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STRUCTURAL CHANGES OF LIVER MICROSOMES IN RAT DURING NEONATAL LIFE : INFLUENCE ON THE GLUCURONIDATION RATES OF VARIOUS SUBSTRATES

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The Vmax of the membrane bound UDP-glucuronosyltransferase (UDP-GT) towards group 1 substrates (4-nitrophenol, 2-naphthol) was particularly higher in young rats than in adults. On the contrary, activity towards group 2 substrates such as borneol or testosterone was not detectable in fetus liver. The developmental pattern of UDP-GT was related to changes in lipid composition of microsomes, namely in the content in lysophosphatidylcholine which rose at birth. The phospholipid-cholesterol molar ratio also increased 2 fold from the 16th day of fetal life to the 4th day after birth. Measurement of the steady state anisotropy of 1,6-diphenyl-1,3,5-hexatriene (DPH) as well as determination of the order parameter S and the rotation cone angle of the fluorescent probe strongly suggested an increase in membrane fluidity in rat liver microsomes during ontogenesis.

UDP-glucuronosyltransferases (UDP-GT) (EC 2.4.1.17) represent a family of enzymes that are involved in the final transformation of drugs. They catalyze the binding of glucuronic acid moiety to various aglycones classified, at least, into two groups according to their physicochemical properties (1). As these enzymes are tightly associated to microsomal membranes, their catalytic regulation depends on the lipid environment as well as on the crystal-liquid state transition of the lipid bilayer (2, 3).

During the development of rat, the activity of hepatic enzymes involved in phospholipid biosynthesis are modified (4), thus leading to changes in microsomal lipid composition (5). Therefore the expression of the

ABBREVIATIONS:
DPH, 1,6-diphenyl-1,3,5-hexatriene ; CHOL, cholesterol ; LPC, Lysophosphatidylcholine ; PC, phosphatidylcholine ; PE, Phosphatidylethanolamine ; PI, Phosphatidylinositol ; PL, phospholipids ; PS, phosphatidylserine ; SPM, sphingomyelin ; UDP-GT, UDP-glucuronosyltransferase.

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membrane-bound drug-metabolizing enzymes may be modulated by variations in their lipid surrounding (6).

The aim of this present study is to define the evolution of the lipid composition of microsomes in rat liver during the neonatal life, and to determine, by fluorescence polarization spectroscopy, the parameters which characterize the lipid organisation in the membranes. The results were related to the corresponding maximal glucuronidation rates of xenobiotics and endogeneous compounds during this period.

MATERIALS AND METHODS

Pregnant Wistar rats were purchased from IFFA Credo, Saint-Germain-sur-l'Arbresle (France). Fetuses at 16, 18, 20 and 22 days of gestation and newborn animals aged 1, 2, 4 and 6 days were killed by decapitation. The livers were pooled by litter and the microsomes were prepared according to the method of Cresteil et al. (7). The purity of the microsomal fraction and especially the degree of contamination by plasma membranes was checked by classical marker enzyme measurements and by electron microscopy. Protein content was determined by the Lowry's method (8).

UDP-glucuronosyltransferase activity. The enzyme activity was measured on a Cobas (Roche Bioélectronique) fast analyzer centrifuge by the method of Mulder and Van Doorn (9) with 4-nitrophenol, 2 naphthol, testosterone (Merck, Darmstadt, Germany), terpineol (Fluka, Buchs, Switzerland), borneol, 4-hydroxybiphenyl (Aldrich, Beerse, Belgium) and 5-hydroxytryptamine (Sigma, Saint-Louis, U.S.A.) as aglycones. Vmax were calculated from double reciprocal plots according to Segal (10) using the kinetic model of a rapid equilibrium, random bireactant system.

Lipid analysis. After extraction from microsomal membranes (11), the phospholipids were separated by thin layer chromatography on silicagel, 0.25 mm thick (Merck, Darmstadt, Germany), according to Skipski et al. (12). After detection by iodine vapour exposure, each individual phospholipid (PL) was scraped off and mineralized for 2 hours with 70 % v/v perchloric acid. Lipid quantification was achieved by phosphorus determination (13). Membrane cholesterol (CHOL) content was estimated by the method of Klose et al. (14).

Fluorescence polarization measurements. Microsomal membranes (0.5 mg protein) were incubated for 30 minutes at 37°C with 2 μ mol 1,6-diphenyl-1,3,5-hexatriene (DPH, Koch-Light, Colnbrook Berks, England). The anisotropy of the steady state fluorescence emission was recorded on a SPF 500 Kontron spectrofluorimeter equipped with a polarization accessory made in our laboratory and coupled to an Apple II computer for data processing. The limiting anisotropy (r^{∞}), the rotation cone angle of the probe ($A\omega$) and the order parameter (S) were calculated according to Kinosita et al (15). The life time and the time resolved anisotropy were measured on apparatus made in our laboratory, using the classical single photon technique.

RESULTS

UDP-glucuronosyltransferase activity at Vmax. Table I shows the values of Vmax obtained for glucuronidation of aglycones in young rats.

Table I. Vmax values of glucuronidation of various substrates by liver microsomes in rat as the function of age

	Gestational time (days)		After birth (days)		Male adults (days)
	18	22	2	6	49
Group 1 Substrates					
4-nitrophenol	7.50	82.0	98.0	78.0	83.0
2-naphthol	24.75	147.0	373.0	133.0	90.0
5-hydroxytryptamine	1.80	13.0	-	15.3	30.0
Groupe 2 Substrates					
Borneol	ND*	ND	-	3.8	14.0
Terpineol	ND	ND	-	2.1	11.5
Testosterone	ND	ND	-	1.2	5.0
Hydroxybiphenyle	0.6	3.0	-	15.1	21.0

*ND : not detectable ; Vmax was expressed as nmol substrate transformed per min and mg microsomal protein.

Transformation of group 2 substrates was almost undetectable in fetus liver, but developed postnatally. On the contrary, group 1 substrates which were glucuronidated at a great extent before birth, reached afterwards a maximum 2 days after birth. Interestingly, except for 5-hydroxytryptamine, it appeared that young rats were able to conjugate 4-nitrophenol and 2-naphthol faster than adult animals (Table I).

Membrane lipid composition during rat development. Table I represents the evolution of PL and CHOL contents of hepatic microsomes in rat before and after birth. Although the total PL concentration was rather stable throughout the experiment, but at a lesser extent than that found in adult animals, the CHOL content decreased continuously as a function of age, especially 4 days after birth. The consequence was an increase in the PL-CHOL molar ratio during the same period. Moreover, membrane PL was qualitatively changed (Table II). The sphingomyelin (SPM) content was quite stable but that of PC decreased until the 1st day after birth and then increased to adult values. The amount in lysophosphatidylcholine was gradually enhanced in function of age with a maximum level one day after birth. As phosphatidylserine (PS), phosphatidylinositol (PI) and phosphatidylethanolamine (PE) concentrations were also modified, it could be concluded that important changes in both the composition and quantity of

Table II. Microsomal lipid composition in rat liver as a function of age

Age (days)	PL CLASSES (%)					Total PL (nmol/mg protein)	CHOL (nmol/mg protein)	PL/CHOL (molar ratio)
	LPC	SPM	PC	PI+PS	PE			
Gestation time								
16	8	12	51	17	12	263 ± 24[a]	104 ± 15[a]	2.5
18	11	14	48	15	12	211 ± 24	82 ± 9	2.6
20	14	14	46	14	12	267 ± 15	70 ± 4	3.8
22	13	15	45	14	13	210 ± 9	68 ± 3	3.1
After birth								
1	15	16	45	13	11	210 ± 9	67 ± 3	3.1
2	13	14	49	11	13	296 ± 36	59 ± 7	5.0
4	10	15	51	11	13	251 ± 27	45 ± 5	5.6
6	6	12	56	11	15	270 ± 24	53 ± 5	5.1
Adult male (49 days)	5	10	57	10	18	319 ± 9	79 ± 3	4.1
Adult female (49 days)	4	10	58	13	15	324 ± 10	63 ± 2	5.1

[a] mean ± SD n = 4

microsomal lipids occured during the perinatal development of rat.

Estimation of the membrane lipid viscosity. The anisotropy $\bar{r}$ and the mean life time (τ) of DPH embedded into the microsomal membrane decreased continuously, 38 % and 23 % respectively, from the 18th day of the fetal life to the 6th day after birth (Table III). The adult value for τ was the same but that for $\bar{r}$ was higher than data found with newborn animals.

By comparing the two sets of values, it could be concluded that variations in the life time of DPH in microsomes were probably due to important

Table III. Viscosity parameters of hepatic microsomal membranes on rat during development : $\bar{r}$, mean anisotropy ; τ , mean life time ; r^{∞}, limiting anisotropy ; $A\omega$ (d°), rotation cone angle ; S, order parameter.

	($\bar{r}$)	(τ)	(r^{∞})	($A\omega$, d°)	(S)
Gestation time (days)					
18	0.237	7.68	0.155	42	0.65
20	0.215	7.26	0.150	42	0.64
22	0.225	7.32	0.158	41	0.66
After birth (days)					
1	0.195	7.32	0.113	48	0.56
2	0.199	6.36	0.177	47	0.57
4	0.155	6.60	0.060	52	0.41
6	0.154	6.72	0.056	59	0.39
Adult male (49 days)	0.225	6.48	0.102	50	0.60
Adult female (49 days)	0.217	6.54	0.095	51	0.54

changes in the emission properties of the probe. Successive flash excitation of DPH was therefore undertaken in order to measure the time resolved anisotropy, which is non dependent on the life time, and also the limiting anisotropy (r^{∞}), the cone angle of rotation of DPH ($A\omega$) and the order parameter (S). The values obtained are also reported in Table III. r^{∞} and (S) gradually decreased in function of time, as the PL-CHOL molar ratio increased. The maximal decrease was 66 % ; in the same conditions, $A\omega$ was enchanced by 40 %.

DISCUSSION

In this work we have focused attention on the importance of fluorescence polarization measurements after pulse excitation of DPH on the estimation of lipid viscosity. These data allowed the calculation of S which provides information with a good degree of accuracy, on the molecular motions occuring in heterogenic media such as biological membranes. However this technic remains semi quantitative as the localization of the probe, the local perturbation that it makes and the existence of rigid and non rigid areas in the membranes may be estimated. Despite these drawbacks, determination of S should replace favorably the microviscosity parameter, according to Shinitzky et al. (16). In the case of microsomes, the only measurements of $\bar{r}$ after continuous excitations could not be compared between themselves as the DPH life time was modified by 23 % throughout the experiment (Table III). The time resolved anisotropy data showed a decrease in the S value due to an increase in microsome fluidity with age. Comparison with liposomes of similar PL-CHOL molar ration led to the conclusion that a 100 % increase in Pl-CHOL molar ratio could account for such a fluidity (results not shown). The increase in LPC content at birth could also participate in membrane fluidity (17). Triglycerides (18) or insaturated fatty acids (19) cause the same effects. On the other hand, CHOL is well known to enhance the rigidity of the lipid membranes (20).

These changes in membrane viscosity and in lipid composition, especially in lysophosphatidylcholine content could account for UDP-GT activation

towards 4-nitrophenol and 2-naphthol since these phospholipids have been found, in reconstituted systems, to increase the activities at Vmax of the pure enzyme (21). This situation probably also occurs in vivo since it has been reported that, in rat fed a protein deficient diet, the increase in UDP-GT activity (4-nitrophenol) was the consequence of high level in lyso-phosphatidylcholine in the liver microsomes (22). It appeared that, according to the substrate used, the susceptibility of the enzyme to the lipid environment was different, thus confirming the heterogeneity of UDP-GT. Finally, the development of UDP-GT activity, which is under hormonal control (23), could also be modulated by the lipid composition and organization of the microsomal membranes.

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