Biochem. J. (1986) 239, 41–45 (Printed in Great Britain)

Glucocorticoids increase the fluidity of the fetal-rat liver microsomal membrane in the perinatal period

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Dexamethasone, a synthetic glucocorticoid, was administered to pregnant rats during the last week of pregnancy in order to examine its effects on the fluidity of the developing fetal-rat liver microsomal membrane. This early prenatal exposure to dexamethasone, which preceded the natural appearance of fetal corticosteroids, markedly accelerated the normal perinatal course of fluidization of this membrane. The lipid apparent microviscosity, which was determined by measurement of fluorescence polarization, decreased in 21-days-old treated fetuses to values that were indistinguishable from those of untreated newborn rats. This dexamethasone-mediated acceleration of membrane fluidization was associated with an increase in the index of unsaturation of the fatty acyl moiety of microsomal lipids. Dexamethasone caused a significant increase in the microsomal content of polyunsaturated fatty acids (arachidonic and linoleic acid), which was accompanied by a decrease in content of monoenoic fatty acids (oleic and palmitoleic acid). This early exposure *in utero* to dexamethasone precociously induced the changes in fatty acid composition of fetal-rat liver microsomal lipids that normally occur between the last day of pregnancy and the first day of extra-uterine life. These results suggest that endogenous glucocorticoids play a major role in the perinatal fluidization of the rat liver microsomal membrane.

INTRODUCTION

During the fetal and perinatal phases of development, significant changes occur in the levels of many cellular proteins, including enzymes, antigens and transport proteins. Studies in laboratory animals and man described both increases and decreases in a variety of enzymic activities that accompany the transition from fetal life to adulthood (Neims *et al.*, 1976; Greengard, 1977; Dutton, 1978). Fetal-rat liver serves in its early developmental stages as a haematopoietic organ (Jacquot, 1971). During the last week of pregnancy, the fetal liver acquires its adult differentiated characteristics and new proteins are synthesized and inserted in the newly formed membranes of the endoplasmic reticulum. The physical properties and lipid composition of the microsomal membrane are major determinants of the activity of these proteins (Eletr *et al.*, 1973; Duppel & Ullrich, 1976; Stubbs & Smith, 1984).

The lipid apparent microviscosity ($\bar{\eta}$) of the microsomal membrane is much higher in fetal- than in adult-rat liver (Kapitulnik *et al.*, 1979). Similar developmental differences exist for human liver microsomes (E. Weil, R. Rabinowitz, M. Krausz & J. Kapitulnik, unpublished work), as well as for the plasma membrane of chick heart (Kutchai *et al.*, 1976). The transition from the 'rigid' fetal microsomal membrane to the adult 'fluid' type started in rat liver at about 18 days of pregnancy and was almost fully completed within 24 h after birth (Kapitulnik *et al.*, 1980). This temporal pattern of perinatal fluidization of the hepatic microsomal membrane was reminiscent of the timetable for corticosteroid excretion by the fetal-rat adrenal. The corticotropic activity of the fetal hypophysis and the excretion of fetal corticosteroids start on day 17 and reach a maximum on day 19 of pregnancy (Holt & Oliver, 1968; Kamoun, 1970; Cohen, 1973; Milković *et al.*, 1973). The main goal of the present study was to find out whether this surge of fetal corticosteroids is the endogenous trigger of the processes leading to the perinatal fluidization of the rat liver microsomal membrane. Administration of the synthetic glucocorticoid dexamethasone to pregnant rats before the natural initiation of fetal corticosteroid excretion, markedly increased the fluidity of the fetal-rat liver microsomal membrane. The values of $\bar{\eta}$ obtained for the dexamethasone-treated fetuses were very similar to those normally observed in untreated animals after birth.

MATERIALS AND METHODS

Dexamethasone sodium phosphate (Ikacor, 4 mg/ml) was purchased from Ikapharm (Ramat Gan, Israel). 1,6-Diphenyl-1,3,5-hexatriene (DPH) was obtained from Aldrich (Milwaukee, WI, U.S.A.). Cholesterol, phospholipid and fatty acid standards, as well as the Fiske–SubbaRow reducing agent, sodium taurocholate and cholesterol oxidase were purchased from Sigma (St. Louis, MO, U.S.A.). All chemicals were ACS reagent grade or the best available grade, and all solvents were h.p.l.c. grade.

Treatment of rats with dexamethasone

Sabra rats (originated from the Wistar strain) were obtained from the Animal Facility of the Hebrew

Abbreviations used: DPH, 1,6-diphenyl-1,3,5-hexatriene; $\bar{\eta}$, lipid apparent microviscosity; PUFA, polyunsaturated fatty acids.

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University Medical School. They were maintained in cages with corn-cob bedding, on 12 h-light/12 h-dark cycles and were given free access to rat chow and water. Female rats, which went through previous cycles of pregnancy and delivery, were mated (overnight) with males. The presence of spermatozoa in the vagina was checked on the following morning and, if positive, this day was considered day 1 of gestation. Pregnant rats received daily intraperitoneal (i.p.) injections of 0.4 mg of dexamethasone on days 14–18 or 15–19 of pregnancy, and fetuses were delivered by caesarean section on day 19 or 21 of pregnancy respectively. Control pregnant rats received simultaneous injections of 0.1 ml of normal saline (0.9% NaCl), and fetuses were delivered on days 19 and 21 of pregnancy.

Preparation of microsomes

Fetuses and newborn (up to 24 h old) rats were decapitated and their livers excised. The livers of littermates were pooled together and immediately homogenized in 3 vol. of cold 0.15 M-KCl/0.05 M-Tris/HCl, pH 7.5. The microsomal fraction was isolated by differential centrifugation (Lu & Levin, 1972) and resuspended in 0.15 M-KCl/10 mM-EDTA. Microsomes were obtained after an additional centrifugation at 100000 *g* and suspended in 0.25 M-sucrose. Microsomal protein was determined (Lowry *et al.*, 1951), with bovine serum albumin as a standard. All further analyses were performed on microsomal suspensions stored at −20 °C, except for the measurement of fluorescence polarization, which was done on fresh microsomal preparations.

Determination of lipid apparent microviscosity ($\bar{\eta}$)

The $\bar{\eta}$ of rat liver microsomes was determined by measurement of fluorescence polarization (Shinitzky & Barenholz, 1974) using the fluorophore DPH (0.5 mM in tetrahydrofuran). Steady-state fluorescence-polarization values for membranes include contributions from both the rate and range of motion of the fluorescent probe (Stubbs & Smith, 1984). Fluorescence polarization of DPH is largely a measure of lipid order rather than rate of motion. However, the lipid structural-order parameter derived from these measurements, which reflects the degree of organization of the lipid in the membrane, is considered a useful parameter of 'fluidity' (Van Blitterswijk *et al.*, 1981). A 1 μl portion of the DPH solution was mixed vigorously with 2 ml of the microsomal suspension (0.4 mg of protein/ml), and the mixture was incubated for 1 h at 37 °C. Fluorescence polarization was measured at 37 °C with a Perkin–Elmer MPF 44A spectrofluorimeter equipped with special polarizers (Lakowicz, 1983). Excitation and emission wavelengths were 365 and 430 nm respectively.

Determination of lipids

Microsomal lipids were extracted into chloroform/methanol (2:1, v/v) (Folch *et al.*, 1957). Cholesterol was determined by a cholesterol oxidase method (Barenholz *et al.*, 1978). Total phospholipids were determined by phosphorus analysis using a slight modification of the method of Fiske & SubbaRow (Bartlett, 1959). The content of individual phospholipids was determined by two-dimensional t.l.c. (Yavin & Zutra, 1977) followed by phosphorus analysis.

The fatty acid composition of the extracted microsomal lipids was determined by g.l.c. of the fatty acid methyl esters. Aliquots of the chloroform/methanol lipid extracts were dried under N_2 and treated with 1 ml of a solution of 0.4 M-KOH in methanol (3 h at 37 °C). The mixture was then acidified with HCl and the fatty acid methyl esters were extracted into heptane and separated by g.l.c. on a column packed with 15% EGSS-X on 100–120 mesh Chromosorb (Eldan, Jerusalem, Israel). The column temperature was 195 °C, nitrogen was the gas carrier, and flow was set at 40 ml/min.

The calculated values are presented as means ± S.E.M., and differences between means were evaluated by the Student's *t* test.

RESULTS

Fluidity of the liver microsomal membrane

The lipid apparent microviscosity ($\bar{\eta}$) of the fetal-rat liver microsomal membrane decreased during the last week of pregnancy from 0.209 ± 0.001 Pa·s [2.09 ± 0.01 P (poise)], on day 19, to 0.164 ± 0.003 Pa·s (1.64 ± 0.03 P) on day 21 of pregnancy (Table 1). Treatment of pregnant rats with daily i.p. injections of 0.4 mg of dexamethasone between days 14 and 18 of pregnancy markedly decreased the $\bar{\eta}$ of the fetal microsomal membrane on day 19 of pregnancy to 0.149 ± 0.006 Pa·s (1.49 ± 0.06 P) (Table 1). Dexamethasone administration between days 15 and 19 of pregnancy decreased the $\bar{\eta}$ in 21-day-old fetuses to 0.123 ± 0.005 Pa·s (1.23 ± 0.05 P) (Table 1).

Lipid composition of the liver microsomal membrane

The microsomal phospholipid content of the fetal-rat liver increased by 47% between day 21 of pregnancy and day 1 after birth, whereas no significant change was observed in the cholesterol content (Table 2). Prenatal exposure to dexamethasone during days 15–19 of pregnancy did not affect either phospholipid or cholesterol content of liver microsomes from 21-day-old fetuses (Table 2). The relative content of phosphatidylcholine and phosphatidylethanolamine, which represent about 70% of the rat liver microsomal phospholipid mass, did not change between day 21 of pregnancy and day 1 after

Table 1. Effect of dexamethasone on the fluidity of the fetal-rat liver microsomal membrane

$\bar{\eta}$ values for fetal-rat liver microsomes were determined by measurement of fluorescence polarization as described in the Materials and methods section. Pregnant rats received daily i.p. injections of 0.4 mg of dexamethasone on days 14–18 or 15–19 of pregnancy, and fetuses were delivered on day 19 or 21 of pregnancy respectively. Values are expressed as means ± S.E.M. Values in parentheses indicate the numbers of litters studied. Differences between means of dexamethasone-treated and control groups are significant at $P < 0.001$ on both day 19 and 21 of pregnancy.

		Lipid apparent microviscosity ($\bar{\eta}$) (Pa·s)	
Treatment	Day of pregnancy...	19	21
Control		0.209 ± 0.001 (3)	0.164 ± 0.003 (6)
Dexamethasone		0.149 ± 0.006 (3)	0.123 ± 0.005 (8)

Table 2. Effect of dexamethasone on the phospholipid and cholesterol content of the fetal-rat liver microsomal membrane

Daily i.p. injections of 0.4 mg of dexamethasone were administered to pregnant rats on days 15–19 of pregnancy. Phospholipid and cholesterol content of liver microsomes were measured as described in the Materials and methods section. Values are expressed as means ± S.E.M. *n* indicates the number of litters studied (fetuses on day 21 of pregnancy and newborn rats within 24 h after birth). * indicates that differences between means of untreated fetuses and newborn rats are significant at $P < 0.001$.

	Content (nmol/mg of protein)		
	Fetus	Fetus + dexamethasone	Newborn
Phospholipid	230 ± 6	229 ± 8	338 ± 16*
Cholesterol	70 ± 4	70 ± 3	60 ± 1
Phospholipid/cholesterol molar ratio . . .	3.3 ± 0.2	3.3 ± 0.1	5.7 ± 0.3*
n . . .	6	8	3

Table 3. Effect of dexamethasone on the composition of fetal-rat liver microsomal phospholipids

For details of dexamethasone treatment, see Table 2. Microsomal phospholipids were analysed by t.l.c. as described in the Materials and methods section. Values indicate relative content (% of total) of each individual phospholipid and are expressed as means ± S.E.M. *n* indicates the number of litters studied (fetuses on day 21 of pregnancy and newborn rats within 24 h after birth). * indicates that differences between means of untreated fetuses and newborn rats are significant at $P < 0.005$.

	Relative content (%)		
Phospholipid	Fetus	Fetus + dexamethasone	Newborn
Phosphatidylcholine	54.7 ± 4.3	52.7 ± 0.9	50.2 ± 0.8
Phosphatidylethanolamine	17.8 ± 1.9	20.8 ± 0.9	21.7 ± 0.4
Phosphatidylinositol	8.1 ± 0.5	5.6 ± 0.8	4.7 ± 0.2*
Phosphatidylserine	4.3 ± 0.6	6.2 ± 0.5	8.5 ± 0.4*
Sphingomyelin	5.1 ± 0.3	5.2 ± 0.4	5.7 ± 0.3
Lysophosphatidylcholine	4.5 ± 2.7	3.4 ± 0.7	3.2 ± 0.3
Lysophosphatidylethanolamine	2.6 ± 1.4	4.2 ± 0.2	3.9 ± 0.2
Diphosphatidylglycerol	2.7 ± 0.6	1.9 ± 0.4	2.2 ± 0.4
n . . .	3	7	3

birth (Table 3). During this period the content of phosphatidylinositol significantly decreased, whereas that of phosphatidylserine increased. Prenatal exposure to dexamethasone did not affect the phospholipid composition of the fetal liver microsomes (Table 3).

The most remarkable developmental changes in rat liver microsomal lipids were observed in the profile of their fatty acyl moieties (Table 4). At birth, arachidonic acid ($C_{20:4}$) became the major fatty acyl component of rat liver microsomal lipids. Its relative content was 2-fold higher in newborn as compared with 21-day-old fetal liver, and did not change further between birth and adulthood (results not shown). In contrast, there were significant perinatal decreases in the content of the monoenoic oleic acid ($C_{18:1}$) and palmitoleic acid ($C_{16:1}$) (Table 4). The net result of these perinatal changes in the fatty acid profile of microsomal lipids was a 52% increase in their relative content of polyunsaturated fatty acids (PUFA) (Fig. 1). Thus a higher index of unsaturation (which accounts for the total number of double bonds in the unsaturated fatty acids) and a lower melting point (which is determined by both the degree of unsaturation and chain length of the fatty acids) were obtained for the fatty acids of newborn as compared with fetal liver microsomal lipids (Fig. 1). In 21-day-old fetuses of dexamethasone-treated rats there were significantly higher levels of arachidonic and linoleic acids, and lower levels of oleic and palmitoleic acids, than in fetuses from untreated animals (Table 4). Prenatal exposure to dexamethasone significantly increased the relative content of PUFA (Fig. 1), and thus increased the index of unsaturation and decreased the melting point of liver microsomal fatty acids from 21 day-old fetuses to values which were similar (differences were not statistically significant) to those obtained after birth in untreated newborn animals (Fig. 1).

DISCUSSION

Dexamethasone, a synthetic glucocorticoid that crosses the placenta freely (Zarrow *et al.*, 1970), markedly accelerated the normal perinatal course of fluidization of the fetal-rat liver microsomal membrane when administered to pregnant rats before the natural initiation of

Table 4. Effect of dexamethasone on the fatty acid content of fetal-rat liver microsomal lipids

For details of dexamethasone treatment, see Table 2. The methyl esters of the fatty acids were analysed by g.l.c. as described in the Materials and methods section. Values indicate relative content (% of total) of each individual fatty acid and are expressed as means ± S.E.M. *n* indicates the number of litters studied (fetuses on day 21 of pregnancy and newborn rats within 24 h after birth). Differences between means of untreated and dexamethasone-treated fetuses, or between means of untreated fetuses and newborn rats, are significant at: $*P < 0.001$; $**P < 0.01$.

	Relative content (%)		
Fatty acid	Fetus	Fetus +dexamethasone	Newborn
Myristic ($C_{14:0}$)	2.6±0.4	4.4±0.6	1.8±0.2
Myristoleic ($C_{14:1}$)	1.4±0.2	1.9±0.2	0.6±0.1
Palmitic ($C_{16:0}$)	24.0±0.4	22.9±0.8	26.1±0.1**
Palmitoleic ($C_{16:1}$)	6.1±0.2	4.7±0.2*	2.4±0.2*
Stearic ($C_{18:0}$)	17.4±0.4	16.7±0.5	18.8±0.2
Oleic ($C_{18:1}$)	23.0±0.3	17.0±0.3*	11.7±0.8*
Linoleic ($C_{18:2}$)	9.9±0.3	12.4±0.2	11.0±0.9
Arachidonic ($C_{20:4}$)	15.6±0.2	20.0±0.6*	27.7±1.2*
n...	6	8	3

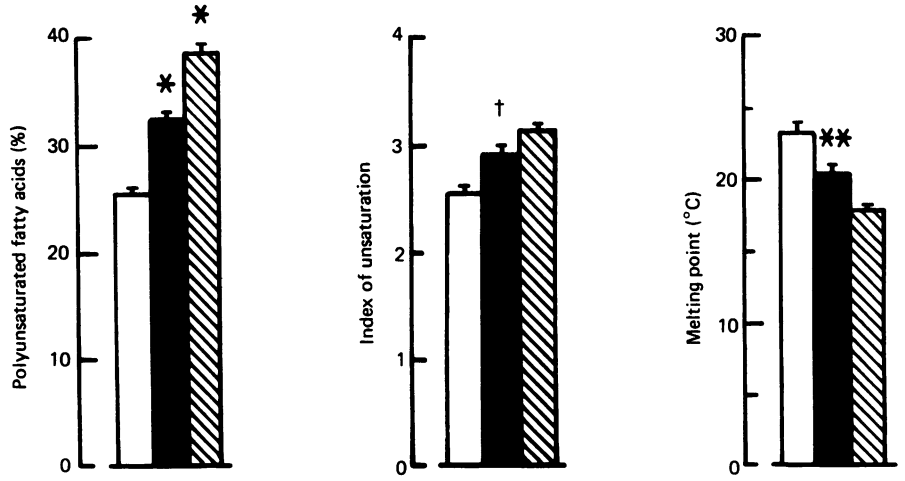

Fig. 1. Effect of dexamethasone on unsaturation characteristics of the fatty acyl moiety of fetal-rat liver microsomal lipids

□, Fetus on day 21 of pregnancy, untreated; ■, fetus on day 21 of pregnancy, dexamethasone-treated (between days 15 and 19 of pregnancy); ▨, 1-day-old newborn. Values were calculated from the data in Table 4 and are expressed as means ± S.E.M. When so indicated, a given mean value is significantly different from the preceding value at: $*P < 0.001$; $**P < 0.002$; $\dagger P < 0.02$.

fetal corticosteroid excretion. Thus the values of $\bar{\eta}$ for microsomes of 21-day-old dexamethasone-treated fetuses were indistinguishable from those which we have previously reported for untreated newborn rats (Kapitulnik *et al.*, 1979). This dexamethasone-induced precocious fluidization of the fetal-rat liver microsomal membrane suggests that endogenous glucocorticoids play a major role in the perinatal processes leading to membrane fluidization. Similarly, dexamethasone administration to pregnant rats before the natural surge of fetal corticosteroids, precociously induced the synthesis of microsomal UDP-glucuronosyltransferase in fetal-rat liver (Wishart & Dutton, 1977). Thus glucocorticoids appear to be involved not only in regulating the perinatal synthesis of microsomal enzymes, but also in controlling the formation of the membranes of the smooth endoplasmic reticulum of rat liver, and may therefore play an important role in modulating the activity of these newly synthesized enzymes.

Membrane fluidity is reduced by cholesterol, whereas certain phospholipids, such as phosphatidylcholine, increase it; others, such as sphingomyelin, decrease fluidity (Shinitzky & Barenholz, 1978). Another major factor that affects membrane fluidity is the degree of unsaturation of the fatty acyl moiety of membranal lipids (Shinitzky & Barenholz, 1978; Stubbs & Smith, 1984). In the present study the newborn-rat liver microsomes showed a higher phospholipid/cholesterol molar ratio and a higher index of unsaturation of fatty acids than did those of 21-day-old fetuses. The accelerated rate of

fluidization of the fetal-rat liver microsomal membrane observed in dexamethasone-treated animals was associated with an increase in the relative content of PUFA in microsomal lipids, but there was no change in the phospholipid/cholesterol molar ratio. As a result of the early exposure *in utero* to dexamethasone, before the natural appearance of fetal corticosteroids, both fetal microsomal membrane fluidity and the index of unsaturation of the microsomal fatty acids had already reached before birth the values normally obtained only after birth. These results suggest that the natural perinatal fluidization of the rat liver microsomal membrane results from the increased unsaturation of fatty acids observed during this period of development. A similar relationship, between changes in fluidity (as measured by fluorescence polarization) and fatty acid composition, has been previously reported (York *et al.*, 1982; Castuma & Brenner, 1983).

The glucocorticoid-mediated increase in the index of unsaturation of the fatty acyl moiety of microsomal lipids, which is associated with the perinatal fluidization of the fetal-rat liver microsomal membrane, resulted from a major increase in the content of arachidonic acid and a smaller increase in linoleic acid content. Glucocorticoids stimulate the release and synthesis of polypeptides (e.g. lipomodulin and macrocortin), which inhibit phospholipase A_2 (Blackwell *et al.*, 1980; Hirata *et al.*, 1980), the enzyme responsible for the release of arachidonic acid from the C-2 position of phospholipids. This inhibition of arachidonic acid release by glucocorticoids, which is responsible for the anti-inflammatory action of these agents (Blackwell & Flower, 1983), may also account for the perinatal increase in arachidonic acid content of microsomal lipids. In addition, since arachidonic acid inhibits the desaturation of oleic to linoleic acid (Brenner, 1974), a decrease in the free levels of arachidonic acid may reduce this inhibition, and would probably account for the changes in oleic and linoleic acid content observed in the perinatal period.

The glucocorticoid-mediated perinatal fluidization of the rat liver microsomal membrane may have far-reaching implications. It has been suggested that changes in membrane fluidity may contribute to developmental changes in the uptake of sugars, amino acids and urea by chick heart cells (Kutchai *et al.*, 1976), as well as to the perinatal changes in latency of hepatic membrane-bound enzymes (Dutton & Leakey, 1981; Winsnes, 1971). Glucocorticoids, which are administered during pregnancy for the prevention of respiratory-distress syndrome in prematurely delivered infants (Ballard & Ballard, 1979), may therefore increase the fluidity of hepatic and extrahepatic membranes. This may in turn affect the functions of membranal proteins that play a critical role in fetal development.

This work was supported by a grant from the Joint Research Fund of the Hebrew University and Hadassah. We thank Dr. Y. Barenholz for his help and advice and Ms. Ana Fibach for preparing the manuscript.

Received 1 April 1986/2 June 1986; accepted 11 June 1986

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