Pergamon

Biochemical Pharmacology, Vol. 49, No. 4, pp. 503–509, 1995.

0006-2952/95 $9.50 + 0.00

0006-2952(94)00434-X

HORMONAL INFLUENCES OF DETOXICATION IN THE RAT OVARY ON ENZYMES IN COMPARISON WITH THE LIVER

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(*Received* 30 *May* 1994; *accepted* 12 *September* 1994)

Abstract—Variations in the total capacity of the rat ovary to metabolize xenobiotics during different phases of the estrous cycle were studied. The level of the conjugating enzymes, phenol UDP-glucuronosyltransferase (pUDPGT; EC 2.4.1.17), phenol sulfotransferase (pST; EC 2.8.2.1) and glutathione transferases (EC 2.5.1.18) was determined in the ovary and compared with the corresponding hepatic activities. In addition, catalase (EC 1.11.1.6) and NAD(P)H: quinone oxidoreductase (EC 1.6.99.2) two other detoxifying enzymes, were assayed. In order to study the hormonal influences on detoxifying enzymes, mature rats were characterized with respect to their stage in the estrous cycle. Immature rats were treated with pregnant mare's serum gonadotropin (PMSG) for 2 or 3 days to enrich the ovaries in preovulatory follicles or corpora lutea, respectively. The present study demonstrates that ovarian pUDPGT and pST activities are increased 936% and 175%, respectively, in ovaries enriched in corpora lutea compared to ovaries from untreated immature rats. Increases in these activities in mature rats during the metestrous stage of the estrous cycle compared to the proestrous stage were also noted. In the liver pUDPGT activity is increased significantly (1.6-fold) in immature rats with ovaries enriched in preovulatory follicles compared to untreated rats. Both ovarian pST and pUDPGT activities increased in mature rats treated with PMSG ("hyperstimulated"), while in the liver only pST was increased by such treatment. Ovarian glutathione transferase activity proved not to be dependent on the hormonal fluctuations associated with the estrous cycle. However, in the liver of mature rats treated with PMSG, this activity increased 2-fold compared to the untreated immature rats. The catalase activity found in the ovarian mitochondrial fraction was approx. 10-fold higher than in the cytosolic fraction, independent of the hormonal status. Moreover, we found a significant 1.4-fold increase in peroxisomal catalase activity in the mitochondrial fraction of immature rats treated with PMSG, both when enriched in preovulatory follicles and in corpora lutea. In the liver cytosolic catalase activity decreased several-fold in immature rats following PMSG treatment. We did not find any variations in ovarian NAD(P)H: quinone oxidoreductase activity during the estrous cycle, whereas in the liver this activity decreased in the luteal phase, as it did in mature rats treated with PMSG. From this study and earlier investigations in our laboratory, we conclude that cyclic variations due to hormones of the estrous cycle of the major 7,12-dimethylbenz(a)anthracene (DMBA)-metabolizing phase I enzymes in the ovary are not accompanied by increases in the activities of the corresponding phase II enzymes. An increased steady-state level of reactive intermediates around the time of ovulation may thus increase the risk of cellular damage, e.g. in the oocyte, during this period.

Key words: ovary; estrous cycle; gonadotropin; liver; detoxication; conjugation

Xenobiotic metabolizing enzymes play an important role in the biotransformation of hydrophobic compounds to more hydrophilic substances which can be excreted from the body. The first step in this transformation, i.e. bioactivation, is often carried out by the microsomal cytochrome P-450-dependent monooxygenase system. The products formed are in general more reactive than the parent substance itself. For the deactivation of these products, several detoxication enzymes, called phase II enzymes, are required. These phase II enzymes render the products more hydrophilic by conjugation and/or hydrolysis [1].

It has previously been reported by this laboratory that the activity of rat ovarian cytochrome P-450-dependent DMBA† monooxygenase activity is regulated by gonadotropic hormones [2]. Moreover, we have shown that ovarian granulosa cells exposed to DMBA produce reactive metabolites able to cause mutations in neighbouring cells [3]. Together, these results indicate that an increase in phase I enzyme activity is not counterbalanced by an increase in phase II enzyme activities.

The ovary is a target for the gonadotropic hormones produced by the pituatary gland: FSH

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† Abbreviations: pUDPGT, phenol UDP-glucuronosyltransferase; pST, phenol sulfotransferase; PMSG, pregnant mare's serum gonadotropin; DMBA, 7,12-dimethylbenz(a)anthracene; FSH, follicle stimulating hormone; LH, luteinizing hormone; UDPGA, UDP-glucuronic acid; UDPGT, UDP-glucuronosyltransferase; PAPS, 3′-phosphoadenosine 5′-phosphosulfate; CHAPS, 3-[(3-cholamidopropyl) dimethylammonio]-1-propane sulfonate; CDNB, 1-chloro-2,4-dinitrobenzene; PAH, polycyclic aromatic hydrocarbon; CYP11A1, cytochrome P450 side-chain cleavage; DHEA ST, dehydroepiandrosterone sulfotransferase; ACTH, adrenocorticotropic hormone; ST, sulfotransferase.

and LH [4]. FSH stimulates the biosynthesis of estrogen and the induction of LH receptors in the ovary. The function of LH is to stimulate the final maturation and the rupture of the follicle prior to ovulation and the formation of the corpus luteum, which synthesizes progesterone. During the estrous cycle the concentrations of the gonadotropins and of steroid hormones in the ovary vary in a cyclic manner [5, 6]. In the present investigation we have studied the possible influence of these gonadotropins and steroid hormones on phase II enzyme activities. The rats were treated with PMSG, which exhibits the biological activities of both FSH and LH (1:1) [7]. While the ovary synthesizes steroids from cholesterol, the liver is the major organ with respect to further metabolism and inactivation of these hormones.

One major pathway of xenobiotic conjugation involves glucuronidation by UDPGT [8]. The affinity of this enzyme for most substrates is not very high, but its maximal capacity is pronounced. In contrast, a conjugation enzyme demonstrating low maximal activity, but high affinity for its substrate is ST [9]. Sulfoconjugation is the major form of conjugation of many small xenobiotics and endogenous compounds, such as steroids and lipids [9]. STs also play an important role in the regulation of the concentrations of free steroid hormones in cells. STs catalyse the sulfation of steroids with different substrate specificites. Steroid sulfates demonstrate a lower affinity for their receptors than do free steroids [10].

There are multilple forms of UDPGTs and STs with different substrate affinities [11, 12]. In this work we have studied pUDPGT and pST in the ovary during the estrous cycle of the rat, as well as after exogenous administration of gonadotropin (i.e. PMSG).

Another important conjugation pathway is that catalysed by the glutathione transferases. Glutathione transferases form thioesters between electrophilic centres in xenobiotics and/or their metabolites and the endogenous nucleophil glutathione [13]. Glutathione transferase can also metabolize endogenous compounds, such as steroid hormones [14]. It is known that the levels of certain isozymes of glutathione transferase are hormonally regulated in rat liver and adrenal glands. Staffas *et al.* showed that in the liver, glutathione transferase activity towards CDNB increases in female rats after hypophysectomy compared to control animals. This activity is decreased almost to control levels by treatment with thyroxine, cortisone and growth hormone [15]. In adrenals the glutathione transferase subunit 4 is increased after hypophysectomy and down-regulated by ACTH. The up-regulation of this subunit by hypophysectomy also occurs in liver, although to a lesser extent, and is not down-regulated by ACTH [16].

Reactive species of oxygen are formed during ovulation. Such species may be released in connection with follicle rupture, since they are necessary for ovulation [17]. Reactive oxygen can also be generated by the cytochrome P450 system. Catalase plays an important role in protecting the cell from oxygen radicals. In granulosa cells from porcine ovaries, Peterson *et al.* found an increase in catalase activity during the luteal phase [18]. It has also been shown that in the post-mitochondrial pellet (containing peroxisomes) of the whole rat ovary, catalase activity increases upon PMSG treatment [18].

The metabolism of xenobiotics by cytochrome P450-dependent monooxygenases also produces reactive quinones. NAD(P)H: quinone oxidoreductase is able to reduce such quinones to relatively stable hydroquinones which are subsequently conjugated by UDPGT and ST [19, 20].

The aim of the present study has been to examine the influence of the hormones of the estrous cycle, e.g. gonadotropins and estrogens, on the balance between the bioactivating (phase I) and detoxicating (phase II) enzymes in the rat ovary. An enhancement of the ratio phase I to phase II activities may increase the steady-state level of reactive intermediates in the cell and thus increase the risk of cytotoxicity and/or mutagenicity. The ovarian phase II enzyme activities investigated here were: pUDPGT, pST, glutathione transferases, catalase and NAD(P)H: quinone oxidoreductase. These enzymes were studied during the different phases of the estrous cycle, and/or upon treatment with gonadotropic hormones. For comparison, these enzyme activities were also measured in the liver.

MATERIALS AND METHODS

Chemicals. PMSG, 1-[1-^{14}C]naphthol (7.1 mCi/mmol), UDPGA, PAPS, dithiothreitol, cytochrome c, dicoumarol, Triton X-100 and BSA were purchased from Sigma Chemical Co. (St Louis, MO, U.S.A.). CHAPS and NADPH were from Boehringer Mannheim (Bromma, Sweden). CDNB, hydrogen peroxide, menadione and Folin-ciocalteaus Phenolreagenz were from E. Merck (Darmstadt, F.R.G.). All other chemicals were obtained from frequently used local suppliers.

Animals and treatment. Immature (26-day-old) female Sprague–Dawley rats were treated with PMSG as described previously [21], to synchronize rats in their preovulatory and luteal phases.

Determination of the stage in the estrous cycle was performed by daily observations of vaginal smears [2]. Progesterone concentrations were measured by radioimmunoassay (Diagnostic Products Corporation, Los Angeles, CA, U.S.A.) in the serum of all rats (data not shown).

Mature rats (230 g) were treated with PMSG to increase the proportion of large follicles ("hyperstimulated") [22].

All rats were housed under controlled temperature, humidity and light conditions. The rats were killed between 8 and 10 a.m. by cervical fracture. The ovaries and livers were immediately removed. The ovaries were placed in ice-cold 150 mM KCl, 1 mM EDTA and 20 mM Tris–HCl (pH 7.6) and were pooled, weighed and homogenized. Livers were handled similarly, except that they were put into ice-cold 0.25 M sucrose and homogenized individually.

Subcellular fractionation of ovaries and livers. The homogenates were centrifuged at 900 g_{av} for 10 min to sediment nuclei and debris. The resulting ovary and liver supernatant fractions were then centrifuged

Table 1. Effects of PMSG on the specific activities of microsomal phenol UDP-glucuronosyltransferase and cytosolic phenol sulfotransferase in ovaries and livers of female rats

Organ	State of maturity	Hormonal state	pUDPGT	pST
Ovary	Immature	Untreated	100 ± 35	100 ± 0.18
		Preovulatory	567 ± 46***	112 ± 8.2
		Luteal	936 ± 70***	175 ± 23*
Liver	Immature	Untreated	100 ± 6.5	100 ± 2.0
		Preovulatory	167 ± 2.8***	112 ± 13
		Luteal	136 ± 37	129 ± 33
	Mature	"Hyperstimulated"†	98.1 ± 19	255 ± 6.8***

The activities of the different hormonal stages are compared with those of the immature rats (100%), which were 0.23 nmol/min/mg protein for pUDPGT and 5.10–11 pmol/min/mg protein for pST in the ovary. In the liver the specific activities for pUDPGT in immature rats was 15.3 nmol/min/mg protein and for pST 55.6 pmol/min/mg protein.

All values are means ± SD. In the ovary, the means of pUDPGT are triplicates from one preparation and the values of pST are from at least two different preparations. Each preparation involves 15 animals. In the liver, the values show means for three animals.

* $P < 0.05$; *** $P < 0.001$ compared to the value for the immature group. †PMSG-treated rats.

at 9500 g_{av} and 13,300 g_{av}, respectively, for 10 min to yield a mitochondrial fraction. These mitochondrial pellets were washed, resuspended in buffer and used to measure peroxisomal catalase. In order to obtain cytosolic and microsomal fractions, the post-mitochondrial supernatants were centrifuged at 100,000 g_{av} in the case of the ovary and at 105,000 g_{av} in the liver. The cytosolic fractions were used in assays for pST, glutathione transferases, catalase and NAD(P)H:quinone oxidoreductase.

The ovarian microsomal pellet was washed once and resuspended in the same buffer. The microsomal pellet from the liver was washed and resuspended in 150 mM Tris–Cl (pH 8.0). UDPGT was measured in these fractions.

Samples were stored at −70° until use (no longer than 6 months).

Enzyme assays. The following enzyme activities were measured under optimal conditions with respect to saturating substrate concentration, cofactor concentrations, pH and temperature and in the linear range in regard to protein amount and time of incubation. These conditions were determined here for the ovary and taken from appropriate reports in the literature in the case of liver.

Microsomal pUDPGT activity was measured radiometrically according to Hjelle and Otani [12, 23]. A protein concentration in the range of 0.5–1.0 mg and a final substrate concentration of 500 μM [^{14}C]-1-naphthol (0.04 μCi), with 6.25 mM saccharic acid-1,4-lactone and 5 mM $MgCl_2$ in 100 mM Tris–HCl and 8 mM CHAPS, pH 7.4, were employed. The reaction was started by 3 mM UDPGA and stopped by the addition of 0.13 M trichloroacetic acid and 0.2 M glycine (pH 2.7). Sulfation of 1-naphthol was measured using a method described by Hjelle [12] and Bamforth [24], with certain modifications. Ovarian or liver protein (0.5–1.0 mg) was incubated with 11 μM [^{14}C]-1-naphthol (0.04 μCi) and 17 μM PAPS (final concentration) in a 5 mM potassium phosphate buffer containing 5 mM dithiothreitol (pH 6.5) at 37°. The assay was terminated by addition of 0.13 M trichloroacetic acid and 0.2 M glycine (pH 2.7). The mixture was then extracted once with chloroform and the radioactivity in the aqueous and chloroform phases determined [24]. Cytosolic glutathione transferases were measured spectrophotometrically with CDNB as substrate according to Habig *et al.* [25]. Peroxisomal and cytosolic catalase activities were measured as described by Bergmeyer *et al.* [26]. Cytosolic NAD(P)H:quinone (accepting) oxidoreductase activity was monitored spectrophotometrically by following the reduction of cytochrome c, with menadione as electron acceptor [27].

Protein concentrations were determined using the method of Lowry *et al.* [28], with BSA as standard.

Data are expressed as means ± SD. Analysis of statistical significance was performed using Student's *t*-test.

RESULTS

Microsomal pUDPGT activity during the estrous cycle and upon administration of gonadotropic hormones

The present results are the first documentation of the specific activities of pUDPGT and pST in the ovary. Thus, the optimal assay conditions for ovarian microsomal pUDPGT were determined using mature rats treated with PMSG (data not shown).

The specific activity of ovarian pUDPGT in immature rats with and without treatment of gonadotropins is shown in Table 1. This activity was found to be increased 5.7-fold in ovaries enriched in preovulatory follicles and 9.4-fold in the luteal phase.

In Fig. 1 ovarian pUDPGT activities of mature rats during different phases of the estrous cycle and after treatment with PMSG are shown. The activities

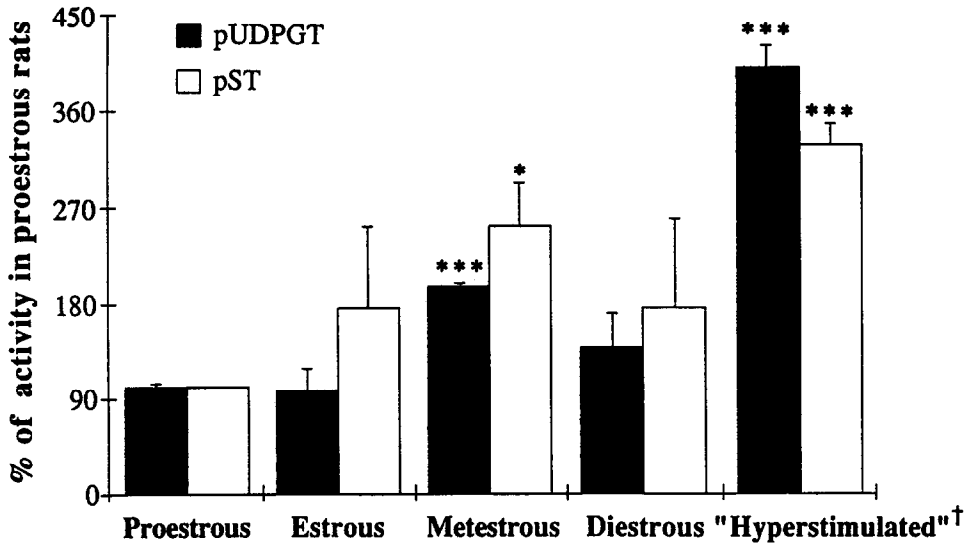

Fig. 1. Ovarian microsomal pUDPGT and cytosolic pST activities in mature rats in the different phases of the estrous cycle and after gonadotropin treatment. Activities in the different hormonal states are compared with those in proestrous (100%), which was 0.36–0.55 nmol/min mg protein for pUDPGT and 1.09–5.60 pmol/min mg protein for pST. All values are means ± SD from three preparations in the case of pUDPGT and two different preparations in the case of pST. Each preparation involves at least three animals. *P < 0.05; ***P < 0.001 compared to the value for the proestrous group. †PMSG-treated rats.

are presented as percentages of the activity in the proestrous phase, which was 0.36–0.55 nmol/min/mg protein, i.e. of the same order of magnitude as in immature rats. There was a 2.0-fold increase in pUDPGT activity during the metestrous phase, i.e. in the luteal phase. The activity of pUDPGT in mature rats treated with PMSG increased significantly (approx. 4.0-fold) compared to that in the proestrous phase and approx. 2-fold compared to the highest endogenous activity.

In Table 1 the specific activities of pUDPGT in the liver are shown and compared to the corresponding activities in immature rats. We found a 1.7-fold increase in the hepatic UDPGT activity of immature rats treated with PMSG, i.e. in the preovulatory phase of the estrous cycle.

Cytosolic pST activity during the estrous cycle and upon administration of gonadotropic hormones

The optimal conditions for ovarian cytosolic pST were determined using mature rats treated with PMSG (not shown).

Table 1 shows the cytosolic pST activity in the ovary of immature rats stimulated by gonadotropic hormones. Specific activities are expressed as percentages of the activity of immature rat ovaries, which was 5.1–11 pmol/min/mg protein. There was a 1.8-fold increase in ovarian pST activity in immature rats 3 days after PMSG treatment, i.e. in the corpora lutea, whereas ovaries enriched in preovulatory follicles showed a 1.1-fold increase in this activity.

Ovarian pST activities in mature rats stimulated with gonadotropins during the estrous cycle are shown in Fig. 1. The activities are expressed as percentage of the corresponding activity in the proestrous phase, which is 1.1–5.6 pmol/min/mg protein, i.e. substantially lower than in immature rats. Furthermore, rats in the proestrous phase exhibited between 2.5-fold and 1.7-fold lower specific activity than during the rest of the estrous cycle (Fig. 1). pST was increased after ovulation and, thus, after the LH peak both in immature rats stimulated by gonadotropins and in untreated mature rats. The activity of pST in PMSG-treated rats was 3.3-fold higher than during the proestrous phase, but only 1.4-fold higher than during the rest of the estrous cycle.

In Table 1 the specific activities of pST in the liver are shown. No change was seen in immature rats treated with gonadotropins; however, there was a 2.6-fold increase in mature rats treated with PMSG.

Cytosolic glutathione transferase and NAD(P)H: quinone oxidoreductase activities upon treatment with gonadotropic hormones

Ovarian glutathione transferase activity was unchanged during the different hormonal states examined, which is consistent with previous findings [2], whereas in the liver there was a 2.2-fold increase in cytosolic glutathione transferase activity towards CDNB in mature rats treated with PMSG (Table 2).

The specific ovarian NAD(P)H:quinone oxidoreductase activity in PMSG-treated immature and mature rats was unchanged compared with that of untreated immature rats, consistent with the results previously shown [2]. In the liver there was a 2.7-fold decrease in this enzyme activity after ovulation,

Table 2. Effects of PMSG on cytosolic glutathione transferase and NAD(P)H:quinone oxidoreductase activities in ovaries and livers female rat

Organ	State of maturity	Hormonal state	Glutathione transferase	NAD(P)H:quinone oxidoreductase
			(μmol/min/mg)	
Ovary	Immature	Untreated	0.52 ± 0.04	0.12 ± 0.01
		Preovulatory	0.53 ± 0.01	0.12 ± 0.02
		Luteal	0.52 ± 0.01	0.10 ± 0.01
	Mature	"Hyperstimulated"†	0.41 ± 0.04	0.18 ± 0.02
Liver	Immature	Untreated	0.58 ± 0.07	0.54 ± 0.09
		Preovulatory	0.64 ± 0.03	0.36 ± 0.04
		Luteal	0.72 ± 0.09	0.20 ± 0.06*
	Mature	"Hyperstimulated"†	1.30 ± 0.16**	0.28 ± 0.01*

All values are means ± SD. The means in the ovary were calculated from at least two different subcellular preparations each involving at least 15 animals. The values for the liver were from three animals.

* $P < 0.05$; ** $P < 0.01$ compared to the immature group. †PMSG-treated rats.

Table 3. Effects of PMSG on cytosolic and peroxisomal catalase activities in ovaries and livers of female rats

Organ	State of maturity	Hormonal state	Catalase (μmol/min/mg) Cytosolic	Peroxisomal
Ovary	Immature	Untreated	9.99 ± 1.1	96.0 ± 15
		Preovulatory	9.57 ± 0.6	129 ± 9.0*
		Luteal	9.61 ± 0.3	130 ± 5.0*
	Mature	"Hyperstimulated"†	8.20 ± 2.8	113 ± 2.0
Liver	Immature	Untreated	256 ± 20	1080 ± 170
		Preovulatory	88.0 ± 21***	1240 ± 38
		Luteal	91.0 ± 26***	1230 ± 280
	Mature	"Hyperstimulated"†	108 ± 39***	1130 ± 270

All values are means ± SD. The means in the ovary were calculated from at least two different subcellular preparations each involving at least 15 animals. The values for the liver were from three animals.

* $P < 0.05$; *** $P < 0.001$ compared to the value for the immature group. †PMSG-treated rats.

i.e. in the luteal phase. There was also a decrease in this activity in mature rats stimulated with PMSG (Table 2).

Effects of gonadotropins on cytosolic and peroxisomal catalase activities

The specific activities of cytosolic and peroxisomal catalase are shown in Table 3. No differences in ovarian catalase activities were found in connection with the different hormonal states. It can be seen in Table 3 that the specific activity of catalase in the mitochondrial fraction was higher than that of the cytosolic fractions in all of the groups.

In the liver cytosolic catalase activity decreased in the preovulatory follicle and corpus luteum groups by 3-fold and 2-fold, respectively, compared with immature rats, whereas peroxisomal catalase activities were unchanged. Hepatic catalase activities were also higher in peroxisomes than in the cytosol in all groups.

DISCUSSION

An investigation of the influence of hormones on enzymes involved in xenobiotic metabolism in the rat ovary and, especially, on enzymes involved in the metabolism of products formed of the microsomal cytochrome P450-dependent monooxygenase system has been performed here. For comparison, the corresponding activities were assayed in liver the main site for metabolism of xenobiotics as well as of steroid hormones.

pUDPGT and pST are two of the most quantitatively important conjugation enzymes involved in detoxication. In this study we found that in the ovary these enzymes are regulated by the hormones participating in the estrous cycle of the rat. We detected an increase in the activities of these conjugating enzymes in ovaries enriched in corpora lutea, i.e. in the period after the endogenous LH peak when the concentration of progesterone is maximal.

As previously shown, DMBA-metabolizing cytochrome P450-dependent monooxygenase activity in rat ovary is increased during the proestrous and estrous phases, as well as by exogenous gonadotropin stimulation [22, 29]. The present investigation demonstrates that conjugating enzyme activities are higher during the luteal phase, i.e. the metestrous phase. This means that there is no coordinate hormonal regulation on phase I activation activity and the phase II activities pST and pUDPGT.

In this connection, it is of interest to consider the development of mitochondrial CYP11A1 activity in the ovary upon gonadotropin treatment. CYP11A1 is the rate-limiting enzyme in steroidogenesis. Expression of this enzyme is hormonally regulated in preovulatory follicles, but constitutive in corpora lutea [30]. Induction of CYP11A1 by gonadotropins has been demonstrated using the PMSG model [31]. This could mean that the increase in the activities of the conjugating enzymes pUDPGT and pST in the ovary of mature and immature rats treated with PMSG is correlated to increased CYP11A1 activity and, thus, elevated steroid synthesis.

The different forms of UDPGTs have overlapping substrate specificity. Mammalian cells up-regulate certain cytochrome P450 and UDPGT isoenzymes in response to many inducers [32]. pUDPGT is inducible by PAH-type inducers, e.g. 3-methylcholanthrene [33]. There is a possibility that the increase in pUDPGT activity found in the ovaries of rats enriched in corpora lutea could be coordinated with the induction of xenobiotica metabolizing cytochrome P450s, other than DMBA metabolizing cytochrome P450.

The pUDPGT enzyme metabolizes planar phenols particularly well and it is already known that hepatic pUDPGT does not metabolize steroids [34]. Our experiments demonstrate that hepatic pUDPGT activity does not vary during the estrous cycle, which could mean that induction of this enzyme by steroid(s) is not possible in the liver.

A study of the inhibition of human hepatic steroid and phenol STs with commonly used drugs has been carried out by Bamforth *et al.* [24]. These investigators found that pST activity is not inhibited by most of the drugs used, while ST activity with DHEA and estrone as substrate was inhibited by half of these drugs. It has also been shown that human liver DHEA ST does not conjugate the substrates of two forms of pST [35]. In our studies, we have found an elevation of hepatic pST activity in mature rats treated with PMSG ("hyperstimulated"). Future studies should include measurements of different estrogen STs in this PMSG model to clarify possible correlations between the activities of pST and estrogen ST.

Tissue damage is required in the ovarian follicle to achieve ovulation [17]. At the same time free oxygen radicals are produced. For protection of the ovarian cells from cytotoxicity during this process, more catalase may be needed. From our results we can see that in the rat ovary cytosolic catalase activity does not increase during or after ovulation, whereas catalase activity in the mitochondrial fraction is increased in the ovaries of the immature PMSG-treated rats as compared with untreated rats. This increase in peroxisomal catalase activity caused by gonadotropins is also seen in porcine ovaries [18] where it is correlated to increased levels of CYP11A1. The authors suggest that the increase in catalase might protect against radicals generated by CYP11A1 and ferredoxin [18]. However, we found that ovarian catalase activity is unchanged in mature rats treated with PMSG. Interestingly, cytosolic catalase activity does not vary in the ovaries, but decreases in the liver before ovulation.

The different subunits of glutathione transferase can be separated by HPLC. It has been found that the relative amounts of different ovarian glutathione transferase subunits vary during the estrous cycle (E. Toft *et al.*, unpublished results), although total glutathione transferase activity towards CDNB does not change. In the liver this activity is increased in mature rats treated with PMSG.

Ovarian NAD(P)H:quinone oxidoreductase activity does not change during the estrous cycle, whereas a decrease in this enzyme is detected in the liver of rats in the luteal phase and in the mature rats treated with PMSG. From these and previous results [2] we can conclude that ovarian NAD(P)H:quinone oxidoreductase is not hormonally regulated.

The gonadotropic hormones have different effects on the hepatic enzymes studied in this work. In the livers of mature "hyperstimulated" rats pUDPGT and peroxisomal catalase activities are unchanged in comparison with immature rats. In the same rats pST and glutathione transferase activities increase and NAD(P)H:quinone oxidoreductase and cytosolic catalase activities decrease. These findings show that the gonadotropins increase the conjugation capacity of the hepatic cell but decrease its protection against oxygen radicals.

There exist, of course, many detoxicating mechanisms in the cell in addition to those examined in this study. None the less, the activities studied here are of major importance for the total secretion of xenobiotics from cells. Ovarian cells are exposed to and adapt to highly variable hormone concentrations at the same time as they have to adapt to exposure to different exogenous substances, such as PAHs. Therefore, it is important that the balance between the production and conjugation of reactive metabolites remain unchanged during the estrous cycle. From earlier experiments in our laboratory we have found a high release of reactive metabolites from rat ovarian granulosa cells during the preovulatory phase of the estrous cycle [3]. Taken together, exposure of ovarian cells to PAHs, e.g. DMBA, may result in a higher level of reactive metabolites around ovulation than during the other phases of the estrous cycle.

Acknowledgements—This investigation was supported by the Swedish Environmental Protection Agency. We wish to thank Jarl Olsson for his excellent care and treatment of the animals.

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