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In Vivo Modification of the UDP-Glucuronosyltransferase Functional State in Rat Liver Following Hypophysectomy and Partial or Complete Hormonal Restoration

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The effects of growth hormone on the uridine diphosphate glucuronosyltransferase functional state, biophysical membrane parameters (order parameters and rotational correlation frequency) and the composition in phospholipids were studied in male rat hepatic microsomes. Sham-operated and hypophysectomized animals were injected with two different dosages of growth hormone, mimicking either the male or female growth hormone secretion pattern. Half the animals received thyroxine and cortisol in concentrations chosen to compensate for the lack of thyroid hormones and glucocorticoids in hypophysectomized rats. Growth hormone treatment resulted in a decrease in the latency (that gives a quantification of uridine diphosphate glucuronosyltransferase functional state) of the glucuronidation activities towards various substrates (testosterone, androsterone, bilirubin and 4-nitrophenol). This decrease with growth hormone treatment was particularly evident in hypophysectomized animals that had received cortisol and thyroxine supplementation treatment. These modifications were strongly correlated with modifications in the microsomal membrane lysophospholipid content and to a lower extent with microsomal membrane fatty acid composition. The cytosolic phospholipase A_2-dependent increase in the lysophospholipid content in the endoplasmic reticulum is probably a major determinant in the regulation of the functional state of glucuronoyltransferases in response to high dosage growth hormone treatment.

Key words: fatty acid composition, lysophospholipids, membrane fluidity, somatotropin, UDP-glucuronosyltransferases.

Abbreviations: ANDRO, androsterone; ANOVA, analysis of variance; BIL, bilirubin; CT, cortisol and thyroxine; DBI, double bond index; GH, growth hormone; LPC, lysophosphatidylcholine; LPE, lysophosphatidylethanolamine; LPL, lysophospholipid; MUFA, monounsaturated fatty acid; 4-NP, 4-nitrophenol; NS, nitroxide stearic acid; PC, phosphatidylcholine; PE, phosphatidylethanolamine; $cPLA_2$, cytosolic phospholipase A_2; PUFA, polyunsaturated fatty acid; SFA, saturated fatty acid; TESTO, testosterone; UDPGA, UDP-glucuronic acid; UGT, UDP-glucuronosyltransferase.

Microsomal uridine diphosphate glucuronosyltransferases (UGTs) [EC 2.4.1.17] comprise a family of closely related enzymes that conjugate a wide variety of lipophilic compounds with glucuronic acid, enhancing their water solubility and then facilitating their excretion into bile or urine. Glucuronidation represents a major metabolization pathway of endogenous and exogenous compounds (*1*, *2*).

As UGTs are deeply embedded in the microsomal membrane, the apparent activity depends on the amounts of enzyme as well as on the interactions between enzymes and their lipidic environment. Therefore, the activity of UGT can vary more than 100-fold as a function of the lipid used to reconstitute the pure enzyme (*3*). In hepatocytes or in intact microsomes, the glucuronidation activity is low, but it can be increased by adding pore-forming agents or detergents to the microsomal vesicles (*4*, *5*). This biochemical property of UGTs in the restriction of activity under native conditions is designated as the latency, as is the case for enzymes embedded in the endoplasmic reticulum (ER) membranes such as glucose 6-phosphatase (*6*). Therefore, the regulation of the functional state of UGTs can be considered as another possible way for cells to regulate their own capability to synthesize glucuroconjugates of lipophilic compounds.

Several factors including diet, gender, age or pregnancy-delivery events have been shown to modify the latency of UGTs (*7*–*10*). The latency reflects only the average degree of constraint of native UGTs, their activity being measured both under native and non-constrained conditions (*10*). Yet, despite extensive investigation, the regulation of the functional state of UGTs at the molecular level is still the subject of debate. In fact, different alternative models have been proposed to account (i) for the existence of biochemical constraints on UGTs under native conditions in microsomes (*11*) and (ii) for their modulation depending on biochemical factors such as uridine diphosphate-glucuronic acid (UDPGA), uridine diphos-

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phate-*N*-acetylglucosamine (UDP-*N*-acetylglucosamine), and uridine monophosphate (UMP), which are compartimentalized (*12*). More recently, a third model for latency has been proposed: the UGT oligomeric model, which presents dimers or higher oligomers of UGTs as putative transporters of UDP-*N*-acetylglucosamine in membranes (*13*). Modifications of UGT latency with the pregnancy-delivery event have been observed with concomitant changes in membrane viscosity (*10*), but no statistical correlation has been strictly established between these two membrane parameters. On the contrary, the difference in the functional state of 4-nitrophenol UGT in male and female liver is not related to a sex difference in membrane fluidity (*7*). So, the structural link between the UGT functional state and membrane fluidity is not fully understood and seems rather controversial.

In a previous work, we showed that growth hormone (GH) could differentially modify the amount of different UGTs in rat liver, particularly UGT 1A1, which conjugates bilirubin (*14*). Besides, we showed also that GH can deeply modify the fatty acid composition of the hepatic microsomal membranes of male rats inducing a so-called "feminization" process (*15*). GH induces a reduction in the proportion of monounsaturated fatty acids (MUFAs) and an increase in polyunsaturated fatty acids (PUFAs), particularly in arachidonic acid. Therefore, as GH is a pleiotropic hormone implicated in the regulation of numerous metabolic processes including lipid metabolism, its effect on UGT latency needs to be determined precisely.

In the present work, we examined the effects of GH treatment and hypophysectomy associated or not to hormonal restoration with thyroid hormone and glucocorticoid on the latencies of several UGTs in male rats. Two different concentrations of GH were used, mimicking either the male or female secretion pattern of GH. Since the microsomal fatty acid composition is deeply modified by these treatments (*15*), the dependence of latency on the modification of the composition of specific fatty acids was studied using multidimensional statistics. This method revealed the probable biophysical effect of some specific fatty acids on the passive diffusion kinetics of UGT substrates depending on the activity considered. Although measurements of viscosity parameters in membranes, which were performed in parallel, revealed a significant correlation with modifications in fatty acids composition, importantly, none of the viscosity markers observed are related to latency change. The only global structural change induced *in vivo* in ER membranes by such physiological treatments corresponds to significant modifications in the amounts of lysophospholipids (LPLs), which are strongly correlated with the modification of UGT latency, regardless of the UGT activity considered.

MATERIALS AND METHODS

Chemicals—Unlabelled 4-nitrophenol (4-NP), testosterone (TESTO), androsterone (ANDRO), bilirubin (BIL), 4-nitro[U-^{14}C]phenol (437 MBq/mmol), coenzymes and biochemicals were purchased from Sigma-Aldrich Chimie (St-Quentin Fallavier, France). [4-^{14}C]testosterone (1.67–2.22 GBq/mmol) was provided by NEN Life Sciences (Les Ulis, France). [4-^{14}C]Androsterone was synthesized enzymatically in the laboratory from [4-^{14}C]testosterone as previously described (*14*).

Animals—Male Sprague-Dawley rats, hypophysectomized or sham-operated at 6 weeks of age were purchased from Iffa-Credo (L'Arbresle, France). The animals were weighed every day after their arrival in the laboratory. They were individually housed under conditions of controlled temperature (20–22°C) and photoperiod (14 h of light/10 h of darkness) and were fed a standard diet *ad libitum*. The rats were allowed to recover for two weeks from hypophysectomy and sham-operation, and then, were randomly divided into 12 groups of 8 according to a factorial arrangement. The effectiveness of hypophysectomy was verified by the absence of weight gain over this period. The animals were given (at 12-h intervals) a s.c. injection of recombinant human growth hormone (GH1: 0.25 IU/kg of body weight; GH2: 2 IU/kg of body weight) twice daily, while GH controls (GH0) received only vehicle (25 mM sodium carbonate buffer, pH 9.5, 0.9% NaCl). Recombinant human growth hormone was a generous gift from Sanofi-Synthélabo (Labège, France). Half the animals received daily subcutaneously injection of cortisol (400 μg/kg of body weight) and L-thyroxine (T_4) (50 μg/kg of body weight) (Sigma-Aldrich Chimie) as described elsewhere (*16*, *17*). This T_4 concentration was found to render hypophysectomized rats euthyroid (*18*). The remaining animals received only vehicle (0.9% NaCl). This cortisol/thyroxine treatment (CT) started 2 days prior to GH treatment and lasted throughout the experimental period. Weight gain during the treatment period showed that hormone replacement was effective.

After 16 days of treatment, the animals were slaughtered between 09:00 and 11:00 h a.m. to avoid any circadian variation; blood was collected and the liver was immediately perfused with Ringer solution *via* the portal vein, excised, weighed and placed in liquid nitrogen before storage at –80°C. Microsomal membranes were prepared as described previously (*14*).

Measurements of UDP-Glucuronosyltransferase Activities and Estimation of Their Latency—UGT activities towards 4-NP, TESTO, ANDRO and BIL were measured both in "native" and fully detergent-activated microsomes. For each substrate, the optimal concentration of detergent leading to the activation of glucuronidation activities (Triton X-100 in all cases except for BIL glucuronidation activity for which digitonin was used) was determined in a preliminary step by testing each treatment (hypophysectomy or GH2 treatment) (*14*). The common optimal dose of detergent for all groups of animals was the one that gave a maximal increase of UGT activity regardless of the treatment group.

UGT activity towards 4-NP was assayed radiochemically by the procedure of Tukey *et al.* (*19*) with 250 μM of substrate, 2.5 mg of microsomal protein and Triton X-100 (0.15 mg/mg microsomal protein) at 37°C for 30 min. UGT activities towards TESTO and ANDRO were assayed radiochemically as described in a previous work (*14*) with 100 μM of substrate, Triton X-100 (0.4 and 0.5 mg/mg of microsomal protein for TESTO and ANDRO glucuronidation, respectively), 1 mM UDPGA and 0.5 mg of microsomal protein, for 30 min at 37°C. UGT activity towards BIL was assayed as described by Van Roy &

Heirwegh (*20*). Incubations were carried out with 3.75 mM UDPGA, 1 mg of microsomal protein, 400 μM of substrate and 2% digitonin for 30 min at 37°C. Determination of BIL glucuronide in the incubation mixtures was done spectrophotometrically at 530 nm using the diazonium salt of ethyl anthranilate (*20*). All glucuronidation rates were found to be linear with respect to incubation time and amount of microsomal protein.

$$\frac{A_{det} - A_{nat}}{A_{det}} \times 100$$

Calculations of latency were done for each animal and each substrate as described elsewhere (*21*): where A_{det} represents UGT activity measured in the presence of detergent (activated conditions) and A_{nat} represents UGT activity without detergent (native conditions).

Fatty Acid Analysis—The influence of different treatments on the fatty acid composition of total microsomal phospholipids was analysed in a previous work (*15*). In the present study, these results were used again to establish statistical correlations with other variables, such as UGT latency and membrane physical parameters. Results obtained in the same set of experiments on sham-operated CT-treated male rats treated with GH0, GH1, and GH2 were combined with those obtained in animals presented in the previous study in order to obtain a greater number of individuals for establishing correlation with latency variables and biophysical parameters.

ESR Measurements—Membrane lipids were probed with 5-, 7-, and 10-nitroxide stearic acids (5-, 7-, and 10-NS) under conditions of 2% (by volume) spin label solution (10^{-2} M in DMSO) in microsomes for the measurement of order parameter S (with 5- and 7-NS probes that undergo anisotropic motion, and 10-NS probe, which is located in the intermediate zone of the phospholipidic layer). Membrane lipids were probed with the 16-NS probe, which is in the hydrophobic zone, under conditions of 1% (by volume) spin label solution (10^{-2} M in DMSO) in microsomes for the measurement of rotational correlation frequency ν. ESR experiments were performed using an E-109 Varian-X Band spectrometer (Varian, Les Ulis, France) at 9.14 GHz. Measurements were made at increments of 2°C between 0 and 40°C and the sample temperature was monitored with a thermocouple. The spectra were then digitized on a HP 9847 A (Hewlett-Packard, Les Ulis, France), and the spectral characteristics were analysed on a HP 9825 T calculator. From these measurements, values calculated at 37°C were obtained by linear regression for measurements done with the 5-, 7-, and 16-NS probes or by third degree polynomial regression for measurements done with the 10-NS probe.

Phospholipid Composition of the Microsomal Membrane—Total microsomal lipids were extracted according to Bligh and Dyer (*22*). Quantification of phospholipids was achieved by the measurement of inorganic phosphorus by the Bartlett procedure (*23*). The lipidic extract was then submitted to separation on a silica cartridge as described previously in order to isolate phospholipids from other lipid fractions (*15*). The different phospholipid classes were separated by HPLC as described elsewhere (*24*) on a Varian model 9010 apparatus using a Lichro-CART column (244 mm × 4 mm) filled with Lichrosorb Si 60 (5 μm) (Merk, Darmstadt, Germany) and a Cunow-model 10 light diffusion detector (Eurosep Instruments, Cergy, France). Separation was achieved using a ternary gradient: A, hexane; B, 2-propanol/chloroform 4/1 (v/v); C, 2-propanol/water 1/1 (v/v). The following gradient was used: from 0 to 20 min, elution with a linear gradient from A/B/C 42:52:6 (by volume) to A/B/C 33:52:15 (by volume); from 20 to 55 min, isocratic elution with A/B/C 33:52:15 (by volume); from 55 to 57 min, linear gradient elution to A/B/C 28:52:20 (by volume), which was maintained isocratically to 90 min. The flow rate was 1 ml/min. Retention times were confirmed using authentic standards.

Statistical Analysis—Treatment effects on UGT latencies were determined by analysis of variance (ANOVA) using the SAS GLM procedure (*25*) on a completely randomized design with a 2 × 3 × 2 factorial arrangement of treatments (hypophysectomy, GH and CT, respectively). Treatment effects on biophysical and phospholipid composition parameters were determined by ANOVA on a restricted number of animals. Data concerning phospholipidic composition expressed in percentage were transformed into arcsine (x) to correct for unequal variances. When a three-factor ANOVA was used (complete additive model: hypophysectomy *GH*CT), some first order and second order interactions between treatments most often proved to be highly significant. Thus, the effect of each treatment was also tested in the following reduced groups of animals: GH treatment in sham-operated CT-untreated, sham-operated CT-treated, hypophysectomized CT-untreated, and hypophysectomized CT-treated rats, separately; hypophysectomy in GH-untreated animals; and CT treatment in sham-operated GH-untreated rats. Within these different groups, the statistical analysis of differences between the means was performed using the Student-Newman-Keuls test. In order to examine further the conditions of hormonal restoration of control values after hypophysectomy, a one-factor ANOVA was done followed by mean comparisons between the 12 groups of animals using the Student-Newman-Keuls test.

Canonical analyses were made by the SAS CANCOR procedure. This statistical method was used in order to test the dependence level (or canonical correlations) existing between different sets of variables, such as UGT latencies on one hand and biophysical spectroscopic parameters or fatty acid/phospholipid composition on the other hand (*26*, *27*). This procedure tests a series of hypotheses that each canonical correlation and all smaller canonical correlations are zero in the population. Besides, this multivariate technique provides multiple regression analysis to explain in more detail the canonical correlation analysis. So, it is possible to examine the linear regression (based on a least-squares criterion) of each variable that belongs to the dependent variable set on the opposite set of variables that belong to the regressor variable group. This canonical analysis allows comparison of a great number of variables belonging to different sets and making apparent the simple correlations existing eventually between two variables belonging to two different sets of variables. When these correlations were found to be significant, simple correlations were then calculated between the different variables. Simple total or partial correlations were determined using the SAS CORR procedure.

Table 1. **Hepatic microsomal membrane fatty acid composition of all groups of animals.**

Fatty acids	Sham-operate						Hypophysectomized					
	CT–			CT+			CT–			CT+		
	GH0	GH1	GH2	GH0	GH1	GH2	GH0	GH1	GH2	GH0	GH1	GH2
14:0	0.28 ± 0.06	0.25 ± 0.05	0.22 ± 0.02	0.21 ± 0.02	0.18 ± 0.04	0.23 ± 0.08	0.41 ± 0.09	0.47 ± 0.22	0.44 ± 0.14	0.28 ± 0.04	0.24 ± 0.09	0.25 ± 0.03
16:0	20.59 ± 0.86	17.58 ± 1.99	14.48 ± 0.39	18.95 ± 1.03	16.60 ± 2.79	19.22 ± 2.08	20.72 ± 0.75	22.22 ± 1.05	20.43 ± 1.15	20.04 ± 0.80	18.47 ± 1.68	15.50 ± 1.97
16:1 *n*-7	1.77 ± 0.35	1.33 ± 0.44	0.67 ± 0.25	1.23 ± 0.16	0.84 ± 0.24	0.73 ± 0.20	0.94 ± 0.20	1.16 ± 0.53	0.98 ± 0.30	0.81 ± 0.14	0.49 ± 0.22	0.35 ± 0.06
18:0	21.06 ± 1.19	25.14 ± 1.90	28.36 ± 1.22	23.70 ± 0.87	26.89 ± 2.65	27.11 ± 2.08	18.66 ± 1.44	17.68 ± 2.13	19.76 ± 0.90	23.89 ± 0.73	24.70 ± 1.77	25.74 ± 2.51
18:1 *n*-9	6.42 ± 0.91	6.02 ± 0.71	6.05 ± 0.75	6.84 ± 0.39	6.35 ± 0.71	6.33 ± 0.75	10.42 ± 0.57	9.79 ± 0.57	8.01 ± 0.69	8.75 ± 1.01	8.52 ± 0.81	7.19 ± 0.66
18:1 *n*-7	4.10 ± 0.48	3.40 ± 0.35	2.94 ± 0.57	3.88 ± 0.53	3.72 ± 0.59	3.51 ± 0.54	2.42 ± 0.23	2.86 ± 0.21	3.31 ± 0.73	2.67 ± 0.22	2.61 ± 0.33	2.43 ± 0.27
18:2 *n*-6	12.28 ± 1.40	11.36 ± 1.27	11.68 ± 0.94	12.38 ± 1.02	11.54 ± 1.31	11.75 ± 1.12	20.01 ± 1.27	19.10 ± 1.07	15.61 ± 1.68	13.14 ± 0.46	13.01 ± 0.74	12.64 ± 0.65
18:2 *n*-3	0.09 ± 0.04	0.12 ± 0.04	0.11 ± 0.05	0.08 ± 0.02	0.08 ± 0.03	0.09 ± 0.02	0.23 ± 0.05	0.20 ± 0.04	0.17 ± 0.06	0.08 ± 0.02	0.11 ± 0.04	0.09 ± 0.02
20:3 *n*-6	1.33 ± 0.17	1.23 ± 0.17	0.98 ± 0.15	0.91 ± 0.20	0.93 ± 0.12	0.70 ± 0.16	2.55 ± 0.34	2.35 ± 0.64	1.41 ± 0.20	1.34 ± 0.12	1.04 ± 0.17	0.66 ± 0.10
20:4 *n*-6	21.99 ± 0.78	22.69 ± 1.57	24.20 ± 1.23	21.77 ± 0.82	23.55 ± 1.55	24.57 ± 1.19	14.23 ± 1.22	15.78 ± 1.89	19.39 ± 1.85	21.72 ± 1.17	22.75 ± 1.77	23.60 ± 1.45
20:5 *n*-3	0.56 ± 0.08	0.68 ± 0.07	0.59 ± 0.06	0.40 ± 0.07	0.44 ± 0.06	0.48 ± 0.08	0.83 ± 0.26	0.56 ± 0.17	0.66 ± 0.11	0.37 ± 0.04	0.41 ± 0.04	0.26 ± 0.09
22:4 *n*-6	0.39 ± 0.03	0.38 ± 0.05	0.32 ± 0.02	0.32 ± 0.02	0.33 ± 0.04	0.30 ± 0.02	0.19 ± 0.03	0.24 ± 0.05	0.22 ± 0.03	0.30 ± 0.04	0.33 ± 0.02	0.33 ± 0.05
22:5 *n*-6	0.58 ± 0.11	0.74 ± 0.23	0.48 ± 0.06	0.63 ± 0.16	0.66 ± 0.17	0.39 ± 0.16	0.26 ± 0.13	0.26 ± 0.08	0.36 ± 0.17	0.34 ± 0.11	0.29 ± 0.06	0.32 ± 0.12
22:5 *n*-3	0.89 ± 0.12	0.76 ± 0.21	0.59 ± 0.08	0.54 ± 0.12	0.49 ± 0.13	0.47 ± 0.05	0.60 ± 0.13	0.65 ± 0.09	0.55 ± 0.14	0.47 ± 0.09	0.60 ± 0.03	0.48 ± 0.08
22:6 *n*-3	6.72 ± 0.88	7.04 ± 0.82	7.21 ± 0.72	6.41 ± 0.45	6.83 ± 0.77	6.26 ± 0.88	4.37 ± 0.67	5.44 ± 0.72	6.45 ± 0.74	4.72 ± 0.38	5.27 ± 0.34	5.78 ± 0.52

Data are expressed as the means of eight individual determinations ± SD. Analysis of the samples was done as described in MATERIALS AND METHODS. CT, cortisol and thyroxine treatment. CT–, CT-untreated animals. CT+, CT-treated animals. GH, growth hormone treatment; GH0, no GH treatment; GH1, low dosage treatment; GH2, high dosage treatment. Fatty acid composition of total phospholipids of liver microsomes is expressed as relative proportions of total fatty acids.

RESULTS

In order to point out statistical correlations, we show in the first two tables data obtained in this study and a previous one performed using the same animals (*15*). Table 1 summarises the hepatic microsomal membrane fatty acid compositions obtained for all groups of animals. In Table 2, the phospholipid compositions of hepatic microsomal membranes in sub-groups of animals are shown.

Effects on UGT Latency of GH and CT Treatments in Sham-Operated Animals, and Hormonal Restorations in Hypophysectomized Ones—Latency represents the percentage of constrained UGT in untreated microsomes. The latency of four UGT activities was studied in the different animal groups (Fig. 1). ANOVA on all animal groups showed that GH has a depressing effect, which is significant: $p < 0.0001$ for the BIL, TESTO and 4-NP glucuronidation latencies, and $p < 0.05$ for the ANDRO glucuronidation latency. This GH effect is more important than the effect of hypophysectomy or CT treatment.

When ANOVA was performed on sub-groups of animals (Fig. 1), the GH effect appears much more important in animals that had been treated concomitantly with CT. So, in sham-operated animals, the GH effect is significant only for TESTO glucuronidation latency with a 31% reduction with GH2 treatment ($p < 0.05$); when sham-operated animals were treated with CT, the GH effect was significant for all studied latencies. In this latter group, TESTO glucuronidation latency was reduced by 21 and 40% ($p < 0.001$), BIL glucuronidation latency by 10 and 22% ($p < 0.001$), 4-NP glucuronidation latency by 16 and 21% ($p < 0.01$) and ANDRO glucuronidation latency by 20 and 24% ($p < 0.01$) with GH1 and GH2 treatments, respectively. In hypophysectomized rats, the GH effect was significant only for BIL glucuronidation latency with a slight reduction with GH2 treatment; when hypophysectomized animals were treated concomitantly with CT, the GH effect was again significant for all studied latencies, but primarily at high dosages of GH. In this GH2-treated group, BIL glucuronidation latency was reduced by 22% ($p < 0.001$), 4-NP glucuronidation latency by 53% ($p < 0.001$), ANDRO glucuronidation latency by 34% ($p < 0.05$), and TESTO glucuronidation latency was reduced by 56% ($p < 0.01$).

Hypophysectomy had only a slight significant enhancing effect of 8% ($p < 0.05$) on BIL glucuronidation latency, which was reversed by most of the treatments used.

In sham-operated animals, the CT treatment had an increasing effect on 4-NP (+14%, $p < 0.05$) and on ANDRO (+30%, $p < 0.05$) glucuronidation latencies.

Canonical Relationships between UGT Latencies, Fatty Acid Composition and Spectroscopic Parameters of the Microsomal Membrane—In order to obtain a more comprehensive biochemical as well as structural characterization of UGT latency, canonical correlations between latencies, microsomal fatty acid proportions and membrane spectroscopic parameters were studied.

Canonical analysis shows evidence for a statistical link existing between UGT latencies and the microsomal fatty acid composition ($R^2 = 0.53$, $p = 0.0001$), and, more particularly, the MUFA proportion (Table 3). Nevertheless, the relationships between fatty acid composition and UGT latencies vary qualitatively from one substrate to another

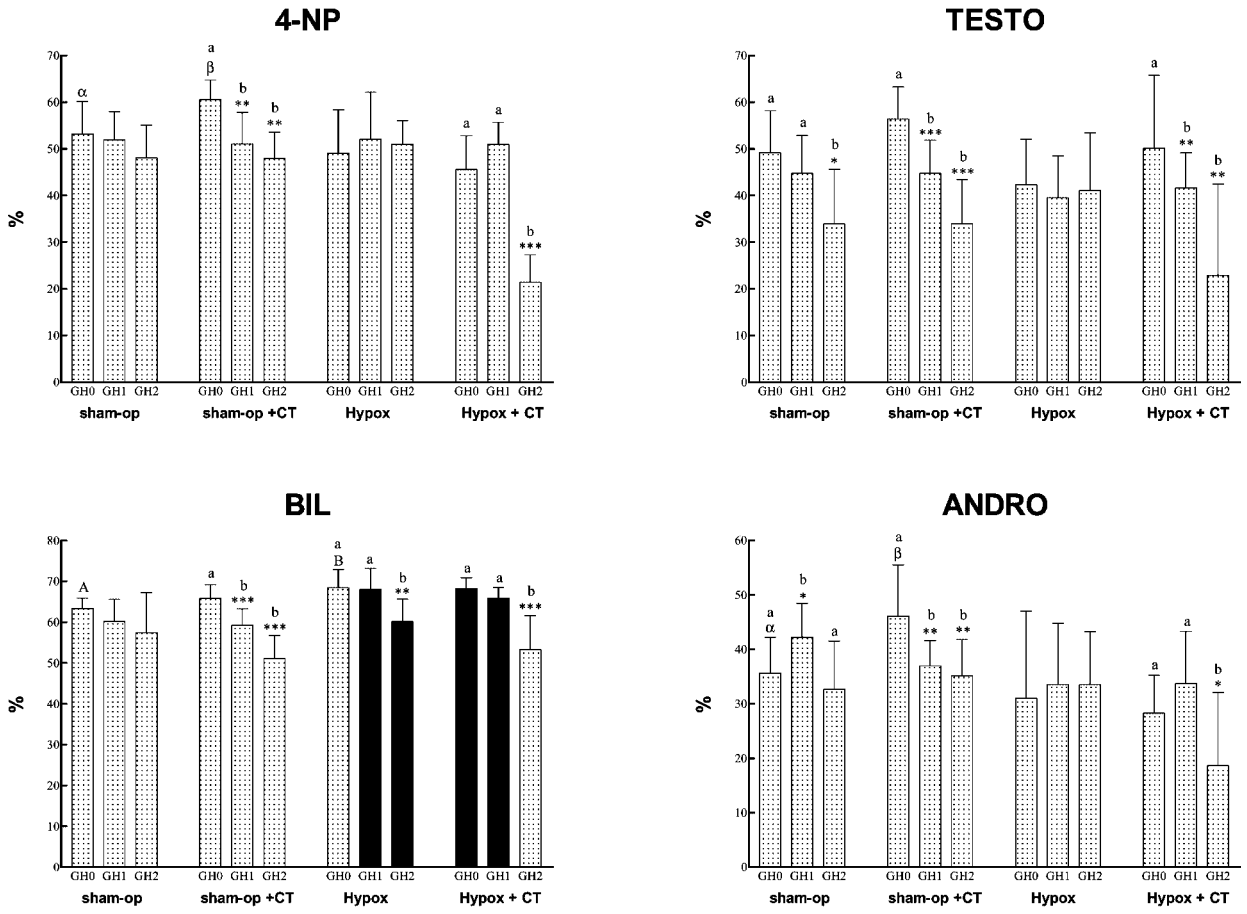

Fig. 1. **Effect of GH treatment, hypophysectomy and cortisol/thyroxine treatment on various UGT latencies.** Latencies were calculated as described in "MATERIALS AND METHODS." Values are the means of eight individual determinations ± SD. Abbreviations: CT, cortisol/thyroxine treatment; GH, growth hormone treatment; GH0, no GH treatment; GH1, low dosage treatment; GH2, high dosage treatment; hypox, hypophysectomized rats; sham-op, sham-operated rats. *, **, and *** show a significant effect of GH in the following groups of animals: sham-operated untreated rats, sham-operated CT-treated rats, hypophysectomized CT-untreated rats and hypophysectomized CT-treated rats with $p < 0.05$, $p < 0.01$, and $p < 0.001$, respectively. a and b show significant differences between GH treatments within each group at $p < 0.05$ (Student-Newman-Keuls test). α and β show a significant effect of CT treatment at $p < 0.05$. A and B show a significant effect of hypophysectomy at $p < 0.05$. Black bars represent the necessary combination of hormonal treatments of hypophysectomized rats for a restitution of the control values (statistically not different from sham-operated untreated rats) with Student-Newman-Keuls tests.

Table 2. **Phospholipid composition of hepatic microsomal membranes obtained on restrained groups of animals. Effects of GH2 and CT treatments.**

Phospholipids	Sham-operated		Sham-operated + CT		Hypophysectomized + CT	
	GH0	GH2	GH0	GH2	GH0	GH2
PC	69.6 ± 1.9	70.2 ± 4.7	57.0 ± 12.8	73.2* ± 2.6	47.8 ± 11.1	56.6 ± 9.9
PE	17.9[a] ± 4.1	15.1 ± 2.0	31.2[b] ± 9.1	15.9* ± 3.6	40.6 ± 9.3	28.6* ± 7.4
PI + PS	8.86 ± 2.74	10.7 ± 4.9	8.87 ± 4.77	7.95 ± 1.53	7.90 ± 4.68	9.16 ± 5.47
SM	2.48 ± 0.37	2.3 ± 1.18	1.68 ± 1.11	1.61 ± 0.55	1.73 ± 0.58	2.65 ± 1.35
PG	0.43 ± 0.18	0.52 ± 0.11	0.28 ± 0.08	0.31 ± 0.09	0.90 ± 0.61	0.92 ± 0.42
LPE	0.29 ± 0.13	0.43 ± 0.12	0.34 ± 0.10	0.38 ± 0.11	0.59 ± 0.38	0.79 ± 0.30
LPC	0.45 ± 0.16	0.75 ± 0.24	0.55 ± 0.20	0.61 ± 0.19	0.44 ± 0.54	1.24** ± 0.45
LPL	0.75 ± 0.28	1.18* ± 0.21	0.89 ± 0.27	0.98 ± 0.25	0.86 ± 0.20	2.03** ± 0.72

Data are expressed as the means of four individual determinations ± SD. Analysis of the samples was done as described in "MATERIAL AND METHODS." CT, cortisol and thyroxine treatment; CT–, CT-untreated animals; CT+, CT-treated animals; GH, growth hormone treatment; GH0, no GH treatment; GH2, high dosage treatment; PC, phosphatidylcholine; PE, phosphatidylethanolamine; PI, phosphatidylinositol; PS, phosphatidylserine; SM, sphingomyeline; PG, phosphatidylglycerol; LPE, lysophosphatidylethanolamine; LPC, lysophosphatidylcholine; LPL, lysophospholipids (LPE + LPC). * and ** show a significant effect of GH2 treatment with $p < 0.05$ and $p < 0.01$, respectively. a and b show a significant effect of CT with $p < 0.05$. Phospholipid composition is expressed as relative proportion (percentage) of total phospholipids.

Table 3. **Canonical analyses between the different sets of dependent variables (biophysical and latency parameters) and regressor variable sets (fatty acids proportions, fatty acid synthetic parameters, biophysical and phospholipid concentration parameters) on data obtained on all animals.**

Canonical analyses										
	Dependent variable sets									
	Biophysical parameters				Latency parameters					
Regressor variable sets	Canonical correlations (R^2) and p-value	Multiple correlations (R^2) and their p-value for dependent variable		*Most significant regressors*	Canonical correlations (R^2) and p-value	Multiple correlations (R^2) and their p-value for dependent variable				*Most significant regressors*
		5-NS	16-NS			4-NP	TESTO	BIL	ANDRO	
Fatty acid proportions										
	0.425	0.238	0.424	*1 18:1n-9*	0.525	0.417	0.388	0.483	0.337	*1 16:1n-7*
	0.0001	0.0001	0.0750	*2 22:5n-3*	0.0001	0.0001	0.0002	0.0001	0.0021	*2 18:1n-9*
	***	***	NS	*3 22:6n-3*	***	***	***	***	**	*3 16:0*
Fatty acid synthetic parameters										
	0.240	0.228	0.164	*1 DBI*	0.322	0.265	0.223	0.296	0.157	*1 MUFA*
	0.0234	0.0030	0.0404	*2 Σn-3*	0.0001	0.0005	0.0038	0.0001	0.0532	*2 DBI*
	*	**	*	*3 20:4/18:2*	***	***	**	***	NS	*3 PUFA*
										4 Σn-3
Biophysical parameters										
	–	–	–		0.049	0.0005	0.008	0.024	0.296	*1 5-NS*
	–	–	–	–	0.4919	0.8037	0.6781	0.3201	0.0774	*2 16-NS*
	–	–	–	–	NS	NS	NS	NS	NS	

Squared correlation coefficients and their p-values are given for canonical analyses as well as for multiple regressions derived from canonical analyses. The most significant regressors that explain canonical correlations are presented in descending order

(Table 4). If MUFAs correlate positively with most of the studied UGT latencies, negative correlations between latencies and fatty acids differ as a function of the UGT substrate. 4-NP glucuronidation latency is negatively correlated with the sum of the fatty acids from the *n*-6 family, TESTO glucuronidation latency is negatively correlated primarily with the PUFA proportion and the *n*-6 family, ANDRO glucuronidation latency is positively correlated with the 22:5*n*-6 proportion but not with the MUFA proportions, and BIL glucuronidation latency is negatively correlated mainly with the 18:0 and 20:4*n*-6 proportions, the 20:4*n*-6/18:2*n*-6 ratio, the double bond index (DBI) and content in PUFAs. BIL glucuronidation latency shows the greatest correlations with fatty acid composition variables, while ANDRO glucuronidation latency is far less correlated with these variables (Table 4). In fact, when partial correlations are calculated by excluding the influence of LPL content on UGT activity latencies (see below), no significant difference with the total correlations could be shown.

The statistical correlation between UGT latencies and microsomal fatty acid proportions needs to be more precisely defined in a membrane structural context since global membrane biophysical parameters, such as viscosity, are related to lipidic composition. Furthermore, the canonical analysis shows evidence for a strong relationship existing between the studied membrane spectroscopic parameters and the microsomal fatty acid composition (R^2 = 0.42, p = 0.0001) (Table 3). This validates the use of these parameters to account for the biophysical state of the membrane. Among the different treatments used in this study, hypophysectomy appears to affect those parameters in the most important way, determining a membrane rigidization with a reduction of the rotational correlation frequency and an enhancement of the order parameter measured with the 5- and the 7-NS probes (Fig. 2). The other treatment effects are mostly erratic and it is more difficult to arrive at reliable interpretations. Besides, canonical analysis done with 96 animals between UGT latencies on one hand and spectroscopic parameters measured with the 5- and 16-NS probes on the other hand shows no significant relationships between these two sets of variables (R^2 = 0.049, p = 0.49) (Table 3).

Order parameter measurements with 7- and 10-NS probes were carried out with a reduced number of animals (28 instead of 96 for 5- and 16-NS measurements). GH1-treated animals and hypophysectomized CT-untreated animals treated with GH2 were excluded from this study because of the insufficient amount of information they provide with respect to the latency study. Canonical analysis done with 28 animals between UGT latencies and spectroscopic parameters measured with the 5-, 7-, 10-, and 16-NS probes (Table 5) shows no significant correlation (R^2 = 0.578, p = 0.08) between spectroscopic parameter measurements and glucuronidation activity latencies.

Relationship between UGT Latencies and the Microsomal Phospholipid Composition—The microsomal phospholipid composition was studied in different groups of animals that showed great UGT latency variations as a function of GH treatment (24 animals). So this study was restricted to sham-operated and hypophysectomized CT-

Table 4. **Correlations between 4-NP, TESTO, BIL, and ANDRO glucuronidation activity latencies and hepatic microsomal membrane fatty acid composition.**

Fatty acids	UGT activity latency							
	4-NP		TESTO		BIL		ANDRO	
	ρ	*p*	ρ	*p*	ρ	*p*	ρ	*p*
16:0	+0.05	0.61	**+0.43**	**0.0001**	**+0.45**	**0.0001**	–0.03	0.74
16:1 *n*-7	**+0.42**	**0.0001**	**+0.35**	**0.0007**	**+0.28**	**0.006**	**+0.22**	**0.041**
18:0	–0.17	0.1	**–0.29**	**0.006**	**–0.43**	**0.0001**	+0.08	0.46
18:1 *n*-7	**+0.27**	**0.007**	+0.19	0.07	–0.09	0.35	+0.13	0.21
18:1 *n*-9	+0.04	0.67	+0.13	0.22	**+0.5**	**0.0001**	+0.02	0.84
18:2 *n*-6	+0.07	0.52	–0.01	0.89	**+0.34**	**0.0007**	–0.14	0.17
20:3 *n*-6	**+0.28**	**0.006**	+0.13	0.22	**+0.48**	**0.0001**	**–0.21**	**0.04**
20:4 *n*-6	**–0.21**	**0.03**	–0.17	0.1	**–0.5**	**0.0001**	+0.02	0.83
22:4 *n*-6	–0.06	0.55	+0.19	0.06	–0.17	0.09	+0.06	0.59
22:5 *n*-6	**+0.27**	**0.007**	+0.18	0.09	–0.08	0.44	**+0.31**	**0.003**
22:5 *n*-3	**+0.25**	**0.01**	**+0.22**	**0.04**	**+0.25**	**0.01**	**+0.07**	**0.48**
22:6 *n*-3	+0.15	0.15	–0.03	0.78	**–0.4**	**0.0001**	**+0.25**	**0.01**
Σ*n*-6	**–0.28**	**0.006**	**–0.37**	**0.0003**	**–0.21**	**0.04**	–0.18	0.08
Σ*n*-3	**+0.25**	**0.01**	**–0.01**	**0.89**	**–0.22**	**0.03**	**+0.23**	**0.03**
SFA	–0.16	0.13	+0.05	0.63	–0.19	0.07	–0.04	0.69
MUFA	**+0.23**	**0.03**	**+0.24**	**0.02**	**+0.49**	**0.0001**	+0.01	0.96
PUFA	–0.08	0.47	**–0.38**	**0.0002**	**–0.35**	**0.0005**	–0.04	0.72
20:4/18:2	–0.13	0.23	–0.07	0.5	**–0.41**	**0.0001**	–0.08	0.46
DBI	–0.03	0.75	–0.10	0.37	**–0.41**	**0.0001**	–0.08	0.43

Correlations were established with 96 animals. Fatty acid proportions and glucuronidation activity latencies were measured as described in "MATERIAL AND METHODS." ρ represents the correlation coefficient and p the probability of a null correlation. Σ*n*-6, sum of the fatty acids from the *n*-6 family; Σ*n*-3, sum of the fatty acids from the *n*-3 family; 20:4/18:2, arachidonate to linoleate ratio; SFA, saturated fatty acids; DBI, double bond index [sum (% of each unsaturated fatty acid x number of double bonds in the same fatty acid)]. Bold values indicate a significant correlation

treated rats that had been treated with GH0 or GH2. Sham-operated CT-untreated rats receiving GH0 or GH2 treatment were added to this study (Table 2). These groups were also chosen in order to get an almost continuous distribution in the UGT latency values to achieve regression studies and correlations between UGT latencies and microsomal phospholipid composition using satisfying statistical conditions.

GH2 treatment decreased the proportion of phosphatidylethanolamine (PE) in sham-operated (–49%, $p < 0.05$) and hypophysectomized (–29%, $p < 0.05$) rats that were concomitantly treated with CT (Table 2). On the contrary, GH2 treatment enhanced significantly the proportion of phosphatidylcholine (PC) only in sham-operated CT-treated rats (+28%). In sham-operated animals, CT treatment enhanced the PE proportion by 74% ($p < 0.05$).

Minor classes of microsomal phospholipids were not affected by GH treatment, except LPLs, and particularly lysophosphatidylcholine (LPC), whose proportion was enhanced by 57% in sham-operated rats and by 180% in hypophysectomized CT-treated rats.

Canonical analysis performed between microsomal phospholipid composition and UGT latencies shows evidence for a strong correlation existing between these two sets of variables ($R^2 = 0.79$, $p = 0.001$) (Table 5). Significant individual correlations were further established between all different UGT latencies and LPL proportions ($p < 0.005$) (Fig. 3). On the contrary, no significant correlation was found between these latencies and proportions in the major phospholipids. Only BIL glucuronidation latency was correlated with PE proportion ($p < 0.05$). No significant statistical relationship was found between phospholipid composition and spectroscopic parameters ($R^2 = 0.61$, $p = 0.47$) except with the 7-NS probe measurement ($R^2 = 0.58$, $p = 0.04$).

When we performed ANOVA on the latency parameter using substrates as factors, and the proportions of PUFAs and the ratio between the proportions of saturated fatty acids (SFA) and PUFAs (SFA/PUFA ratio) as covariates, it appeared that the LPL content was highly significant ($p < 0.0005$) whatever substrate considered and had prominent effects on 3 (4-NP, TESTO, and ANDRO) of the 4 substrates considered. In contrast, the effects of the covariate PUFAs and SFA/PUFA ratio were less significant ($p < 0.025$) on the latency parameter when the substrate was not taken as a specific factor in the statistical model. These effects were related to the latency for only 4-NP, TESTO and BIL when considering PUFAs, and for only 4-NP and TESTO when considering the SFA/PUFA ratio. Clearly, this means that latency can be considered to be a composite parameter that is mainly explained by the overall concentration in lysophospholipids, whatever UGT considered, but also to a lesser extent by the composition in some fatty acids when considering some specific substrates.

DISCUSSION

The present study was undertaken in order to determine whether GH, which was previously shown to differentially modify the amounts of various hepatic UGT isoforms in microsomal vesicles (*14*, *28*), can also modify

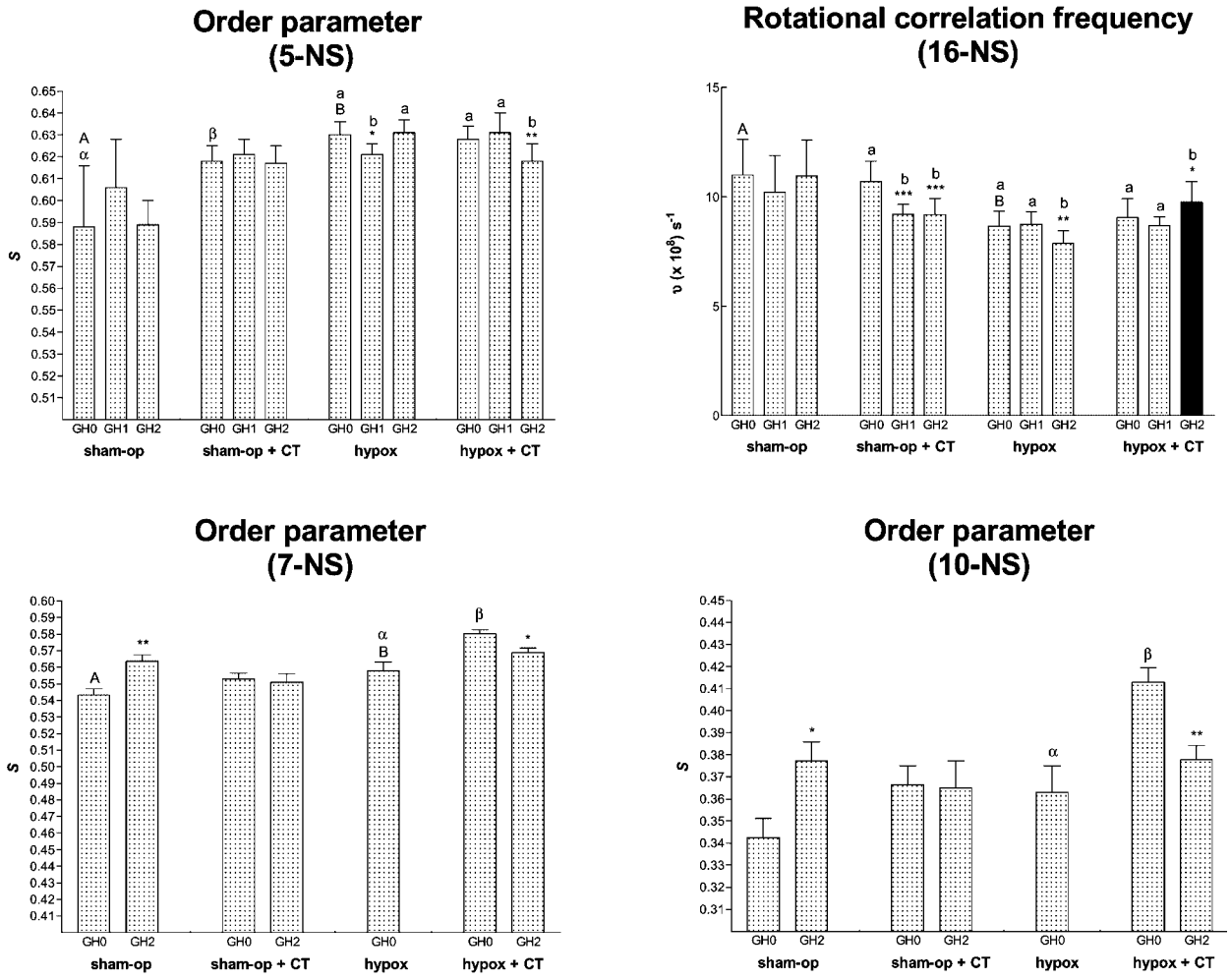

Fig. 2. **Effect of GH treatment, hypophysectomy and cortisol/thyroxine treatment on the microsomal membrane order parameter (measured at various membrane depths with various probes: 5-, 7-, and 10-NS) and the rotational correlation frequency (measured with the 16-NS probe).** Spectroscopic parameters were measured as described in "MATERIALS AND METHODS." Values are the means of eight individual determinations ± SD for the 5- and 16-NS probe measurements (96 animals); values are the means of four individual determinations ± SD for the 7- and 10-NS probe measurements (28 animals). Abbreviations: see Fig. 1.

their latency *in vivo*. Therefore, using different physiological disruptions resulting from hypophysectomy and hormonal restorations based on GH and cortisol/thyroxine treatments, a more precise analysis based on a convenient multidimensional statistical procedure can be obtained on the possible relationships existing between UGT latency, lipid composition and the membrane physical state. Two different dosages of GH were used in order to mimic either the male GH secretion pattern (GH1) in hypophysectomized rats or the female pattern (GH2) in intact male rats. Indeed, in rats the GH secretion pattern appears to be a deciding factor for the regulation of several hepatic features such as cytochromes P450 and glutathione S-tranferases (*18*, *29*, *30*), as well as for microsomal fatty acid composition (*15*).

Link between LPL Content in ER Membranes and UGT Latency—Enzyme activities were measured in both native and fully detergent-activated microsomes in order to calculate latency from the different UGT1 and UGT2 isoenzymes studied in this work. In the last decade, pore-forming agents such as alamethicin and α-toxin have been used to permeabilize microsomes instead of detergents. Using all-*trans*-retinoic acid (RA) and 5,6-epoxy-RA as UGT substrates, Little *et al.* (*31*) have obtained similar patterns of activation of UGT with Brij-58 or alamethicin, although the activities obtained with detergent were lower than with alamethicin. Under our conditions, it is possible that 4-NP, TESTO and BIL glucuronidation activities are underestimated when compared to previously published values (*32*), probably due to a partial inhibitory effect of detergent on these activities that should be revealed only by comparison with activation conditions using pore-forming agents. Nevertheless, thanks to robust statistical analyses such as ANOVA and canonical analysis, we were able to analyse unambiguously the effect of physiological factors on the variation of latency determined under detergent-activation conditions. Latency is an important functional parameter of UGTs and can be related to the broader notion of "functional potential" that was previously defined to charac-

Table 5. **Canonical analyses between the different sets of dependent variables (biophysical and latency parameters) and regressor variable sets (fatty acids proportions, fatty acid synthetic parameters, biophysical and phospholipid concentration parameters) on data obtained on a reduced number of animals chosen for a maximal range of latency variations with continuously distributed values.**

Canonical analyses	Dependent variable sets											
	Biophysical parameters						Latency parameters					
Regressor variable sets	Canonical correlations (R^2) and p-value	Multiple correlations (R^2) and their p-value for dependent variable				*Most significant regressors*	Canonical correlations (R^2) and p-value	Multiple correlations (R^2) and their p-value for dependent variable				*Most significant regressors*
		5-NS	7-NS	10-NS	16-NS			4-NP	TESTO	BIL	ANDRO	
Fatty acid proportions												
	0.935	0.737	0.871	0.541	0.691	*1 18:1n-7*	0.869	0.676	0.669	0.698	0.552	*1 16:0*
	0.0720	0.0828	0.0028	0.5503	0.1572	*2 22:5n-3*	0.1216	0.0455	0.0500	0.0295	0.2521	*2 22:6n-3*
	NS	NS	**	NS	NS	*3 22:5n-6*	NS	*	*	*	NS	*3 16:1n-9*
Fatty acid synthetic parameters												
	0.722	0.583	0.611	0.239	0.332	*1 Σn-3*	0.609	0.496	0.485	0.518	0.278	*1 Σn-3*
	0.2620	0.0150	0.0088	0.6534	0.3591	*2 SFA/PUFA*	0.0192	0.0191	0.0231	0.0124	0.3650	*2 PUFA*
	NS	*	*	NS	NS	*3 PUFA*	*	*	*	*	NS	*3 SFA/PUFA*
Biophysical parameters												
	–	–	–	–	–	–	0.578	0.319	0.120	0.088	0.296	*1 7-NS*
	–	–	–	–	–	–	0.0829	0.0560	0.5485	0.6999	0.0774	*2 5-NS*
	–	–	–	–	–	–	NS	NS	NS	NS	NS	*3 16-NS*
Phospholipid concentration												
	0.612	0.413	0.579	0.409	0.141	*1 PC*	0.789	0.520	0.785	0.662	0.436	*1 LPL*
	0.4653	0.2924	0.0429	0.3033	0.9609	*2 PG*	0.0013	0.0236	0.0001	0.0009	0.0913	*2 LPE*
	NS	NS	*	NS	NS	*3 LPL*	**	*	***	***	NS	*3 LPC*

Squared correlation coefficients and their p-values are given for canonical analyses as well as for multiple regressions derived from canonical analyses. The most significant regressors that explain canonical correlations are presented in descending order.

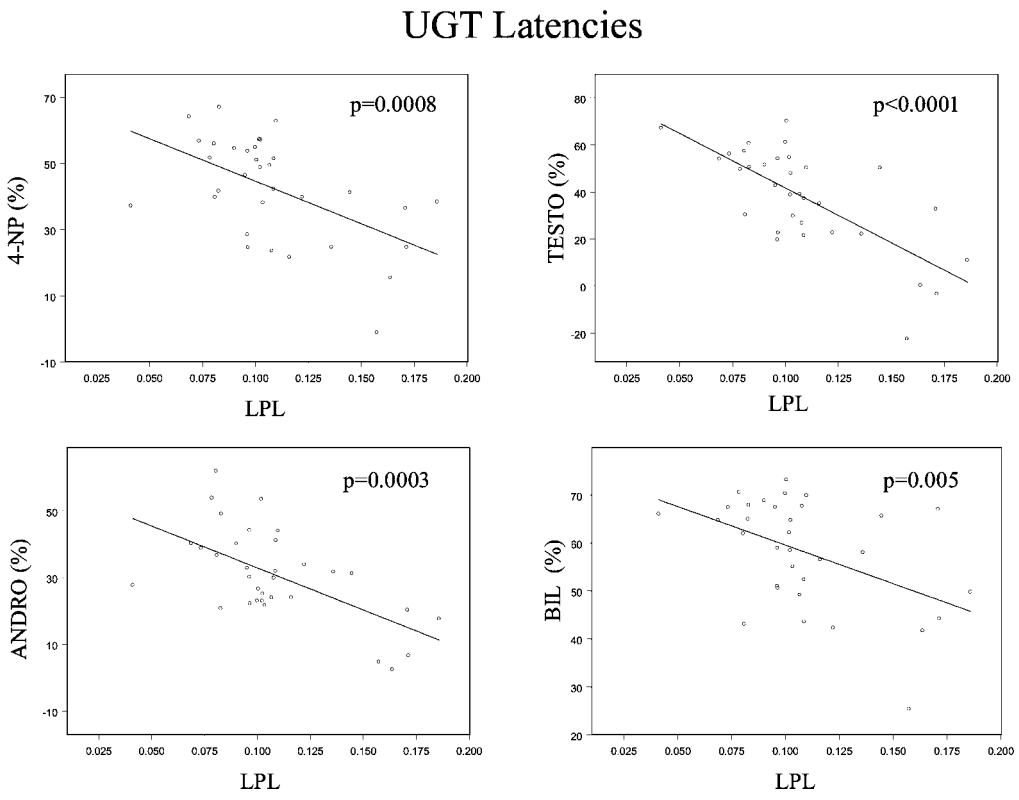

Fig. 3. **Variation of the different latencies on 4-NP, TESTO, BIL, and ANDRO UGT activities with hepatic microsomal LPL contents expressed as arsine(x) calculated variables.** The linear regressions are plotted as continuous lines.

terize membrane functioning (*33*). In microsomes, UGT activity is not fully expressed until physical or chemical treatments, such as high pressure, detergents or pore-forming agents, are used to activate these enzymes (*11*). The biochemical basis for this constraint is controversial (*11*). The compartmental model is based on the assumption that UGTs are oriented with their active sites within the lumen of the ER, thus preventing passive diffusion of UDPGA to the active site of the enzyme (*34–36*). Different authors have reported the existence of a transporter for UDPGA (*12*, *37*) that is rate-limiting for glucuronidation in native microsomes as well as in the intact ER of permeabilized hepatocytes (*38*). Such UDPGA and UDP-*N*-acetylgalactosamine transporters have recently been identified, cloned and characterized (*39*), but the involvement of the proteins in the glucuronidation of xenobiotics is still unclear. A second model assumes that UGTs can exist in different conformational states that have variable kinetic properties and are dependent on lipid-protein or protein-protein interactions (*11*). In their oligomeric model, Ikushiro *et al.* (*13*) have invoked a dimeric organization for UGTs implicated in the transport of UDPGA under native conditions.

Here, we show that GH treatment has a significant depressing effect on UGT latency, whatever activity is considered (Fig. 1). So, when compared to control, UGTs from GH-treated animals can be considered as more "active." This GH effect is dependent on the GH dosage used and is greatly amplified by parallel CT treatment. This indirect and general effect of GH on UGT activities is independent from the effect observed on the transcription of these enzymes by us (*14*) and others (*28*), and which has been shown to be isoform specific. Indeed, the GH effect on UGT latency seems to counterbalance its effect on UGT expression. For example, when bilirubin glucuronidation is considered, GH treatment decreases the bilirubin glucuronidation activity significantly by 30% in sham-operated animals under activated conditions, which implies that the activities measured under activated conditions are correlated to the amount of total UGT protein (*14*), while under native conditions, which reflect the amount of enzymes together with their functional state, this decrease is no longer significant (results not shown).

The cholesterol content was not modified by the different treatments used in these experiments, as previously shown (*15*), and, therefore, cannot be involved in latency modifications. The most evident relationship existing between latency and microsomal composition as a function of the treatment used is the inverse relationship between all the studied latencies and the LPL content of microsomes (Fig. 3). The increased LPL content in ER membranes can be related to an increase in the activity of cytosolic phospholipase A_2 ($cPLA_2$) with GH treatment, as it has been shown in hepatocyte cultures (*40*). However, the synergetic effect of CT treatment on the increase of LPL with GH treatment remains to be explained. CT treatment could interfere with LPL metabolism, possibly by preventing LPL reacylation activity (*41*).

LPL are phospholipids whose carbon chain has been hydrolyzed at the *sn*2 position and, consequently, they possess important detergent properties. For this reason,

LPL are commonly used in biochemical studies as activators of pure, delipidated UGTs (*3*, *42*) as well as UGTs embedded in microsomes (*9*). In response to GH treatment, this increase in microsomal content of LPL could easily explain the decrease in UGT latencies we have observed. However, the amount of LPC usually used for the *in vitro* activation of UGTs, which represents about 50% of the final phospholipid content in microsomal suspension (*43*), is far greater than the amount obtained in our study after GH treatment, which represents at most only 3% of the microsomal phospholipid content. LPC is known to affect bulk physical properties of the membrane *via* modifications of its curvature strain and then may influence protein-mediated functions of the membrane, as it has been shown for the functionality of the insulin receptor (*44*). In particular, LPC has a large polar head and a thin hydrophobic acyl chain tail and can be pictured as an inverted cone with its base at the polar interface (*45*, *46*) reducing the energetic cost associated with a convex monolayer membrane deformation (*47*). The UGT conformational state could then be affected by modifications of membrane physical properties. Although compounds that relieve negative curvature strain on the membrane, such as LPC, have a rather inhibitory effect on membrane functionality (*44*), LPL could nevertheless have a facilitating effect on the transport of UDPGA through the ER membrane as shown previously (*48*, *50*). Interestingly, it has been shown by preincubation of embryo extracts with phospholipase that LPL are released concomitantly with the activation of two membrane transferases: the collagen glucosyl and galactosyltransferases, which share common biochemical properties with UGTs (*51*). Probably, according to the model of UGT oligomer formation (*13*), the native UGT dimeric or oligomeric organization involved in the active transport of UDPGA could be significantly affected by a modification of the physical properties of the ER membrane due to an increase in LPC content.

Specific Link between a Given UGT Latency and the Membrane Composition in Some Specific Fatty Acids: a Minor Additional Mechanism for Modulating Glucuronidation—Beside the overall effect of LPLs on the UGT functional state regardless of the activity considered, some significant correlations do exist between a given UGT latency and the microsomal composition in specific fatty acids. If MUFAs seem to be implicated in correlation with most of the studied latencies, some specific fatty acids also have preferential relationships with only one glucuronidation latency. This relationship implicating specific fatty acids may be related to the transport/partitioning of lipophilic substrates of UGTs that diffuse across the ER membrane. So, it was previously shown that the partition of bilirubin between albumin and membrane depends on its fatty acid composition, independent of its bulk phase fluidity (*52*). Some of the substrates used in this study are glucuronidated by different UGT isoforms (TESTO, 4-NP and to a lesser extent BIL) with different biochemical characteristics. The possible functional relationship between fatty acid composition and substrate transport or diffusion kinetics through the ER membrane could then be a function of the lipophilic characteristics of the substrate, but not necessarily a function of the conformational characteristics of the UGT isoforms under study.

Nevertheless, if a correlation is well demonstrated between the latency calculated for a given UGT and the content of some specific fatty acids, this quantitative relationship is not related to parallel variations in biophysical parameters such as order parameter and rotational correlation frequency (Tables 3 and 5). Indeed, UGT latency is essentially affected by GH treatment when membrane spectroscopic parameters are primarily affected by hypophysectomy, among the treatments we used. However, as awaited, membrane spectroscopic parameters are well correlated with membrane fatty acid composition. The fact that there is no evidence for a relationship between the membrane biophysical parameters we have measured and enzyme latency has been reported by others considering the (Ca^{2+}-Mg^{2+}) ATPase (*53*). Spectroscopic parameters such as the order parameter or the rotational correlation frequency give a general idea of the biophysical state of the whole membrane but, most of the time, may not account for the biophysical state of the possible microenvironment surrounding membrane proteins such as UGTs. Such microdomains in microsomal membranes characterized by different compositions in fatty acids have been well evidenced by Leikin and Shinitzky (*54*) for the Δ6-desaturase.

In conclusion, the UGT functional state appears to be modulated by hormonal status, more particularly GH, which determines significant modifications in LPL content in hepatic ER membranes. Hence, UGT latency adjustment results mainly from LPLs, and to a considerably lesser extent from specific fatty acids. Such structural modifications could have a significant effect on the lateral pressure profile of membranes, which underlies curvature stress as theoretically studied recently by Cantor (*55*, *56*), leading to a disruption of the "curvature stress homeostasis" (*57*). This effect could then result in an alteration of the UDPGA transport towards the active site controlled under normal conditions by UGT dimers or oligomers (*13*) or by UDPGA transporters (*39*). The effects of $cPLA_2$ stimulated release of arachidonate induced by high dosages of GH on the glucuronidation activities in hepatocyte cultures is now under investigation in this laboratory.

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