Effect of Dexamethasone on the Fatty Acid Composition of Total Liver Microsomal Lipids and Phosphatidylcholine Molecular Species

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ABSTRACT: Dexamethasone depresses Δ6 and Δ5 and increases Δ9 desaturase and synthase activities. Therefore, we investigated the effect on the fatty acid composition of microsomal liver lipids and phosphatidylcholine (PtdCho) molecular species. After 15 d of treatment we found a notable decrease in arachidonic acid, a small decrease in stearic acid, and increases of linoleic, oleic, palmitoleic, and palmitic acids in liver microsomal total lipids and PtdCho. The study of the distribution of the PtdCho molecular species indicated that 18:0/20:4n-6, 16:0/20:4n-6, and 16:0/18:2n-6 predominated in the control animals. Dexamethasone, as expected because of its depressing effect on arachidonic acid synthesis and activation of oleic and palmitic acid synthesis, evoked a very significant decrease in 18:0/20:4n-6 PtdCho ($P<0.001$) and an important increase in 16:0/18:2n-6. The invariability of 16:0/20:4n-6 PtdCho could be related to the antagonistic effect of arachidonic and palmitic acid synthesis. PtdCho species containing oleic acid were not significant. The bulk fluidity and dynamic properties of the microsomal lipid bilayer measured by fluorometry using the probes 1,6-diphenyl-1,3,5-hexatriene and 4-trimethylammonium-phenyl-6-phenyl-1,3,5-hexatriene showed no significant modification, probably owing to a compensatory effect of the different molecular species, but changes of particular domains not detected by this technique are possible. However, the extremely sensitive Laurdan detected increased lipid packing in the less-fluid domains of the polar-nonpolar interphase of the bilayer, possibly evoked by the change of molecular species and cholesterol/phospholipid ratio. The most important effect found is the decrease of arachidonic acid pools in liver phospholipids as one of the corresponding causes of dexamethasone-dependent pharmacological effects.

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Polyunsaturated fatty acids derived from linoleic and α-linolenic acids play important roles in the biological functions of mammals, including human beings. The Δ6 desaturase (1) is the key enzyme that regulates polyunsaturated fatty acid biosynthesis at the level of 18-carbon fatty acids. A second step in this regulation at the level of 20-carbon fatty acids is produced by the Δ5 desaturase.

Since 1966, when we discovered that the Δ6 desaturase was depressed in experimental diabetes and could be recovered by insulin injection (2,3), we have enlarged the study regarding the effect of nearly all other hormones, not only upon the Δ6 but also upon the Δ5 desaturase. Several reviews have been published (4,5), and we have found that except for insulin, all hormones for example, glucagon, adrenaline, glucocorticoids (corticosterone, hydrocortisone, dexamethasone, triamcynolone), 11-deoxycorticosterone, aldosterone, estradiol, estriol, testosterone, and ACTH inhibit both enzymes. Progesterone, cortexolone, and pregnanediol were inactive.

When we compared the effects of these hormones *in vivo* upon the hepatic Δ9 desaturase activity, we found (2,3,6), as Gellhorn and Benjamin (7) showed that experimental diabetes depressed Δ9 desaturation of palmitic and stearic acids, and insulin injection recovered the activity. Unlike what happened to the Δ6 and Δ5 desaturases, glucocorticoids, 11-deoxycorticosterone, testosterone, estradiol, and L-triiodothyronine also enhanced Δ9 desaturase activity (Brenner, R.R., unpublished data).

In the case of glucocorticoids, our pioneering experiments done in 1979 (8) showed that *in vivo* hydrocortisone and the synthetic glucocorticoids triamcynolone and dexamethasone were depressors of Δ6 and Δ5 desaturation activity in liver microsomes and activators of Δ9 desaturase, although both synthetic products were more active than hydrocortisone. The strongest effect was found 24 h after the injection. The depressing effect of dexamethasone on [1-^{14}C]eicosa-8,11,14-trienoic acid conversion to arachidonic acid was also shown by direct incubation of the isolated rat hepatocytes or hepatoma tissue culture (HTC) cells with this synthetic glucocorticoid (9). Moreover, *in vivo* experiments demonstrated (10) that this modulation of Δ6 and Δ5 desaturation was produced by the biosynthesis of a cytosolic protein sensitive to trypsin that loosely binds to the microsomes. By incubating rat liver hepatocytes and HTC cells with labeled eicosa-

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Abbreviations: DPH, 1,6-diphenyl-1,3,5-hexatriene; Δτ, differential polarized phase lifetime; ELSD evaporative light-scattering detector; GLC, gas–liquid chromatography; GP, generalized polarization; HPLC, high-performance liquid chromatography; HTC, hepatoma tissue culture; Laurdan-6-lauroyl-2,4-dimethyl aminonaphthalene; PtdCho, phosphatidylcholine; PtdEtn, phosphatidylethanolamine; PtdIns, phosphatidylinositol; r_∞, limiting anisotropy; r_0, fundamental anisotropy; r_s, steady-state fluorescence anisotropy; S, order parameter; τ lifetime; τ_P, phase lifetime; τ_M, modulation lifetime; τ_R, rotational correlation time; TAT, tyrosine aminotransferase; TMA-DPH, 1-(4-trimethylammonium phenyl)-6-phenyl-1,3,5-hexatriene.

8,11,14-trienoic acid in the presence of various corticoids, it was shown that not only dexamethasone but also corticosterone and the mineralocorticoids 11-deoxycorticosterone and aldosterone depressed the biosynthesis of arachidonic acid (11). Similarly, both mineralocorticoids depressed Δ6 desaturation *in vivo* (12). However, the mechanisms of action of each one of these hormones were different, and only the effect of 11-deoxycorticosterone, like dexamethasone, was mediated through a soluble protein of approximately 18 kD present in the liver cytosolic fraction. This protein was induced by the occupancy of the glucocorticoid receptor (13). The inhibitory effect of aldosterone was mediated by a different mechanism. Like dexamethasone (14), 11-deoxycorticosterone induced a cytosolic protein that stimulated Δ9 desaturase activity in rat liver microsomes (15).

Since the above-mentioned corticoids alter the biosynthesis of monoenoic acids and polyunsaturated acids of the n-6 and n-3 families, it was important to recognize the corresponding changes evoked in the composition of tissue lipids. To explore these effects, we investigated the changes produced by a long-term (15 d) administration of dexamethasone on the fatty acid composition of total rat liver microsomal lipids and specifically on phosphatidylcholine (PtdCho), which is the principal component of this membrane and a good change detector in polyunsaturated fatty acids. Moreover, considering that the real effect is evoked in the distribution of the different molecular species of this phospholipid, we studied them as well. In addition, we checked the possible changes in the biophysical properties of the microsomal membrane lipid bilayers by fluorescence techniques.

MATERIALS AND METHODS

Animal treatment. All studies performed with animals were carried out in accordance with accepted international standards. Male Wistar rats weighing between 180 and 200 g were separated into two groups. They were fed a commercial diet (Nutrimento, type 3; Escobar, Argentina). The relative percentages of the fatty acids in the diet were 17.0 palmitic, 0.2 palmitoleic, 5.9 stearic, 25.6 oleic, 1.7 vaccenic, 46.2 linoleic, and 3,4 α-linolenic. One group was used as control. The treated animals were injected daily intraperitoneally for 15 d with 0.5 mg/kg weight dexamethasone phosphate (Sidus, Buenos Aires, Argentina). Thereafter, the injected animals had a mean weight of 165 g, and those in the control group had a mean weight of 250 g. All the animals were killed by decapitation without anesthesia. The liver from each animal was rapidly excised and received in ice-cold homogenizing solution (1:3 wt/vol) which was composed of 0.25 M sucrose, 1 mM EDTA, and 10 mM phosphate buffer (pH 7.2). Microsomes were obtained by differential ultracentrifugation at 100,000 × *g* (Beckman Ultracentrifuge) as described elsewhere (16). They were kept frozen at −80°C. The protein concentration was measured according to the procedure of Lowry *et al.* (17).

To check the effect of the glucocorticoid injection, tyrosine aminotransferase (TAT) activity was determined in the 100,000 × *g* supernatants (cytosol fraction) from both control and dexamethasone-treated rats. All measurements were performed according to the method of Dradmonstone (18). The specific activity in noninduced rat liver cytosol ranged from 5.9 to 8.1 TAT units/mg protein and in treated animals from 32.4 to 44.7 TAT units/mg protein. Only those animals with more than 32.4 TAT units/mg protein were used in the present experiments.

Lipid analysis. Lipids were extracted from microsomes according to the procedure of Folch *et al.* (19). Total lipid content was measured by aliquot evaporation to constant weight. Cholesterol content was determined by the procedure of Huang *et al.* (20) and total phosphorus by the method of Gomori (21).

Phosphatidylcholine (PtdCho) and other phospholipid classes were separated from total lipids by high-performance liquid chromatography (HPLC) using an evaporative light-scattering detector (ELSD) (22). An Econosil silica column of 10 μm and 250 × 4.6 mm from Alltech Associates (Deerfield, IL) was used. Elution was performed at a flow rate of 1 mL/min by a gradient of hexane/isopropanol/dichloromethane (40:48:12 by vol) to hexane/isopropanol/dichloromethane/water (40:42:8:8 by vol) for 15 min followed by additional elution with the latter solvent for 30 min.

Nebulization in the ELSD was set at 90°C drift tube temperature and 2.20 L/min of nitrogen gas flow to the nebulizer. PtdCho peaks were collected manually from the column effluent using a flow splitter. The solvent was evaporated under N_2 and redissolved in methanol/triethylamine (2:1 vol/vol).

The fatty acid compositions of total lipid PtdCho as well as its molecular species were determined by capillary gas–liquid chromatography (GLC) in a Shimadzu Chromatograph, model GC-R9A. A 30 m × 0.25 mm internal diameter with 0.25 μm thickness Omega Wax 250 column (Supelco Inc., Bellefonte, PA) was used. Temperature was programmed for a linear increase of 3°C/min after 3 min at the starting temperature of 185°C and 19 min at the final temperature of 230°C. Helium was used as carrier gas. The chromatographic peaks were identified by comparison of the retention times with those of standards.

PtdCho molecular species separation. The separation of the molecular species was done using the method of Browers *et al.* (23). Resolution of molecular species was performed on two 5-μm endcapped Lichrosphere 100-RP18 columns in series (Merck, Darmstadt, Germany). Isocratic elution was applied using a solvent composed of methanol/acetonitrile/triethylamine (58:40:2 by vol) at a flux of 1 mL/min. The detection and quantification were done in an ELSD using N_2 as nebulizer at a flux of 1.8 L/min and temperature of 100°C (23). A sample of 1 mg of PtdCho was injected; 5 of 100 parts went to the detector and the remaining materials of the peaks were collected and identified by GLC analysis.

Fluorescent probes. 1,6-Diphenyl-1,3,5-hexatriene (DPH) was purchased from Sigma Chemical Co. (St. Louis, MO). 6-Lauroyl-2,4-dimethylaminonaphthalene (Laurdan) and 1-(4-trimethylammonium-phenyl)-6-phenyl-1,3,5-hexatriene

(TMA-DPH) were purchased from Molecular Probes (Eugene, OR).

Preparation and labeling of lipid vesicles. Microsomal lipids (1 mg) in chloroform solution were added to a round-bottomed glass tube. The solvent was evaporated under an N_2 stream, and 1 mL of buffer A (50 mM sodium phosphate pH 7.4) was added. After 15 min at room temperature to allow hydration, samples were vigorously vortexed for 2 min. Then, the lipid suspensions were extruded 11 times through a 100-nm pore polycarbonate filter using a Liposo-Fast extruder (Avestin, Inc., Ottawa, Canada). For labeling, 0.25 mL of the extruded vesicles was mixed with 0.25 mL of a 4-μM suspension of the fluorescent probe (DPH, TMA-DPH, or Laurdan) in buffer A and vigorously vortexed. Then, samples were kept at room temperature for at least 30 min and diluted five times with buffer A before the fluorescence measurements were made. The final concentration of the samples was 0.1 mg/mL of lipids and 4 μM of the fluorescent probe.

Fluorescence measurements. All measurements were made in an SLM 4800 spectrofluorometer in 1 × 1 cm cuvettes. DPH or TMA-DPH steady-state fluorescence anisotropy (r_s), lifetime (τ), and differential polarized phase lifetime ($\Delta\tau$) were measured using an excitation wavelength of 361 nm and observing the total emission at wavelengths >389 nm through a sharp cut-off filter (KV389) according to Lakowicz *et al.* (24,25) with some modification (26–28). For the τ and $\Delta\tau$ measurements, the exciting light was modulated sinusoidally in amplitude at 18 or 30 MHz with a Debye-Sears modulator and vertically polarized with a Glan-Thompson polarizer. For τ, the emission was observed through a Glan-Thompson polarizer oriented 55° to the vertical to eliminate the effect of Brownian rotation (29). The phase shift and demodulation of the emitted light were measured relative to the reference standard 1,4-bis(5-phenyloxazol-2-yl)benzene in ethanol (τ = 1.35 ns) (30) and used to compute the phase (τ_P) and modulation (τ_M) lifetimes of the samples (31). $\Delta\tau$ was obtained from the phase shift between the parallel and perpendicular components of the emission observed with the emission polarizer vertically or horizontally oriented, respectively. Data were interpreted according to the model of hindered wobbling rotation (32). As described elsewhere (33,34), the values obtained for r_s, τ, and $\Delta\tau$ were used to compute (i) the rotational correlation time (τ_R), which is inversely related to the rotational rate and reflects the local viscous resistance to the probe rotation; and (ii) the limiting anisotropy (r_∞), which is related to the order parameter S ($S^2 = r_\infty/r_0$) and reflects the limitation imposed by the local environment to the extent or range of the probe wobbling.

Fluorescence spectra of Laurdan were taken with monochromator bandpasses of 8 nm in excitation and emission as previously described (34–36). All spectra were corrected for background contribution by subtracting the signal of unlabeled samples. Generalized polarization spectra were obtained by measuring the excitation intensity spectra using 440 nm (I_{440}) and 490 nm (I_{490}) for the emission and the emission intensity spectra at 340 nm (I_{340}) and 410 nm (I_{410}) excitation wavelength. Generalized polarization in the excitation (exGP) and emission (emGP) bands were obtained from $exGP = (I_{440} - I_{490})/(I_{440} + I_{490})$ and $emGP = (I_{410} - I_{340})/(I_{410} + I_{340})$, respectively. Laurdan emission lifetime was measured as for DPH and TMA-DPH by exciting at 360 nm and isolating the emission with a KV389 filter.

RESULTS

Effect of dexamethasone on the lipid and fatty acid composition of liver microsomes. The effect of a daily injection of 0.5 mg/kg weight dexamethasone phosphate for 15 d on rat liver microsomal lipid composition is depicted in Table 1. First, it shows that the cholesterol/phospholipid ratio was somewhat increased by the glucocorticoid treatment. However, only very small changes were observed in the relative proportions of the different glycerophospholipids. There was a small decrease in the percentage of PtdCho, whereas the proportions of phosphatidylethanolamine (PtdEtn), phosphatidylinositol, and phosphatidylserine were slightly increased. By far, PtdCho was the predominant phospholipid of the liver microsomal membrane.

The fatty acid composition of rat liver microsomal lipids is shown in Table 2. The 15-d dexamethasone treatment evoked significant increases of palmitic, palmitoleic, oleic, linoleic, and eicosatrienoic n-6 acids and a decrease of stearic and arachidonic acids.

When we analyzed the general fatty acid composition of liver microsomal PtdCho, a similar effect was found as expected (Table 3). The percentages of palmitoleic, oleic, and linoleic acids were increased. Palmitic acid increased and stearic acid decreased, but without statistical significance, whereas arachidonic acid decreased significantly.

Therefore, these results correspond rather well to a decrease of Δ6 and Δ5 fatty acid desaturation activity that inhibits linoleic acid conversion to arachidonic acid and to an increase of the Δ9 desaturation activity that enhances the conversion of palmitic and stearic acids to palmitoleic and oleic acids, respectively, as already demonstrated (8–11,13,15).

Effect of dexamethasone on microsomal PtdCho molecular species. In fact, the fatty acid analysis of the microsomal PtdCho only gives an average composition of the lipid. The

TABLE 1
Percentage Composition of Phospholipids, Cholesterol, and Phospholipid Classes in Liver Microsomal Lipids[a]

	Control	Dexamethasone-treated
Phospholipids	61.5 ± 0.6	63.4 ± 0.2
Cholesterol	4.2 ± 0.2	5.0 ± 0.2
Cholesterol/phospholipid	0.068 ± 0.004	0.079 ± 0.003
PtdCho	80.5 ± 0.4	74.6 ± 0.7
PtdEtn	14.7 ± 0.4	17.6 ± 0.4
PtdIns	3.8 ± 0.5	5.1 ± 0.3
PtdSer	1.0 ± 0.3	2.7 ± 0.9

[a]Results are the mean ± standard error of five animals analyzed separately. PtdCho, phosphatidylcholine; PtdEtn, phosphatidylethanolamine; PtdIns, phosphatidylinositol; PtdSer, phosphatidylserine.

TABLE 2
Fatty Acid Composition (mol%) of Total Microsomal Lipids[a]

Fatty acids	Control	Dexamethasone-treated	*P*
16:0	22.42 ± 0.86	28.21 ± 0.98	<0.01
16:1	0.37 ± 0.08	1.68 ± 0.28	<0.01
18:0	24.62 ± 0.48	21.58 ± 0.70	<0.01
18:1n-9	6.91 ± 0.19	8.89 ± 0.69	<0.02
18:1n-7	2.67 ± 0.23	1.55 ± 0.03	<0.01
18:2n-6	14.68 ± 0.17	15.71 ± 0.38	<0.05
18:3n-6	0.56 ± 0.19	0.46 ± 0.06	
18:3n-3	0.82 ± 0.17	0.72 ± 0.20	
20:3n-9	0.51 ± 0.04	0.40 ± 0.09	
20:3n-6	0.58 ± 0.17	1.27 ± 0.13	<0.02
20:4n-6	21.86 ± 0.71	15.57 ± 0.73	<0.001
22:4n-6	0.38 ± 0.08	0.49 ± 0.06	
22:5n-6	0.27 ± 0.04	0.47 ± 0.03	
22:5n-3	0.85 ± 0.13	0.75 ± 0.06	
22:6n-3	2.50 ± 0.22	2.16 ± 0.10	

[a]Only principal acids were considered. Results are the average of five animals ± standard of the mean.

TABLE 4
Percent Distribution by Weight of PtdCho Molecular Species in Liver Microsomes

Molecular species	Control	Dexamethasone-treated	*P*
18:2/20:4	1.44 ± 0.30	1.18 ± 0.25	
18:2/18:2	1.59 ± 0.28	1.42 ± 0.07	
16:0/22:6	4.32 ± 0.24	4.14 ± 0.48	
18:1/20:4	3.69 ± 0.28	0.30 ± 0.30	<0.001
16:0/20:4	21.83 ± 0.60	22.26 ± 0.56	
18:1/18:2	1.98± 0.16	None detected	
16:0/18:2	12.62 ± 0.53	22.10 ± 1.00	<0.001
18:2/22:6	3.80 ± 0.66	4.68 ± 0.75	
18:0/20:4	36.22 ± 1.03	24.72 ± 1.14	<0.001
16:0/18:1	3.68 ± 0.28	5.66 ± 0.52	<0.01
18:0/18:2	8.83 ± 0.57	13.54 ± 0.64	<0.001

[a]Molecular species were separated by high-performance liquid chromatography as described in the Materials and Methods section. Results are the average of five animals ± standard error of the mean. For abbreviation see Table 1.

real situation in the membrane is that PtdCho constitutes an entire family of different molecular species, each one having different pairs of fatty acids. The molecular species of rat liver microsomal PtdCho and the changes induced by dexamethasone treatment are given in Table 4.

Eleven major molecular species were separated, characterized, and quantitated by the method of Browers *et al.* (23) in both control and treated rats.

As reported in other experiments (34,36,37), the predominant liver microsomal PtdCho species in control rats was found to be 18:0/20:4n-6 followed by 16:0/20:4n-6 and 16:0/18:2n-6.

These three species, constituted by one saturated and another unsaturated acid, represent 70.6% of all molecular species of PtdCho in the microsomes. Therefore, they mainly determine the contribution of this phospholipid to the general biophysical properties of this lipid bilayer.

Dexamethasone treatment evoked changes in the proportion of some PtdCho molecular species (Table 4). The most noticeable modification was a sharp decrease of 18:0/20:4n-6 and an important increase of 16:0/18:2n-6 species. Other changes were a decrease of 18:1n-9/20:4n-6 and an increase of 18:0/18:2n-6 and 16:0/18:1n-9 species. However, the proportion of 16:0/20:4n-6 species was not modified. Despite this last important peak and the small 18:2n-6/20:4n-6 peak, which were not altered, the decrease of the other 20:4n-6 containing peak and the notable increase of 16:0/18:2n-6 and 18:0/18:2n-6 species confirm the changes of polyunsaturated fatty acid mean compositions found in PtdCho (Table 3). They also correspond well to a decrease of dietary linoleic acid conversion to arachidonic acid already shown in glucocorticoid-treated rats (8–11).

No significant changes were found in the 22:6n-3 containing species. The expected small increase of 18:1n-9 containing species was only found in the 16:0/18:1n-9 peak but not in the 18:1n-9/20:4n-6 and 18:1n-9/18:2n-6 peaks.

Effect of dexamethasone on the order and dynamics of microsomal lipids. The effect of dexamethasone administration on the biophysical properties of the microsomal lipids was studied in large unilamellar vesicles of these lipids using three different fluorescent probes. The rotational behavior of DPH and TMA-DPH was studied by using phase and modulation fluorometry (Table 5). These probes sense the order and dynamics of the lipid bilayer at different depths. The neutral DPH probe locates deeply into the bilayer, whereas the amphipathic TMA-DPH is anchored through its positive charge to the phospholipid polar groups and locates the fluorescent moiety more externally than DPH. Thus, TMA-DPH senses a more polar environment, which is indicated by its shorter lifetime in comparison with DPH. The lifetimes of these probes obtained by phase (τ_P) and modulation measurements (τ_M) at 18 MHz excitation frequency are shown in Table 5. No effect of dexamethasone administration was observed, indicating no change in the polarity in both sensed bilayer regions. Similar lifetime values were obtained by measurements at 30 MHz (data not shown). This fact and also the small differences be-

TABLE 3
Fatty Acid Composition (mol%) of Rat Liver Microsomal PtdCho[a]

Fatty acids	Control	Dexamethasone-treated	*P*
16:0	21.31 ± 0.49	23.54 ± 1.24	
16:1	0.36 ± 0.05	1.02 ± 0.14	<0.01
18:0	23.43 ± 0.59	21.83 ± 0.58	
18:1n-9	4.48 ± 0.05	5.73 ± 0.65	
18:1n-7	3.11 ± 0.21	1.81 ± 0.21	<0.01
18:2n-6	13.90 ± 0.33	16.95 ± 0.59	<0.01
18:3n-6	0.78 ± 0.07	0.42 ± 0.13	
18:3n-3	2.06 ± 0.31	1.73 ± 0.19	
20:3n-9	0.54 ± 0.04	0.27 ± 0.01	<0.0001
20:3n-6	0.94 ± 0.12	1.20 ± 0.13	
20:4n-6	24.74 ± 0.32	20.41 ± 1.01	<0.01
22:4n-6	0.24 ± 0.03	0.40 ± 0.06	
22:5n-6	0.29 ± 0.03	0.49 ± 0.04	
22:5n-3	0.62 ± 0.03	0.67 ± 0.07	
22:6n-3	3.20 ± 0.19	3.53 ± 0.29	

[a]Results are the average of five animals ± standard error of the mean. For abbreviation see Table 1.

TABLE 5
Effect of Treatment with Dexamethasone on the Fluorescence Lifetime and Rotational Parameters of TMA-DPH and DPH in Lipid Vesicles of Rat Liver Microsomes[a]

Parameter		Control group[b]	Treated group[b]
TMA-DPH	r_s	0.217 ± 0.002	0.216 ± 0.002
	τ_P (ns)[c]	4.07 ± 0.08	4.12 ± 0.07
	τ_M (ns)[c]	4.58 ± 0.11	4.58 ± 0.10
	τ_R (ns)[c]	1.31 ± 0.02	1.34 ± 0.04
	r_∞[c]	0.161 ± 0.002	0.159 ± 0.01
DPH	r_s	0.107 ± 0.001	0.109 ± 0.002
	τ_P (ns)[c]	7.99 ± 0.08	8.09 ± 0.14
	τ_M (ns)[c]	8.57 ± 0.07	8.55 ± 0.11
	τ_R (ns)[c]	1.29 ± 0.08	1.26 ± 0.02
	r_∞[c]	0.058 ± 0.002	0.061 ± 0.002

[a]TMA-DPH, 1-(4-trimethylammonium phenyl)-6-phenyl-1,3,5-hexatriene; DPH, 1,6-diphenyl-1,3,5-hexatriene.
[b]The values given are the mean ± standard deviation of four animals for the control group and five animals for the treated group. Student's tests do not indicate a significant difference at the 5% probability level for any of the measured parameters.
[c]The indicated values for phase (τ_P) and modulation (τ_M) lifetimes, rotational correlation time (τ_R) and limiting anisotropy (r_∞) were obtained using 18 MHz excitation modulation frequency.

tween τ_P and τ_M indicate that there is no large heterogeneity in the probe distribution into different microdomains in the vesicle bilayer. The fact reasonably allows us to apply the hindered wobbling rotation model (32) assuming a unique rotamer population to calculate the rotational correlation time (τ_R) and limiting anisotropy (r_∞) parameters. The τ_M and r_∞ parameters calculated from measurements at 18 MHz (shown in Table 5) indicate that dexamethasone treatment does not significantly affect the rotational behavior of these probes in the microsomal lipid vesicles.

The effect of dexamethasone administration on the lipid dynamics in vesicles of microsomal lipids was also studied by using the fluorescence properties of Laurdan. This probe locates its fluorescent moiety at the polar-nonpolar interphase of the lipid bilayer, and its emission spectra are highly sensitive to solvent relaxation (38). Small changes in the lipid packing can influence both the amount and mobility of water molecules in the bilayer. An increased water amount at the interfacial region of the bilayer would result in a shorter lifetime and a red-shifted emission of Laurdan fluorescence, whereas an increased mobility of water molecules would produce a red-shifted emission without affecting the fluorescence lifetime (38). Spectral shifts of Laurdan are generally expressed with the generalized polarization (GP) parameter (39). Figure 1 shows the influence of dexamethasone administration on the lipid composition of microsomal membranes and the effect on the GP values calculated from the Laurdan emission spectra at different excitation wavelengths at 25 and 37°C. The observed decrease in GP with increasing excitation wavelength is typical of liquid-crystalline bilayers (40). It is clear from the figure that dexamethasone treatment produces an increase in the GP values when lower excitation wavelengths are used. At lower excitation wavelengths, the less fluid lipid domains should be preferentially selected (40). It is important to note that there was no significant effect of the treatment on the Laurdan fluorescence lifetime because τ_R was 3.81 ± 0.06 and 3.81 ± 0.03 for control and treated groups, respectively; τ_M was 4.21 ± 0.03 and 4.20 ± 0.08 for control and treated groups, respectively, measured at 37°C and 18 MHz, indicating no change in the amount of water in the interfacial region. Thus, the observed increase in GP produced by dexamethasone administration can be attributed to an increased lipid packing, which results in reduced water mobility in the less fluid lipid domains preferentially selected at lower excitation wavelengths.

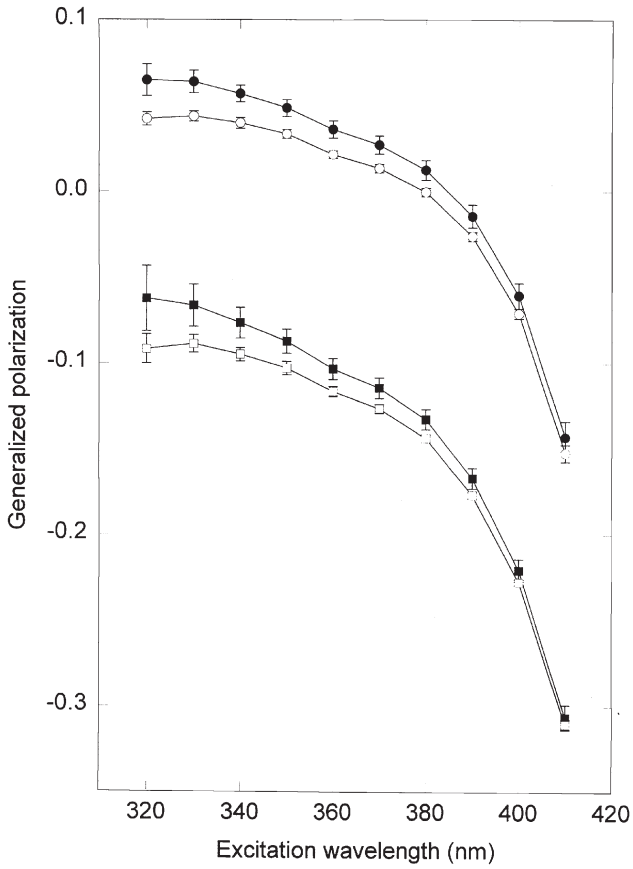

FIG. 1. Effect of dexamethasone administration on the Laurdan generalized polarization in microsomal lipid vesicles. Measurements were made on liver microsomal vesicles of control (○,□) and treated animals (●,■) at 25°C (○,●) and 37°C (□,■) as described in the Materials and Methods section. The values are the mean of four and five animals for the control and treated groups, respectively. The standard deviations are indicated by bars.

DISCUSSION

It is now well accepted that glucocorticoids perform very important functions in glucose and fatty acid metabolism. As already indicated, these hormones depress liver microsomal fatty acid Δ6 and Δ5 desaturase activity and enhance Δ9 desaturase. These changes in the activity of the desaturases are apparently evoked through the biosynthesis of a cytosolic

protein (10,12,13). In the case of Δ9 desaturase, it is important to indicate that the enhancing effect of the hormone has been shown on both [1-^{14}C]palmitic and [1-^{14}C]stearic acid desaturation. This clarification is pertinent because of the two Δ9 desaturases detected in rodents (41); only one, SCD_1 (stearoyl-CoA desaturase 1), is present in liver (42,43), and it is able to desaturate both saturated acids. It could be differently regulated from SCD_2, also found in adipose tissue (44). This last isoform is also an exclusive component of other organs.

The biological changes evoked by the alteration of these fatty acid desaturase activities are, in general, the consequence of the alteration produced in the proportion of polyunsaturated fatty acids of n-6 and n-3 families, mainly arachidonic and docosahexaenoic acids, and the monounsaturated acids of n-9 family (oleic acid) in liver and in other organs.

To evaluate these changes evoked by dexamethasone, a very efficient synthetic glucocorticoid that modifies Δ6, Δ5, and Δ9 desaturase activity (8–10,15), we analyzed the fatty acid composition of liver microsomes after 15 d of treatment. This target membrane readily responds to alterations in the availability of polyunsaturated acids of n-6 and n-3 series. This scheme of evaluation is pertinent because the only source of these acids in our experimental conditions was the biosynthesis from the precursor acids, linoleic and α-linolenic. They are the only n-6 and n-3 acids provided by the diet.

As shown in Tables 2 and 3, significant increases in the proportion of linoleic acid and decreases of arachidonic acid of the n-6 family were found in both total lipids of liver microsomes and PtdCho, the main compound (≅80% of the membrane phospholipids) of this bilayer. These facts are in agreement with and apparently the consequence of the decrease of microsomal Δ6 and Δ5 desaturase activities evoked by dexamethasone treatment. In spite of that, it can be argued that the changes produced in the arachidonic and linoleic acid proportions might be due to a redistribution between microsomes and other organelles; the fact that dexamethasone also evokes similar changes in total lipids of cultures of Morris 7288c rat cells confirms our interpretation (45). Moreover, it has also been shown (14) that the corticoid-11-deoxycorticosterone, which similarly to dexamethasone depresses Δ6 and Δ5 desaturases, evoked similar decreases in arachidonic and increases of linoleic acid proportions in liver microsomes, total liver homogenate, cytosol, and plasma lipids.

No significant decrease was detected in the proportion of docosahexaenoic acid of the n-3 family even though its biosynthesis from α-linolenic acid is also mainly controlled by the same enzymes at the level of 18- and 20-carbon fatty acids. However, a difference is found in relation to arachidonic acid because its synthesis requires further steps of elongation to 22 and 24 carbons, a new desaturation by a poorly characterized Δ6 desaturase, and β-oxidation at the peroxisomes (31) that could modify or mask the effect on the mentioned desaturases. Another difference found in our experiments between the mentioned n-6 and n-3 polyunsaturated fatty acid biosynthesis in the animal is that the rats received a diet very rich in linoleic acid (46.2%) but poor in α-linolenic acid (3.4%). Also, less than 3% of 22:6n-3 is found in the total lipids of the membrane compared to more than 20% of 20:4n-6. As a consequence, the possibility is that arachidonic acid, in addition to being a major component of the membrane, is constantly deacylated and reacylated into PtdCho whereas docosahexaenoic acid is more likely to remain in the membrane. Therefore, it is possible that the effect found on the desaturases and transmitted to the arachidonic acid content would be more easily detected than in the n-3 polyunsaturated fatty acid.

However, we must consider that when we studied the effect of experimental diabetes (29), in which the activity of both liver Δ6 and Δ5 desaturases is decreased, we found that, although the amount of arachidonic acid detected in liver microsomal lipids dropped, a significant increase in docosahexaenoic acid was recorded. This last result has not been properly explained yet, but we suggested (29) it could be due to a specific effect on the decreased oxidation of docosahexaenoic acid. It may indicate that the amount of 22:6n-3 acid in the liver microsomes is regulated by an additional mechanism besides that of 20:4n-6 acid.

A remarkable conclusion from these composition studies is that, at least in rat liver microsomal membranes, 15-d glucocorticoid treatment decreases quantitatively the proportion of the biologically very important arachidonic acid of the n-6 family, but it does not alter the amount of the also very important docosahexaenoic acid of the n-3 series.

The increase evoked by dexamethasone in liver microsomal Δ9 desaturase (15) correlates well with the increase of palmitoleic and oleic acid percentages found in microsomal total lipids and PtdCho fatty acids (Tables 2 and 3). However, whereas the decrease of stearic acid found in total lipids (Table 2) also agrees with that effect, a significant change of this acid was not found in PtdCho fatty acids. As well, the increase in the percentage of palmitic acid in both total lipids and PtdCho would not correlate with an increase of Δ9 desaturation activity. However, this result is not an important objection because the relative amount of palmitoleic acid compared to palmitic is extremely low in these lipids, and any possible effect of increased Δ9 desaturation on the palmitic acid amount would pass undetected. Moreover, an even more important explanation for this result is that the increase of palmitic acid in total microsomal lipids and PtdCho is undoubtedly due to the already demonstrated dexamethasone stimulation of fatty acid synthase activity (46,47). This is apparently evoked through an increase of the synthase mRNA transcription and by posttranscriptional events, too (48).

The increase of synthase mRNA evoked by dexamethasone may be also considered, at least in part, as a consequence of the decrease of Δ6 desaturase activity produced by the same glucocorticoid. The decrease of the Δ6 desaturase activity lowers the synthesis and hepatic content of arachidonic acid that, as it is known, suppresses the synthase mRNA transcription (49). The authors (49) also showed that this effect is

not produced by linoleic acid if it is not previously desaturated by the Δ6 desaturase.

PtdCho molecular species. The important change evoked by dexamethasone *in vivo* on the molecular species of PtdCho, which are basic structural components of rat liver endoplasmic reticulum bilayer membranes, is clearly shown in Table 4.

As already found in other experiments (34,36,37), the highly predominant PtdCho species in the rat liver microsomes were 18:0/20:4n-6, 16:0/20:4n-6, and 16:0/18:2n-6. They were the principal determinants of the contribution of this phospholipid to the general biophysical properties and some of the chemical properties of the bilayer. Similar predominant molecular species were also shown in rat liver microsomal PtdEtn (37). In PtdIns, 18:0/20:4n-6 and 16:0/20:4n-6 were nearly exclusive components (37).

The decrease in the proportion of arachidonic acid and the increase of linoleic acid shown in PtdCho (Table 3) and evoked by dexamethasone are the consequence of the significant decrease of the predominant 18:0/20:4n-6 and small 18:1n-9/20:4n-6 PtdCho molecular species, and the increase of the predominant 16:0/18:2n-6 and also important 18:0/18:2n-6 PtdCho molecular species. However, other minor species containing 18:2n-6 or 20:4n-6 acids as 18:2n-6/20:4n-6 and 18:1n-9/18:2n-6 are little changed. But what is really remarkable is that the very important 16:0/20:4n-6 PtdCho species is not lowered compared to 18:0/20:4n-6. This undoubtedly indicates that the decrease of arachidonic acid biosynthesis evoked by the inactivation of Δ6 and Δ5 desaturases by dexamethasone determines less availability of this acid for modeling the fatty acids at the 2-position of PtdCho molecules, but the other fatty acids at position 1, and other factors as mechanisms of phospholipid synthesis and specificity of enzymes, also determine the final molecular compositions. The effect of glucocorticoids on phospholipase A_2 activity may be an additional factor contributing to the remodeling of PtdCho molecular species.

In the specific case of 16:0/20:4n-6 and 18:0/20:4n-6 species changes, the difference might be due, at least in part, to the different ways 16:0 and 18:0 are affected by dexa-methasone. In the case of 16:0 it has been repeatedly shown that fatty acid synthase is activated by glucocorticoids (46,47).

As expected from the fatty acid composition of microsomal lipids displayed in Tables 2 and 3, no significant modification in the amounts of both 16:0/22:6n-3 and 18:0/22:6n-3 species was evoked by dexamethasone. Although these species are minor components of PtdCho, the reason for no modification is not clear, as previously discussed.

Oleic acid-containing species are not abundant in the liver microsomes of rats fed a linoleic acid rich-diet, and only 16:0/18:1n-9 was nearly doubled by dexamethasone treatment, the consequence of the increase of Δ9 desaturase activity. Indeed, they are unimportant species for the determination of PtdCho contribution to the dynamic properties of the lipid bilayer.

Effect on dynamic properties of microsomal lipid bilayer. The changes in the lipidic composition and PtdCho molecular species of rat liver microsomes and the small increase of the cholesterol/phospholipid ratio evoked by dexamethasone administration showed no significant effect on the rotational behavior of DPH and TMA-DPH in vesicles made with these microsomal lipids (Table 5) in spite of testing different depths of the bilayer. The lack of effect on the fluorescent lifetimes of these probes as well as those of Laurdan indicate no change in the environment polarity of these probes at their different locations. However, as indicated by the increased Laurdan GP at lower excitation wavelengths, dexamethasone produces an increased lipid packing in the preferentially selected less fluid domains, resulting in a decreased mobility of water molecules present in the interfacial region of the bilayer in these domains.

It is not surprising that the increased lipid packing as detected by Laurdan does not result in any detectable change in the rotational mobility of DPH and TMA-DPH. We previously showed that higher sensitivity of Laurdan detected small changes in the lipid packing that were not detected by DPH or TMA-DPH, for instance those produced by streptozotocin diabetes (36) or polyunsaturated fatty acid deficiency (35) in rat liver microsomes. The high sensitivity of Laurdan was attributed to the fact that it senses the rotational mobility of a very small molecule, such as water (35). Small changes in the lipid packing that do not appreciably alter the rotational behavior of relatively large molecules like DPH or TMA-DPH can change the motion rate of smaller molecules such as water. Moreover, DPH and TMA-DPH would sense the motion in the different lipid domains, which, on average, were apparently not greatly changed by dexamethasone administration. On the contrary, Laurdan detects an increased lipid packing after dexamethasone treatment only in the less fluid lipid domains, and this might be related to the change of the molecular species of PtdCho and/or to the small increase of the cholesterol/phospholipid ratio. This effect of dexamethasone in the less fluid lipid domains contrasts with the effects produced in the more fluid domains (i.e., at longer wavelengths) by streptozotocin diabetes (36) (which decreases GP) and polyunsaturated fatty acid deficiency (35) (which increases GP). The relationship between compositional changes and these effects on particular lipid domains is not yet easily interpretable.

The main effect evoked by the glucocorticoid in liver is a decrease of arachidonic acid in the membrane phospholipids that alters the proportion of the molecular species of PtdCho, decreasing the 18:0/20:4n-6 species. However, since the proportion of saturated/unsaturated acid molecules is roughly maintained in spite of a small increase in the cholesterol/phospholipid ratio, no important change is evoked in the bulk fluidity of microsomal bilayer as tested by fluorometric techniques. Only a small packing increase is found in the less fluid lipid domains. However, fluorometric techniques used cannot detect changes in specific particular lipid domains that are very probably produced.

The decrease of arachidonic acid stores in liver evoked by dexamethasone would result in lower availability of this acid for its main biological functions. An inadequate availability

of arachidonic acid is associated with impaired nerve transmission, reduced eicosanoid synthesis, and impaired fetal growth. It exerts vital functioning effects in key steps of cell signaling and the expression of a wide array of genes, including those encoding proteins involved in lipid metabolism, thermogenesis, and cell differentiation.

Moreover, the decrease of arachidonic acid biosynthesis and its pools and the depression of phospholipase A_2 activity (50) evoked by dexamethasone are cooperative factors that prevent generation of arachidonic-acid derived prostaglandins and other eicosanoids and produce the well-known anti-inflammatory effect of glucocorticoids.

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