg of protein (means $\pm$ s.d.). Oleoylanilide treatment was for the following periods (with the further period without treatment in parentheses): (a) 13 days (4 days); (b) 17 days (15 days); 13 days (45 days). Abbreviations used: TG, triacylglycerols; PA, phosphatidic acid; PC, phosphatidylcholine; LPC, lysophosphatidylcholine; PE, phosphatidylethanolamine; tr, trace.

Organ	Lipid classes	Control	Oleoylanilide treatment		
			(a)	(b)	(c)
Liver	TG	2.0 $\pm$ 0.2	1.3 $\pm$ 0.2	3.8 $\pm$ 0.5	4.8 $\pm$ 0.6
	PA	1.5 $\pm$ 0.3	2.4 $\pm$ 0.4	2.9 $\pm$ 0.5	7.9 $\pm$ 0.4
	PC	1.2 $\pm$ 0.2	1.6 $\pm$ 0.2	0.37 $\pm$ 0.01	0.75 $\pm$ 0.01
	LPC	0.08 $\pm$ 0.02	0.08 $\pm$ 0.02	0.05 $\pm$ 0.02	0.08 $\pm$ 0.02
	PE	0.8 $\pm$ 0.2	0.7 $\pm$ 0.2	0.2 $\pm$ 0.08	0.3 $\pm$ 0.07
Lung	TG	2.7 $\pm$ 0.2	0.12 $\pm$ 0.02	1.6 $\pm$ 0.3	2.5 $\pm$ 0.5
	PA	0.9 $\pm$ 0.2	0.07 $\pm$ 0.02	0.8 $\pm$ 0.2	2.4 $\pm$ 0.3
	PC	1.5 $\pm$ 0.2	0.76 $\pm$ 0.08	0.08 $\pm$ 0.02	0.6 $\pm$ 0.1
	LPC	0.04 $\pm$ 0.02	0.03 $\pm$ 0.01	0.04 $\pm$ 0.01	0.08 $\pm$ 0.02
	PE	0.2 $\pm$ 0.02	0.05 $\pm$ 0.01	0.05 $\pm$ 0.01	0.08 $\pm$ 0.02
Adipose tissue	TG	7.2 $\pm$ 0.4	tr	tr	2.1 $\pm$ 0.4
	PA	1.5 $\pm$ 0.3	tr	tr	1.4 $\pm$ 0.3
	PC	0.4 $\pm$ 0.03	tr	tr	0.2 $\pm$ 0.02
	LPC	0.04 $\pm$ 0.01	—	—	0.04 $\pm$ 0.01
	PE	0.2 $\pm$ 0.02	tr	tr	0.08 $\pm$ 0.02

Table 3. *Fatty acid composition of total lipids of microsomes and mitochondria of livers from control rats and those fed with oleoylanilide (OA) or linoleoylanilide (LA)*

The degree of unsaturation was calculated as [% monoenes + 2(% dienes) + 4(% tetraenes) + 6(% hexaenes)]/100.

Fatty acid	Fatty acid composition (%)					
	Microsomes			Mitochondria		
	Control	OA*	LA†	Control	OA*	LA†
C _{12:0}	—	—	—	—	—	—
C _{14:0}	—	—	0.2	0.3	0.3	0.1
C _{16:0}	19.4	17.4	21.6	14.4	15.1	14.2
C _{16:1}	1.0	1.9	—	1.7	—	3.3
C _{18:0}	27.1	27.9	25.4	22.1	23.9	23.6
C _{18:1}	13.2	13.4	14.4	10.4	11.0	12.4
C _{18:2}	10.4	8.4	9.5	18.6	16.4	17.4
C _{20:4}	26.7	27.8	24.9	26.4	27.6	24.0
C _{22:6}	2.1	2.9	3.9	5.7	5.9	5.0
Δ	1.556	1.611	1.564	1.894	1.899	1.752

* Animals fed with oleoylanilide during 13 days, with 4 days without treatment before death.

† Animals fed with linoleoylanilide, prepared by the same procedure as for oleoylanilide and fed under the same scheme.

acyltransferase and lysophosphatidylcholine:lysophosphatidylcholine acyltransferase activities.

The transacylation mechanism was measured through the autoacylation reaction of 1-[1- 14 C]-palmitoyl-*sn*-glycero-3-phosphocholine yielding 1,2-di[1- 14 C]palmitoyl-*sn*-glycero-3-phosphocholine. Table 5 shows the importance of this mechanism in the biosynthesis of pulmonary phospholipids and its lesser importance in liver and adipose tissue lipid metabolism. It is also clear from Table 5 that the

activity of lysophosphatidylcholine:lysophosphatidylcholine acyltransferase was strongly diminished in lung of anilide-treated rats as well as after a long period without anilide administration.

The utilization of palmitoyl-CoA in the acylation reaction of lysophosphatidylcholine is given in Table 6, which also shows clearly the exceptional metabolic distortion of adipose tissue, in which phospholipid synthesis through the use of preformed long-chain acyl-CoA is practically absent. Liver and

Table 4. *Fatty acid biosynthesis in vitro from [1-¹⁴C]-acetyl-CoA by a 150000g supernatant of liver, lung and adipose tissue from control rats and rats fed with oleoylanilide*

Values are expressed as nmol/mg of protein (means $\pm$ S.D.). Oleoylanilide was given for the following periods (with subsequent periods without oleoylanilide feeding shown in parentheses): (a) 13 days (4 days); (b) 17 days (15 days); (c) 13 days (45 days).

Organ	Control	Oleoylanilide treatment		
		(a)	(b)	(c)
Liver	1.6 $\pm$ 0.3	1.7 $\pm$ 0.4	1.4 $\pm$ 0.4	1.0 $\pm$ 0.5
Lung	0.9 $\pm$ 0.3	1.1 $\pm$ 0.3	1.2 $\pm$ 0.4	0.8 $\pm$ 0.2
Adipose tissue	2.5 $\pm$ 0.2	0.8 $\pm$ 0.2	0.8 $\pm$ 0.2	0.5 $\pm$ 0.3

Table 5. *Transacylation reactions in vitro of 1-[1-¹⁴C]-palmitoyl-sn-glycero-3-phosphocholine in supernatants of liver, lung and adipose tissue from control rats and those fed with oleoylanilide*

Values are expressed as nmol/mg of protein (means $\pm$ S.D.). Oleoylanilide was given for the following periods (with subsequent periods without oleoylanilide feeding in parentheses); (a) 13 days (4 days); (b) 17 days (15 days).

Organ	Control	Oleoylanilide treatment	
		(a)	(b)
Liver	13.2 $\pm$ 1.5	16.3 $\pm$ 1.8	10.6 $\pm$ 1.4
Lung	55.5 $\pm$ 2.8	41.2 $\pm$ 2.0	6.0 $\pm$ 1.5
Adipose tissue	14.5 $\pm$ 1.8	9.4 $\pm$ 1.7	3.0 $\pm$ 1.6

Table 6. *Reacylation reactions in vitro of 1-[1-¹⁴C]-palmitoyl-sn-glycero-3-phosphocholine by palmitoyl-CoA in microsomes of liver, lung and adipose tissue from control rats and those fed with oleoylanilide*

Values are expressed as nmol/mg of protein (means $\pm$ S.D.). Rats were fed with oleoylanilide for 13 days, followed by 4 days without this treatment before being killed.

Organ	Control	Oleoylanilide treatment
Liver	13.5 $\pm$ 1.8	16.4 $\pm$ 2.3
Lung	16.5 $\pm$ 1.6	20.1 $\pm$ 2.2
Adipose tissue	14.0 $\pm$ 0.8	0.6 $\pm$ 0.3

lung do not show significant modification of the values from those in the control animals. Acyl-CoA hydrolase activity (Table 7) was simultaneously determined and a general activity increase was detected, showing adipose tissue to exhibit the highest acyl-CoA hydrolysis, in agreement with the absence of acyl-CoA utilization in the acylation reactions. There is, then, a clear difference between transacylation and re-acylation reactions in lung of anilide-treated rats. Both mechanisms are respon-

Table 7. *Acyl-CoA hydrolase activity in vitro determined simultaneously with the reacylation reactions*

Values are expressed as nmol/mg of protein (means $\pm$ S.D.). Rats were fed with oleoylanilide for 13 days, followed by four days without this treatment before being killed.

Organ	Control	Oleoylanilide treatment
Liver	50.9 $\pm$ 4.8	99.9 $\pm$ 7.4
Lung	50.0 $\pm$ 5.2	65.7 $\pm$ 6.8
Adipose tissue	77.8 $\pm$ 6.9	183.5 $\pm$ 9.8

sible for the remodelling of phospholipids in lung, yielding (dipalmitoyl)phosphatidylcholine; however, transacylation was only affected by feeding oleoylanilide, whereas re-acylation was not influenced by this treatment.

Discussion

The concentration of anilides in the renaturated rape-seed oils was very variable, ranging between hundreds and thousands of p.p.m. Taking the different alimentary habits of the population into account as well, in addition to the unknown time for the oil to be ingested, it is difficult to establish both the amount and the dose that was ingested by the toxic syndrome patients. However, the dose of oleoylanilide used in the rat treatment was of a similar order to those of the human patients.

A series of individual assays for various enzymic systems of lipid metabolism in the presence of their direct substrates has been carried out in both control and oleoylanilide-treated rat tissues.

The observations of the present study support the concept that oleoylanilide has different effects in relation to lipogenesis, and in general to lipid metabolism, in lung, liver and adipose tissue and that the most important observations are the strong inhibition of the main enzyme systems involved in lipid synthesis of adipose tissue and the inhibitory effect on key enzymes of lipid pulmonary metabolism, whereas the effect on liver enzyme systems is not marked. These facts suggest that, after intestinal cell transport, the lymphatic system is the most likely way the toxic substance is distributed. Thus lung would be the first target organ and, in this way, adipose tissue would be exposed to the general circulation, whereas liver would be excluded as a main target organ. Also, the anilide treatment of the rats results in a high loss of adipose-tissue stores. These metabolic alterations of the animal experimental model agree with some clinical observations in human patients suffering from the toxic epidemic syndrome, concerning, for example, weight loss, the disappearance of fat tissue and pneumonic symptoms (Noriega, 1982).

It is also well known that (dipalmitoyl)phosphatidylcholine is one of the major functional molecular species of the lung surfactant system (King, 1974; Tierney, 1974; Goerke, 1974; Acebal *et al.*, 1977). These disaturated species of phosphatidylcholine resist collapse in a highly compressible film (Watkins, 1968; King, 1974). Any physiopathological change in either the content or the nature of phosphatidylcholine species would result in a low surface-tension-reducing capacity (Watkins, 1968; Kyriakides *et al.*, 1976). Concerning the biochemical pathways of synthesis of these phospholipid species, the CDP-choline pathway does not produce directly (dipalmitoyl)phosphatidylcholine but unsaturated molecular species (Snyder *et al.*, 1973; Moriya & Kanoh, 1974; Oldenborg & van Golde, 1976; Possmayer *et al.*, 1977; Balibrea *et al.*, 1979) that are remodelled through a previous phospholipase A₂ hydrolysis. Thus, the formed 1-acyl-*sn*-glycero-3-phosphocholine can be re-acylated either directly or through a transacylation mechanism that takes place preferentially in low-energy conditions since the presence of ATP and CoA would support the direct acylation. Thus the decrease in the activity of remodelling enzymes yielding (dipalmitoyl)phosphatidylcholine could be at least one of the molecular bases of the symptoms during the early stages of the toxic syndrome. For this reason, we propose that the marked decrease in the transacylation reaction activity accounts for the physiopathology of the lung on the basis of the participation of this mechanism in the elaboration of surfactant phospholipids (Balibrea *et al.*, 1979).

Finally, fatty acid synthesis is highly diminished, independently of the physiological acetyl-CoA and reducing-equivalents generation. This indicates that the fatty acid synthetase complex is also affected by the presence of the oleoylanilide. This inhibition in a rate-limiting step for the acyl-CoA utilization in lipid synthesis could influence lipogenesis from acetate.

These studies were supported by the National Plan for the study of toxic syndrome in Spain.

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