0022-3565/96/2782-0921$03.00/0
THE JOURNAL OF PHARMACOLOGY AND EXPERIMENTAL THERAPEUTICS
Copyright © 1996 by The American Society for Pharmacology and Experimental Therapeutics
JPET 278:921-925, 1996

Vol. 278, No. 2
Printed in U.S.A.

Ovine Prolactin Increases Hepatic UDP-Glucuronosyltransferase Activity in Ovariectomized Rats[1]

MARCELO G. LUQUITA, VIVIANA A. CATANIA, ENRIQUE J. SÁNCHEZ-POZZI and ALDO D. MOTTINO

Instituto de Fisiología Experimental, Consejo Nacional de Investigaciones Científicas y Técnicas-Universidad Nacional de Rosario, Facultad de Ciencias Bioquímicas y Farmacéuticas, Rosario, Argentina

Accepted for publication April 15, 1996

ABSTRACT

The potential role of prolactin in modulating hepatic UDP-glucuronosyltransferase (UGT) activity was studied. Ovariectomized adult female rats were treated with ovine prolactin (oPRL) at doses of 150, 200, 260 and 310 μg/100 g b.wt. per day, for 4 days. Enzyme assays were performed in native and activated microsomes with *p*-nitrophenol as substrate. Activation was achieved either by including UDP-*N*-acetylglucosamine in the incubation mixtures or by preincubating native microsomes with palmitoyl-lysophosphatidylcholine. Data obtained with UDP-*N*-acetylglucosamine as activator showed that increasing doses of oPRL produced a progressive increase in enzyme activity up to a maximum of about 35% over basal values. Immunoblotting of microsomal protein with anti-UGT antiserum revealed also a dose-dependent increase in the immunoreactive protein. A kinetic method for measuring glucuronidating enzyme content confirmed the result of the immunoblot. oPRL induced minor changes in the physicochemical properties of the microsomal membrane. Consistent with this observation, studies performed with palmitoyl-lysophosphatidylcholine as activator showed no change in UGT latency, suggesting that the functional characteristics of the enzyme were not substantially affected by oPRL. The current data support the conclusion that prolactin may act as a modulator of UGT activity by increasing the amount of enzyme.

UGTs are a group of isozymes of 50 to 60 kDa localized primarily in the hepatic endoplasmic reticulum (Tephly and Burchell, 1990). The UGT superfamily is responsible for the glucuronidation of potentially toxic endobiotics and xenobiotics, thereby facilitating their transport to and excretion in bile (Burchell *et al.*, 1991). In rats, at least three of these isozymes are involved in glucuronidation of a wide variety of planar phenols (Burchell, 1993). Hepatic conjugation of *o*-aminophenol and *p*-nitrophenol, two of these compounds, appears to be influenced by sex hormones (Mulder, 1986) and by pregnancy and lactation (Borlakoglu *et al.*, 1993; Luquita *et al.*, 1994). The UGTs are partially constrained and have complex regulatory properties that depend, in part, on interactions between the enzyme and its membrane environment (Zakim and Dannenberg, 1992). Thus, changes in UGT activity could be associated with changes not only in the amount of enzyme but also in its functional state, with the latter being usually evaluated as the degree of enzyme constraint (latency). We found that the enhancement in *p*-nitrophenol UGT activity during lactation was due to both an increase in the number of catalytic units and an improvement in the functional state of the enzyme. Although the latter finding was also observed in nonlactating mother rats, the increase in the number of catalytic units was associated exclusively with the newborn suckling stimulus (Luquita *et al.*, 1994). This raises the possibility that a modification in prolactin levels or another mechanism related to this stimulus could enhance the amount of UGTs conjugating *p*-nitrophenol.

The present work studies the potential role of prolactin in modulating *p*-nitrophenol UGT activity. For this purpose, enzyme activity and the physicochemical properties of the membrane were evaluated in hepatic microsomes from ovariectomized rats treated with oPRL. Ovariectomized rats have been extensively used to study the regulation of prolactin secretion by the pituitary gland, as well as the effects and mechanism of action of the hormone (Vermouth and Deis, 1974; Tesone *et al.*, 1984; Moy and Lawson, 1992). Ovariectomized rats were also used as a model of post-partum rats (Liu *et al.*, 1992; Liu *et al.*, 1995), because removal of the ovaries decreases estrogen and progesterone levels (Smith and Neil, 1977) and eliminates the endogenous prolactin variations associated with the estrous cycle (Amenomori *et al.*, 1970). Evidence is presented here supporting the hypothesis that prolactin acts as a modulator of *p*-nitrophenol UGT by regulating the amount of enzyme.

Received for publication September 5, 1995.
[1] This work was supported by a research grant from Consejo Nacional de Investigaciones Científicas y Técnicas, Argentina.

ABBREVIATIONS: DPH, 1,6-diphenyl-1,3,5-hexatriene; oPRL, ovine prolactin; PLPC, palmitoyl-lysophosphatidylcholine; UDP-*N*-AG, UDP-*N*-acetylglucosamine; UDPGA, UDP-glucuronic acid; UGT, UDP-glucuronosyltransferase.

Materials and Methods

Chemicals. UDPGA (ammonium salt), UDP-*N*-AG (sodium salt), *p*-nitrophenol, D-saccharic acid-1,4-lactone, PLPC, oPRL and DPH were from Sigma Chemical Co. (St. Louis, MO). All other reagents were of the highest grade commercially available.

Animals. Adult female Wistar rats weighing 200 to 250 g were used throughout. They were fed *ad libitum* on a standard laboratory pellet diet and were allowed free access to water. All animals were ovariectomized at 14 to 15 weeks of age and randomly divided into five experimental groups: control rats (which were sacrificed 25 days after ovariectomy) and rats receiving daily s.c. doses of 150, 200, 260 and 310 μg oPRL/100 g b.wt. (PRL_1, PRL_2, PRL_3 and PRL_4 groups, respectively), injected every 8 hr for 4 consecutive days, starting 21 days after ovariectomy. The last dose was administered 4 to 5 hr before sacrifice. Animals from the control group were given injections of the vehicle for oPRL (0.9% NaCl/25 mM Tris-HCl, buffered to pH 7.6).

Isolation of microsomes. Animals were sacrificed by exsanguination and liver microsomes were obtained as previously described (Catania *et al.*, 1995). Blood samples were allowed to clot at room temperature and the corresponding sera were separated and stored frozen (−20°C) until assayed for endogenous prolactin. The protein content of microsomal preparations was determined by the biuret method (Gornall *et al.*, 1949) and then adjusted to about 12 mg/ml before enzyme assays.

Activation of microsomes with lysophosphatidylcholine. Preincubation of native microsomes with an excess of PLPC serves to abolish possible differences in membrane lipid composition between groups and was used both to evaluate UGT latency and to estimate the number of catalytic units (Dannenberg *et al.*, 1992; Catania *et al.*, 1995). For the latter purpose, V_{max} toward UDPGA was calculated in all experimental groups. The optimal concentration of PLPC was determined as described previously (Luquita *et al.*, 1994), resulting in a PLPC/protein ratio of 0.10:1 (w/w) that was used throughout this work.

Enzyme assay. Procedures to determine *p*-nitrophenol glucuronidation in native, UDP-*N*-AG-activated and PLPC-activated microsomes, as well as the kinetic study of UGT in PLPC-activated preparations, were as reported previously (Catania *et al.*, 1995).

Immunoblot analysis of microsomes. Immunoblotting was performed with antiserum raised against a phenol-conjugating isoform of UGT, as described previously (Catania *et al.*, 1995). Immunoreactive protein bands were quantified by densitometry (CS-9000; Shimadzu, Kyoto, Japan).

Lipid analysis. Microsomal lipids were extracted by the procedure of Folch *et al.* (1957). Phospholipids were separated by thin layer chromatography as described (Neskovic and Kostic, 1968). Total cholesterol was determined by the cholesterol oxidase method (Omodeo-Salè *et al.*, 1984). Lipid phosphate content was measured by the method of Chen *et al.* (1956). Fatty acid composition was determined by gas-liquid chromatography of the corresponding methyl ester derivatives (Metcalfe *et al.*, 1966), as reported previously (Luquita *et al.*, 1994).

Polarization studies. Steady-state fluorescence anisotropy of DPH (r) was measured as described previously (Luquita *et al.*, 1994).

Determination of rat prolactin. Serum prolactin was measured (Carón *et al.*, 1994) to establish whether treatment of animals with oPRL could affect endogenous hormone levels.

Statistical analysis. All data were presented as mean ± S.D. Statistical analysis was performed by using the Newman-Keuls multiple-range test (Tallarida and Murray, 1987), which includes analysis of variance. Values of $P < .05$ were considered to be statistically significant.

Results

UGT activity in UDP-*N*-AG-activated microsomes. Figure 1 shows *p*-nitrophenol UGT activities measured in the presence of UDP-*N*-AG as enzyme activator. Treatment of ovariectomized animals with oPRL produced a progressive increase in the *p*-nitrophenol-glucuronidating capacity up to the dose of 260 μg/100 g b.wt. Administration of a higher dose of oPRL (310 μg/100 g b.wt.) did not further increase enzyme activity.

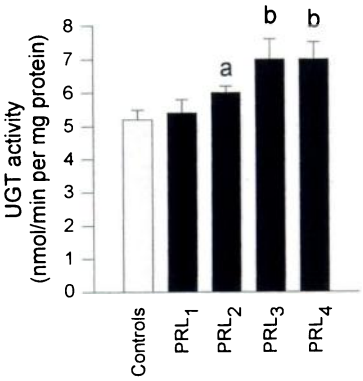

Fig. 1. *p*-Nitrophenol glucuronidation rate in ovariectomized control rats (open bar) and in ovariectomized rats receiving daily doses of 150, 200, 260 and 310 μg oPRL/100 g b.wt. (PRL_1, PRL_2, PRL_3 and PRL_4, respectively) for 4 consecutive days (closed bars). UGT assays were carried out in the presence of UDP-*N*-AG (2 mM) and fixed concentrations of *p*-nitrophenol (0.8 mM) and UDPGA (3.6 mM). Data are expressed as the mean ± S.D. of four or five rats per group. One-way analysis of variance followed by Newman-Keuls test showed a significant group effect ($P < .01$). a, significantly different from controls and PRL_1 ($P < .05$). b, significantly different from controls, PRL_1 and PRL_2 ($P < .01$).

Estimation of the amount of UGT. As shown in figure 2, immunoblot analysis revealed more immunoreactive protein in PRL_2 (about 30% increase), as well as in PRL_3 and PRL_4 (about 60% increase), compared with control females and PRL_1. As stated previously (Dannenberg *et al.*, 1992; Catania *et al.*, 1995), the antiserum used here cross-reacts with multiple isoforms of UGT. Because we were interested specifically in the isozymes conjugating *p*-nitrophenol, it was necessary to use a second approach to compare the amounts of UGT in all experimental groups. The estimation of the V_{max} at infinite UDPGA concentration, with microsomes preincubated with an excess of PLPC (which reflects the sum of all V_{max} values from the individual transferase isoforms), confirmed the results of the immunoblot. As shown in table 1, V_{max} toward UDPGA was higher in PRL_2 (about 16%) and in

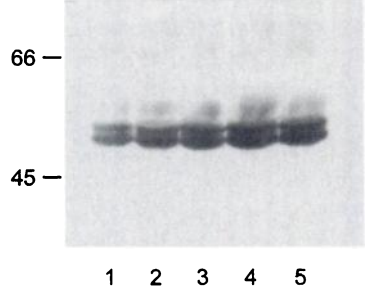

Fig. 2. Western blot analysis of the amount of UGT detected in pooled microsomes from ovariectomized control rats (lane 1) and ovariectomized rats receiving daily doses of 150, 200, 260 and 310 μg oPRL/100 g b.wt. (lanes 2 to 5, respectively) for 4 consecutive days. Equal amounts of total microsomal protein (10 μg) were loaded in all lanes. Densitometry was performed on total immunoreactive UGT as described under "Materials and Methods." The corresponding areas are as follows: controls, 1000; PRL_1, 1018; PRL_2, 1315; PRL_3, 1585; PRL_4, 1623 arbitrary units.

TABLE 1

Apparent kinetic constants toward UDPGA for control and oPRL-treated rats

Kinetic parameters for UDPGA were determined by using variable concentrations of this substrate (0.6–12.0 mM) and a fixed concentration of *p*-nitrophenol (0.8 mM). Microsomes were incubated with PLPC at a detergent/protein ratio of 0.10:1 (w/w). oPRL was administered at daily doses of 150, 200, 260 and 310 μg oPRL/100 g b.wt. (PRL_1, PRL_2, PRL_3 and PRL_4, respectively) for 4 consecutive days. Data are expressed as mean ± S.D. of four to seven rats per group. One-way analysis of variance followed by Newman-Keuls test showed a significant group effect for V_{max} ($P < .01$) but not for K_m ($P > .05$).

	Controls	PRL_1	PRL_2	PRL_3	PRL_4
V_{max} (nmol/min per mg microsomal protein)	52 ± 3	55 ± 4	60 ± 4[a,b]	70 ± 5[c]	73 ± 6[c]
K_m (mM)	1.2 ± 0.2	1.0 ± 0.1	1.2 ± 0.1	1.1 ± 0.2	1.0 ± 0.1

[a] Significantly different from controls ($P < .01$).
[b] Significantly different from PRL_1 ($P < .05$).
[c] Significantly different from controls, PRL_1 and PRL_2 ($P < .01$).

PRL_3 and PRL_4 (about 40%) than in ovariectomized females and PRL_1. K_m was unaffected by prolactin. In agreement with the UGT activities measured in UDP-*N*-AG-activated microsomes (fig. 2), the amount of enzyme (V_{max}) reached a maximum at the dose of 260 μg/100 g b.wt.

Lipid composition and UGT latency. UGT is a membrane-bound system and it is widely accepted that the physicochemical properties of the microsomal membrane represent one of the principal factors regulating its functional state (Zakim and Dannenberg, 1992). Microsomal lipid composition in the aforementioned groups is shown in table 2. The cholesterol/phospholipid molar ratio remained virtually unchanged except in the PRL_4 group, which exhibited a slight increase, compared with controls. No significant difference was found in the relative content of phosphatidylserine, phosphatidylinositol, sphingomyelin, phosphatidylcholine or phosphatidylethanolamine among groups (data not shown). Concerning total fatty acids, prolactin treatment did not significantly affect the unsaturation index, although an apparent decrease in the relative content of the 16:1 and 16:2 fatty acids was detected.

The value of fluorescence anisotropy (r) of DPH in the control group (0.110 ± 0.003) was not affected by prolactin (data not shown). This result is consistent with the slight modification detected in the lipid composition of the membranes.

Lipid composition could modulate the function of UGT *via* direct interactions of individual constituents inserted in the lipidic annulus of the enzyme. The membrane parameters studied here reflect the characteristics of the whole membrane and do not necessarily apply to the enzyme microenvironment. Therefore, it was necessary to introduce a functional study of the enzyme, such as the estimation of its latency. This parameter reflects the average degree of restriction of the isozymes involved in the conjugation of *p*-nitrophenol to express their activities and can be estimated by the percent increase in UGT activity produced by a membrane perturbant (for instance, PLPC) (Catania *et al.*, 1995). We found that oPRL treatment did not affect UGT latency (data not shown), suggesting that hormone-induced modifications in membrane lipid composition do not substantially affect the enzyme functional state.

Rat prolactin. The basal serum level of endogenous prolactin in ovariectomized rats (31 ± 9 ng/ml, $n = 3$) was not affected by the administration of oPRL. The mean hormone concentration for all treated groups was 35 ± 11 ng/ml ($n = 9$). Thus, differences between groups in UGT activity seem to be related exclusively to the different doses of oPRL. Similarly, Liu *et al.* (1992) found that continuous infusion of oPRL did not affect endogenous prolactin levels.

Discussion

Lactation increases bile flow (Klaassen and Strom, 1978; Muraca *et al.*, 1984; Liu *et al.*, 1992) and biliary excretion of bile acids and some xenobiotics (Kilpatrick *et al.*, 1980; Bolt *et al.*, 1984; Liu *et al.*, 1992) and induces changes in some Phase I (Borlakoglu *et al.*, 1993) and Phase II (Luquita *et al.*, 1994; Luquita *et al.*, 1995) detoxifying systems. The enhancement observed in *p*-nitrophenol UGT activity during lactation has

TABLE 2

Lipid composition of microsomal membranes

oPRL was administered at daily doses of 150, 200, 260 and 310 μg oPRL/100 g b.wt. (PRL_1, PRL_2, PRL_3 and PRL_4, respectively) for 4 consecutive days. Data are expressed as mean ± S.D. of four to seven rats per group. One-way analysis of variance followed by Newman-Keuls test showed a significant group effect for cholesterol/phospholipid ratio ($P < .01$) and fatty acids 16:1 and 16:2 ($P < .01$).

	Controls	PRL_1	PRL_2	PRL_3	PRL_4
Cholesterol/phospholipid ratio (mol/mol)	0.11 ± 0.01	0.12 ± 0.02	0.12 ± 0.02	0.12 ± 0.01	0.13 ± 0.01[a]
Fatty acid composition (% weight)					
16:0	18.6 ± 1.7	18.3 ± 2.9	18.5 ± 2.1	18.9 ± 2.9	19.5 ± 2.3
16:1	2.9 ± 1.0	1.4 ± 0.8[b]	1.8 ± 0.2[a]	1.9 ± 0.3[a]	1.3 ± 0.6[b]
16:2	1.8 ± 0.7	1.0 ± 0.3[b]	1.1 ± 0.2[a]	0.9 ± 0.2[b]	0.6 ± 0.1[b]
18:0	32.4 ± 1.5	31.4 ± 2.0	32.8 ± 1.5	32.1 ± 0.8	30.3 ± 2.2
18:1	6.9 ± 0.3	7.5 ± 0.3	7.4 ± 0.4	6.9 ± 0.4	7.0 ± 0.4
18:2	10.1 ± 0.8	11.9 ± 1.4	10.6 ± 1.0	10.4 ± 1.2	10.8 ± 1.2
18:3	2.5 ± 0.9	2.5 ± 0.9	2.2 ± 0.7	1.8 ± 0.5	2.1 ± 0.9
20:3	2.5 ± 0.4	2.1 ± 0.2	2.1 ± 0.6	1.8 ± 0.7	2.7 ± 0.5
20:4	21.0 ± 2.7	22.7 ± 4.8	22.1 ± 3.5	24.0 ± 2.4	24.5 ± 4.0
22:6	1.8 ± 0.7	1.4 ± 0.4	1.4 ± 0.7	1.3 ± 0.5	1.2 ± 0.6
U.I.[c]	1.36 ± 0.05	1.41 ± 0.06	1.38 ± 0.08	1.39 ± 0.07	1.43 ± 0.07

[a] Significantly different from controls ($P < .05$).
[b] Significantly different from controls ($P < .01$).
[c] U.I. (unsaturation index) = $\Sigma n_i x_i$/FA, where n_i is the number of double bonds in each fatty acid, x_i is moles of each fatty acid, and FA is total moles of fatty acid.

been attributed to both a decrease in enzyme constraint and an increase in the number of catalytic units (Luquita *et al.*, 1994). Although changes in enzyme constraint (which seemed to be associated with changes in the physicochemical properties of the microsomal membrane) were independent of lactation, modification of the amount of enzyme appeared to depend exclusively on the newborn suckling stimulus. Because serum prolactin levels are clearly increased during lactation, we studied here the effect of this hormone on hepatic *p*-nitrophenol glucuronidation.

Different approaches allowed us to clarify the action of prolactin. To estimate the actual conjugating capacity of hepatocytes, enzyme activity was first determined in microsomes activated with UDP-*N*-AG. It was suggested that UDP-*N*-AG may be a key physiological activator of the enzyme due to its modulatory effect on rate-limiting transport of UDPGA across the microsomal membrane (Berg *et al.*, 1995; Bossuyt and Blanckaert, 1995). Our data indicate that prolactin produces a dose-dependent increase in UGT activity, reaching the maximal effect at the highest doses. Due to its complex regulatory properties, changes in UGT activity may be attributed to more than one factor.

Both Western blot and PLPC analyses revealed a dose-dependent effect of oPRL on the amount of enzyme. This increase could be due to differences in rates of enzyme synthesis or degradation, which, in turn, could reflect differences in rates of gene transcription or mRNA stability. In this regard, previous reports showed that prolactin increases mRNA encoding hepatic proteins such as phosphoenolpyruvate kinase (cytosolic form) (Zabala and García-Ruiz, 1989) and Na^+/taurocholate cotransport polypeptide (Liu *et al.*, 1995). Thus, it is reasonable to speculate that a similar mechanism may be responsible for the effects of prolactin on UGT expression. Our current data also support the idea that prolactin does not modify enzyme constraint, and thus the dose-dependent improvement in UGT activity seems to be almost exclusively mediated by an increase in the number of catalytic units.

Although other factors resulting from the newborn suckling stimulus could affect UGT activity, the increase (about 40%) in V_{max} previously reported in lactating rats (Luquita *et al.*, 1994) agrees with that observed in the present work. Therefore, prolactin appears to be a principal factor regulating UGT content in hepatocytes from female rats at this reproductive stage. Although moderate, this effect could be reinforced during lactation by a less restrictive environment, as a consequence of the previous pregnancy-delivery event (Luquita *et al.*, 1994). The current data are consistent with those reported previously by Liu *et al.* (1992), describing a significant role of the hormone in regulating biliary bile acid secretion and consequently bile flow. The overall evidence supports a positive modulatory function of prolactin in postpartum hepatic elimination of xenobiotics.

Acknowledgments

We would like to thank Dr. Andrew J. Dannenberg (Department of Medicine, Cornell University Medical College, New York, NY) for providing rabbit anti-UGT antiserum and Dr. Marta Soaje, Elina Guiñazú and Dr. Ricardo P. Deis (Laboratorio de Reproducción y Lactancia, Mendoza, Argentina) for performing rat prolactin determinations. We also express our gratitude to Drs. Alejandro M. Ferri, Alejandro Viale and Joaquín V. Rodríguez and to Graciela P. Rodríguez for their invaluable assistance.

References

Amenomori, Y., Chen, C. L. and Meites, J.: Serum prolactin levels in rats during different reproductive states. Endocrinology **86:** 506–510, 1970.

Berg, C. L., Radominska, A., Lester, R. and Gollan, J. L.: Membrane translocation and regulation of uridine diphosphate-glucuronic acid uptake in rat liver microsomal vesicles. Gastroenterology **108:** 183–192, 1995.

Bolt, M. J. G., Lee, T. M. and Moltz, H.: Altered bile acid physiology during lactation in the rat. Proc. Soc. Exp. Biol. Med. **176:** 164–167, 1984.

Borlakoglu, J. T., Scott, A., Henderson, C. J. and Wolf, C. R.: Alterations in rat hepatic drug metabolism during pregnancy and lactation. Biochem. Pharmacol. **46:** 29–36, 1993.

Bossuyt, X. and Blanckaert, N.: Mechanism of stimulation of microsomal UDP-glucuronosyltransferase by UDP-*N*-acetylglucosamine. Biochem. J. **305:** 321–328, 1995.

Burchell, B.: Molecular biology of the uridine-diphosphate glucuronosyltrasferases. *In* Hepatic Transport and Bile Secretion: Physiology and Pathophysiology, ed. by N. Tavoloni and P. D. Berk, pp. 489–499, Raven Press, New York, 1993.

Burchell, B., Nebert, D. W., Nelson, D. R., Bock, K. W., Iyanagi, T., Jansen, P. L. M., Lancet, D., Mulder, G. J., Roy Chowdhury, J., Siest, G., Tephly, T. R. and Mackenzie, P. I.: The UDP-glucuronosyltransferase gene superfamily: Suggested nomenclature based on evolutionary divergence. DNA Cell Biol. **10:** 487–494, 1991.

Carón, R. W., Salicioni, A. M. and Deis, R. P.: Mifepristone treatment demonstrates the participation of adrenal glucocorticoids in the regulation of oestrogen-induced prolactin secretion in ovariectomized rats. J. Steroid Biochem. Mol. Biol. **48:** 385–389, 1994.

Catania, V. A., Dannenberg, A. J., Luquita, M. G., Sánchez Pozzi, E. J., Tucker, J. K., Yang, E. K. and Mottino, A. D.: Gender-related differences in the amount and functional state of rat liver UDP-glucuronosyltransferase. Biochem. Pharmacol. **50:** 509–514, 1995.

Chen, P. S., Toribara, T. Y. and Warner, H.: Microdetermination of phosphorus. Anal. Chem. **28:** 1756–1758, 1956.

Dannenberg, A. J., Worman, H. J. and Scarlata, S.: Developmental changes in the amount and functional state of UDP-glucuronosyltransferase. Biochim. Biophys. Acta **1116:** 250–255, 1992.

Folch, J., Less, M. and Sloane-Stanley, G. H.: A simple method for the isolation and purification of total lipids from animal tissues. J. Biol. Chem. **226:** 497–509, 1957.

Gornall, A. G., Bardawill, C. J. and David, M. M.: Determination of serum proteins by means of the biuret reaction. J. Biol. Chem. **177:** 751–766, 1949.

Kilpatrick, S. J., Bolt, M. and Moltz, H.: The maternal pheromone and bile acids in the lactating rat. Pharmacol. Biochem. Behav. **12:** 555–558, 1980.

Klaassen, C. D. and Strom, S. C.: Comparison of biliary excretory function and bile composition in male, female and lactating female rats. Drug Metab. Dispos. **6:** 120–124, 1978.

Liu, Y., Ganguly, T., Hyde, J. F. and Vore, M.: Prolactin increases mRNA encoding Na^+-TC cotransport polypeptide and hepatic Na^+-TC cotransport. Am. J. Physiol. **268:** G11–G17, 1995.

Liu, Y., Hyde, J. F. and Vore, M.: Prolactin regulates maternal bile secretory function *post partum*. J. Pharmacol. Exp. Ther. **261:** 560–566, 1992.

Luquita, M. G., Catania, V. A., Sánchez Pozzi, E. J. and Mottino, A. D.: Enhancement of cytosolic and microsomal glutathione *S*-transferase activities in liver and small intestine from female rats during lactation. Pharmacol. Toxicol. **76:** 72–75, 1995.

Luquita, M. G., Sánchez Pozzi, E. J., Catania, V. A. and Mottino, A. D.: Analysis of *p*-nitrophenol glucuronidation in hepatic microsomes from lactating rats. Biochem. Pharmacol. **47:** 1179–1185, 1994.

Metcalfe, L. D., Schmitz, A. A. and Pelka, J. R.: Rapid preparation of fatty acid esters from lipids for gas chromatographic analysis. Anal. Chem. **38:** 514–515, 1966.

Moy, J. and Lawson, D.: Temporal effects of estradiol and diethylstilbestrol on pituitary and plasma prolactin levels in ovariectomized Fischer 344 and Holtzman rats: A comparison of radioimmunoassay and Nb2 lymphoma cell bioassay. Proc. Soc. Exp. Biol. Med. **200:** 507–513, 1992.

Mulder, G. J.: Sex differences in drug conjugation and their consequences for drug toxicity: Sulfation, glucuronidation and glutathione conjugation. Chem. Biol. Interact. **57:** 1–15, 1986.

Muraca, M., Leyten, R. and Fevery, J.: Conjugation and maximal biliary excretion of bilirubin in the rat during pregnancy and lactation and during estroprogestogen treatment. Hepatology **4:** 633–638, 1984.

Neskovic, N. and Kostic, D.: Quantitative analysis of rat liver phospholipids by a two-step thin-layer chromatographic procedure. J. Chromatogr. **35:** 297–300, 1968.

Omodeo-Salé, F., Marchesini, S., Fichman, P. H. and Berra, B.: A sensitive enzymatic assay of determination of cholesterol in lipid extracts. Anal. Biochem. **142:** 347–350, 1984.

Smith, M. S. and Neil, J. D.: Inhibition of gonadotropin secretion during lactation in the rat: Relative contribution of suckling and ovarian steroids. Biol. Reprod. **17:** 255–261, 1977.

TALLARIDA, R. J. AND MURRAY, R. B.: Manual of Pharmacologic Calculations with Computer Programs, Springer-Verlag, New York, 1987.

TEPHLY, T. R. AND BURCHELL, B.: UDP-glucuronosyltransferases: A family of detoxifying enzymes. Trends Pharmacol. Sci. **11:** 276–279, 1990.

TESONE, M., LÜTHY, I. A., LADENHEIM, R. G., CALANDRA, R. S. AND CHARREAU, E. H.: Prolactin regulation of prolactin binding sites in pancreatic islets and adrenal glands of ovariectomized rats. J. Recept. Res. **3:** 711–726, 1984.

VERMOUTH, N. T. AND DEIS, R. P.: Prolactin release and lactogenesis after ovariectomy in pregnant rats: Effect of ovarian hormones. J. Endocrinol. **63:** 13–20, 1974.

ZABALA, M. T. AND GARCÍA-RUIZ, J. P.: Regulation of expression of the messenger ribonucleic acid encoding the cytosolic form of phosphoenolpyruvate carboxykinase in liver and small intestine of lactating rats. Endocrinology **125:** 2587–2593, 1989.

ZAKIM, D. AND DANNENBERG, A. J.: How does the microsomal membrane regulate UDP-glucuronosyltransferases? Biochem. Pharmacol. **43:** 1385–1393, 1992.

Send reprint requests to: Dr. Aldo D. Mottino, Instituto de Fisiología Experimental, CONICET-U.N.R., Facultad de Ciencias Bioquímicas y Farmacéuticas, Suipacha 570, 2000 Rosario, Argentina.