Sex-dependent expression and growth hormone regulation of class Alpha and class Mu glutathione S-transferase mRNAs in adult rat liver

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The sex-dependent expression and growth hormone (GH) regulation of rat liver glutathione S-transferase (GST) was examined using oligonucleotide probes that distinguish between closely related class Alpha (Ya_1, Ya_2, Yc) and class Mu (Yb_1, Yb_2, Yb_3) GST mRNAs [Waxman, Sundseth, Srivastava and Lapenson (1992) Cancer Res. **52**, 5797–5802]. Northern-blot analysis revealed that the steady-state levels of GST Ya_1, Yb_1 and Yb_2 mRNAs are 2.5–3-fold higher in male as compared with female rat liver. In contrast, GST Yc and Ya_2 mRNAs were expressed at a 2–3-fold higher level in female rat liver. Microsomal GST mRNA did not exhibit significant sex-dependent differences in rat liver. Treatment of male rats with GH by continuous infusion suppressed expression of the male-dominant GST Ya_1, Yb_1 and Yb_2 mRNAs to levels at or below those found in female rat liver. This suppressive effect of GH was liver-specific, insofar as GH treatment did not alter kidney GST Ya_1 mRNA levels. Hypophysectomy increased expression of the male-dominant GSTs, particularly in female rats (e.g. 8-fold elevation of GST Ya_1 mRNA). GST Yc mRNA was increased approx. 2-fold in hypophysectomized males, indicating that this mRNA is subject to negative regulation by one or more pituitary-dependent factors. Continuous GH treatment of the hypophysectomized rats suppressed the expression of mRNA of GSTs Ya_1, Yb_1 and Yb_2 when given as a continuous infusion, but not when given by an intermittent (twice daily) GH-injection schedule. Combination of continuous exposure to GH with thyroxine treatment resulted in a more complete suppression of GSTs Ya_1, Yb_1 and Yb_2. In contrast, thyroxine increased the expression of GST Yc in hypophysectomized rats. These studies establish that several Alpha and Mu class GSTs are expressed in a sex-dependent fashion in adult rat liver, where they are regulated by multiple pituitary-dependent hormones through pretranslational mechanisms.

INTRODUCTION

Glutathione S-transferase enzymes (GSTs) catalyse the conjugation of glutathione to structurally diverse endogenous and xenobiotic substrates, including many environmental carcinogens and other electrophilic chemicals [1,2]. Cytosolic GSTs are comprised of two subunits which homo- and hetero-dimerize by non-covalent interactions and can be classified into at least four distinct gene families (designated Alpha, Mu, Pi and Theta) on the basis of their primary structures [3–5]. Individual rat GSTs are designated according to the Y subunit nomenclature, as tabulated in [2]. Corresponding numeric subunit designations are Ya, GST subunit 1; Yc, subunit 2; Yb_1, subunit 3; and Yb_2, subunit 4. A structurally distinct microsomal GST (msGST) that catalyses many of the same conjugation reactions has also been described [6].

GSTs can metabolize, and thereby inactivate, several alkylating agent chemotherapeutic drugs, including melphalan, chlorambucil and 1,3-bis(2-chloroethyl)-1-nitrosourea [7–9]. Overexpression of individual GSTs in tumour cells can consequently confer anti-cancer drug resistance, which may be reversed, at least in part, by the application of suitable GST inhibitors [10,11] (for reviews, see [12,13]). While drug selection pressure is undoubtedly an important factor in development of the stable GST overexpression that characterizes these tumour cells, other factors can lead to reversible changes in GST enzyme levels. These include (a) enzyme induction in response to various drugs and chemicals, and (b) changes in enzyme levels in response to endogenous hormonal factors. While much is known about the responsiveness of individual GSTs to general inducers of drug metabolism, such as phenobarbital, β-naphthoflavone, butylated hydroxyanisole and dexamethasone [14–16], much less is known about the endogenous hormonal factors that regulate GST expression.

Several cytosolic GST activities [17,18], individual GST subunit levels [19,20] and those of their mRNAs [21] vary through post-natal liver development. Sexual dimorphism in the expression of some liver and kidney GSTs has been suggested on the basis of immunochemical studies, but in most cases differences in the expressed levels of individual subunits have been reported to be small (typically approx. 2-fold) [22–25]. A regulatory role for pituitary-dependent factors is suggested by the increase in some liver cytosolic GST activities and proteins in hypophysectomized rats [26]. Whereas the pituitary polypeptide hormone adrenocorticotrophic hormone is a major regulator of adrenal GST activity [26], the pituitary factors involved in the regulation of liver GSTs have not been well defined. With the recent development of methods to monitor the expression of GST mRNAs using gene-specific oligonucleotide probes [16], it is now possible to characterize the responses of individual GST mRNAs to hormonal factors.

Previous studies from this laboratory have shown that the anti-cancer drug cisplatin can have long-term effects on the expression in liver of two specific Alpha-class GSTs and their mRNAs [16]. Thus GST Ya_1 is selectively suppressed and GST Yc is induced in rat liver following cisplatin treatment. Cisplatin has also been shown to feminize the profile of cytochrome *P*-450 and other rat liver microsomal enzymes involved in steroid hormone and drug metabolism [27]. In the case of the cytochrome *P*-450s, cisplatin's effects on liver enzyme expression are restricted

Abbreviations used: GST, glutathione S-transferase; msGST, microsomal GST; GH, growth hormone.

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to the sex-dependent cytochrome *P*-450s and their mRNAs [28], and are a consequence of the gonadal toxicity and the loss of circulating testosterone that is caused by cisplatin treatment [27]. The specific effect of cisplatin and GST Ya_1 and GST Yc mRNA and protein levels [16] therefore suggest that these two GSTs may also be subject to hormonal regulation and sex-dependent expression in rat liver. In the present study, we have used a panel of rat GST-gene-specific oligonucleotide probes [16] to investigate the sexual dimorphism of six individual liver GSTs, and to characterize their regulation by pituitary-dependent hormones that are known to regulate several of the cytochrome *P*-450-linked drug-metabolizing enzymes. Our findings lead us to conclude that several Alpha and Mu class GSTs are expressed in a sex-dependent fashion in rat liver, where they are regulated at a pretranslational level by growth hormone (GH) and thyroxine.

MATERIALS AND METHODS

Animal treatments

Untreated, sham-operated and hypophysectomized post-pubertal male and female Fischer 344 rats (8–9 weeks of age) were purchased from Taconic, Inc. (Germantown, NY, U.S.A.) and maintained under controlled lighting conditions (lights on from 06:00 to 18:00). Rats were hypophysectomized or sham-operated by the supplier at the age of 8 weeks and provided with 5% (w/v) glucose water for the first week after surgery. The completeness of hypophysectomy was verified by the absence of weight gain over a 2–3 week period, during which time no hormone treatments were applied. Hypophysectomized male and female rats were given human or rat GH by continuous infusion and/or daily injections of thyroxine for 7 days at a dose previously shown to restore normal euthyroid levels [29]. In the experiment shown in Figure 2 (see below), human GH was given to hypophysectomized female rats twice daily for 7 days by subcutaneous injection (50 μg of GH/injection). This treatment was verified to stimulate the expression of the male-specific liver cytochrome *P*-450 2C11 [30].

Intact adult male rats were treated for 7 days with human GH (specific activity 2.4 i.u./mg) or rat GH (specific activity 1.8 i.u./mg) (National Hormone and Pituitary Programme) delivered as a continuous infusion using Alzet osmotic minipumps implanted under ketamine anaesthesia [31]. GH treatments were at 20–25 ng of hormone/g of body weight per h, a dose shown to provide a continuous level of circulating rat GH (30–40 ng/ml, corresponding to the physiological range for intact female rats) when administered to hypophysectomized male rats [30]. Killing of rats at the age of 11–13 weeks (i.e. 3–4 weeks after hypophysectomy), and isolation and storage of liver and kidney tissue was carried out as described [27]. All analytical results are expressed as means $\pm$ S.D. for $n = 3$ or 4 individual rats/treatment group.

Oligonucleotide probes and Northern-blotting analysis

Oligonucleotide synthesis, purification and ^{32}P end-labelling were performed as described previously [16,32]. Oligonucleotides used in the present study were as follows: GST Ya_1 (ON-24), Ya_2 (ON-43), Yc (ON-47), Yb_1 (ON-44), Yb_2 (ON-45) and msGST (ON-37) [16]. The rat α-tubulin oligonucleotide probe ON-50 [33] was employed as a control, to verify RNA load consistency and RNA integrity. α-Tubulin mRNA was found to be unaffected by the treatments employed in this study.

Isolation of total RNA from frozen liver and kidney tissue, electrophoresis through formaldehyde-containing agarose gels (10–15 μg of RNA/lane) and Northern transfer to nylon filters followed by u.v. cross-linking were carried out as described elsewhere [32]. The nylon filters were probed with oligonucleotides complementary to individual GST mRNAs with the hybridization temperature (40 °C or 45 °C) and the percentage of formamide (0–20%) adjusted empirically for each oligonucleotide, taking into account its length and percentage G+C content [16]. Nylon filters were washed at the hybridization temperature and then air dried and exposed to Kodak XAR-5 film with two intensifying screens at −80 °C for 2–6 days. Northern blots were stripped and then reprobed sequentially with additional oligonucleotide probes.

RNA quantitation by slot blotting was carried out as described previously [32]. Briefly 0.67, 2 and 6 μg of each RNA sample was applied to individual wells of a Minifold II slot-blot apparatus mounted with a nylon filter. Filters were then u.v. cross-linked, prehybridized, hybridized with individual GST oligonucleotides and then washed using the same conditions employed for Northern blotting. Relative mRNA contents for each experimental group were determined by scanning laser densitometry of autoradiographs of the slot blots and are presented as averages of results for each individual liver RNA sample based on two or three different loadings of the slot blot.

RESULTS

Sex-dependent expression of GST mRNAs in rat liver

Since cisplatin and some of its analogues can modulate the expression of GSTs Ya_1 and Yc and their mRNAs in rat liver [16] in a manner that is similar to the effects that this drug has on several sex-dependent rat liver cytochrome *P*-450s [28], we examined whether these two GST mRNAs are also subject to hormone regulation and sex-dependent expression. Total liver RNA was isolated from male and female rats and analysed on Northern blots probed with oligonucleotides specific for GSTs Ya_1 and Yc, as well as for several other liver-expressed GST mRNAs. Figure 1 demonstrates that the mRNAs encoding GSTs Ya_1, Yb_1 and Yb_2 are expressed in adult male rats at levels that are several-fold higher than those found in adult female rats. In contrast, GST Yc and GST Ya_2 mRNAs are both expressed in females at a higher level than in males (Figure 1). No major sex-dependent differences were observed in the expression of msGST mRNA (see Figure 2e). Slot-blot analysis confirmed these findings and revealed that GST Ya_1, Yb_1 and Yb_2 mRNAs are approx. 2.5–3-fold more abundant in males than in females, while the female-dominant GST Yc mRNA is 2–3-fold higher in females (Table 1).

The pituitary polypeptide hormone GH is known to play a major role in the regulation of several sex-dependent liver enzymes, of which the cytochrome *P*-450s are a well-studied example [34]. Male-specific liver cytochrome *P*-450s are positively regulated by the intermittent (pulsatile) pattern of circulating GH that is present in adult male rats, while several female-specific and female-dominant liver cytochrome *P*-450s are positively regulated by the continuous plasma GH pattern associated with adult females. In order to evaluate whether GH also influences the expression of the sex-dependent liver GSTs, adult male rats were treated with human or rat GH by continuous infusion using an osmotic minipump. This treatment overrides the natural endogenous GH pulse found in male rats, and imparts a continuous profile of circulating GH that closely resembles the one found in females [30]. Northern-blot analysis revealed that this treatment leads to significant decreases in the levels of each of the male-dominant liver GST mRNAs, i.e. Ya_1,

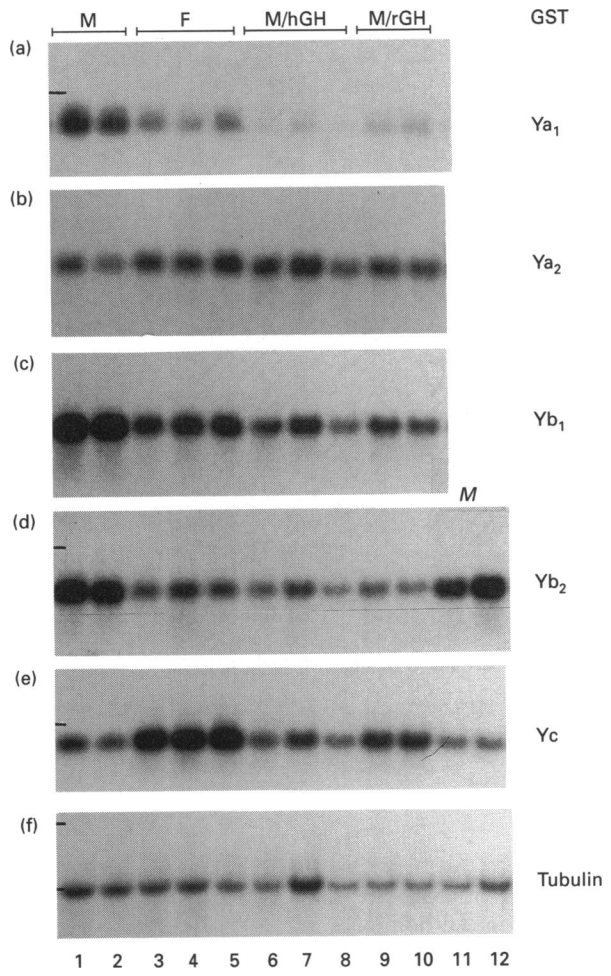

Figure 1 Sex-dependence and GH regulation of GST mRNAs in adult rat liver

Shown is a Northern blot probed for the indicated rat GST mRNAs using gene-specific oligonucleotide probes, as described in the Materials and methods section. Total RNA isolated from livers of individual rats (10 μg/lane) was obtained from males (M; lanes 1, 2, 11 and 12), females (F; lanes 3–5) or from males treated with human GH (lanes 6–8) or rat GH (lanes 9 and 10) by continuous infusion for 7 days using an osmotic minipump. Tubulin mRNA controls shown in panel (**f**) indicate an apparent lower loading of the RNA samples in lanes 8–12 as compared with lanes 1–7. Horizontal markings to the left of panels (**a**), (**d**) and (**e**) indicate the migration of the 18S ribosomal marker. Markings for panel (**f**) indicate 28S and 18S ribosomal markers.

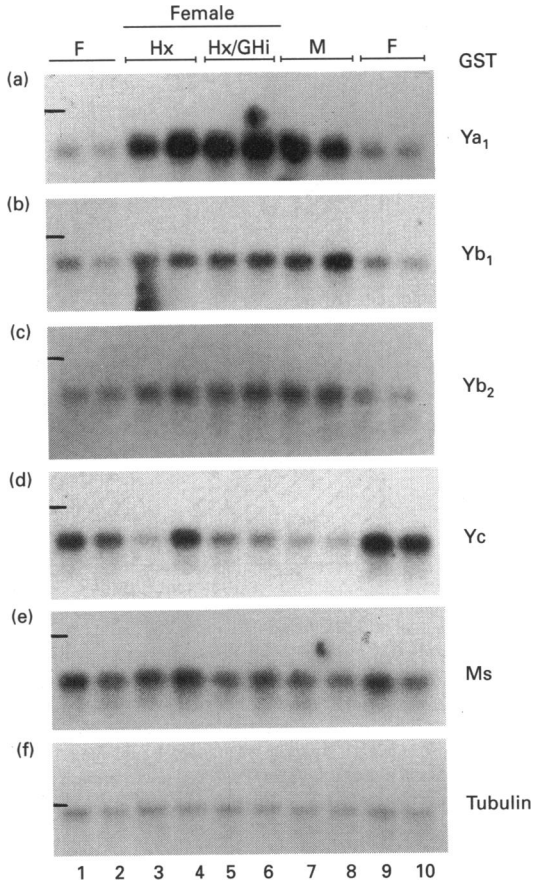

Figure 2 Influence of hypophysectomy and intermittent GH injection on GST mRNA expression

Shown is a Northern blot of rat liver RNA samples probed for the indicated GST mRNAs as described in Figure 1. Abbreviation: Ms, msGST mRNA. Liver RNA samples were prepared from individual normal female rats (F; lanes 1, 2, 9 and 10), male rats (M; lanes 7 and 8), hypophysectomized female rats (Hx; lanes 3 and 4), or hypophysectomized females treated with human GH given twice daily by subcutaneous injection for 7 days (Hx/GHi; lanes 5 and 6). Migration of the 18S ribosomal RNA is marked by a horizontal line at the left-hand side of each panel.

Table 1 Sex dependence of rat liver GST mRNAs

Values determined by slot blotting, and are based on averages of two independent experiments, each involving $n = 3$ or 4 individual male and female rats.

	Relative GST mRNA level	
	Male	Female
GST Ya_1	100 ± 27	40 ± 22
GST Yb_1	100 ± 27	31 ± 10
GST Yb_2	100 ± 29	36 ± 10
GST Yc	100 ± 35	280 ± 50

Yb_1 and Yb_2, and essentially abolishes the differences in their expression between the sexes (Figure 1). This suppressive effect of GH, observed with both human GH and rat GH, is analogous to that described for several male-specific liver cytochrome *P*-450 mRNAs (2C11, 3A2 and 4A2), excepting that the suppression is more complete in the case of the cytochrome *P*-450s [35]. GH had no significant effect on msGST mRNA levels, in accord with the sex-independence of its expression (results not shown).

Several female-dominant and female-specific cytochrome *P*-450 enzymes are positively regulated by continuous GH exposure and their expression in male rats can be increased substantially by continuous GH treatment [34]. Although GST Yc is female dominant in rat liver (see above), continuous GH treatment of male rats had only a small effect in the case of animals treated with rat GH and had no effect when using human GH [cf. Figure 1(e) taking into account tubulin control in Figure 1(f)]. Continuous GH treatment also increased GST Ya_2 mRNA expression to a small extent (Figure 1b). We have previously observed dose-dependent effects of continuous GH treatment on the female-dominant rat liver cytochrome *P*-450 2C7 [33], and it is possible

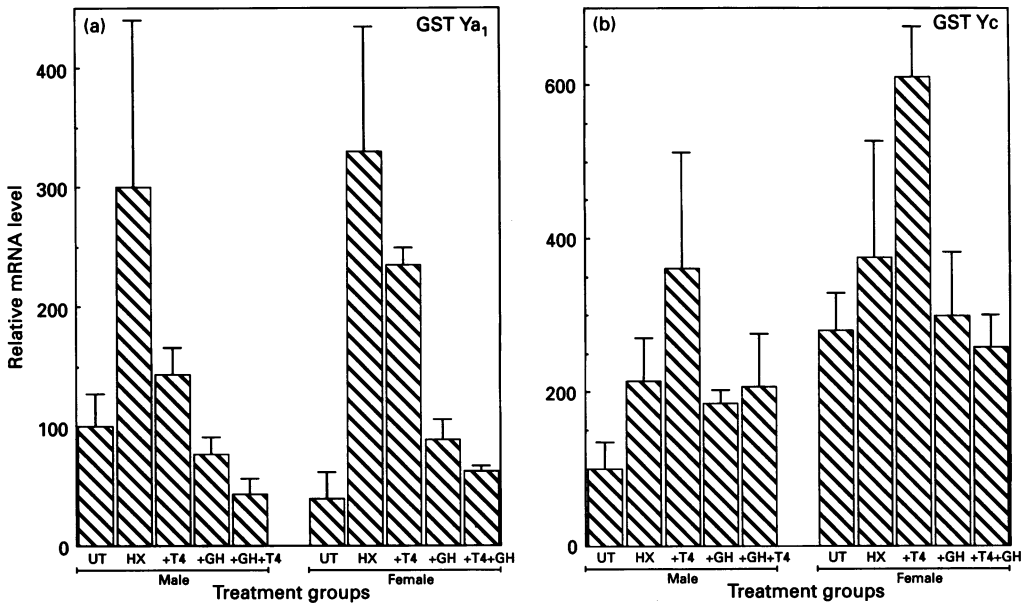

Figure 3 GST Ya_1 (a) and GST Yc mRNA levels (b) in hypophysectomized rat liver: effect of GH and thyroxine (T4) replacement

Shown are relative levels of GST mRNAs determined for groups of untreated (UT) and hypophysectomized (HX) adult male and female rats, as well as hypophysectomized rats treated with thyroxine (T4) and/or GH by continuous infusion, as described in the Materials and methods. Slot-blot analysis of total liver RNA with quantitation by laser densitometry was carried out at three loads of RNA from each of three or four rats/treatment group (mean ± S.D.). Results are based on values obtained from two independent animal experiments, with the exception of the HX + T4 groups, which are based on a single experiment. Values are relative to the mRNA levels present in the untreated adult male group.

that more substantial increases in these female-dominant GST mRNAs would be achieved at higher doses of GH.

Effect of hypophysectomy and GH replacement

Some male-specific liver cytochrome *P*-450s (e.g. cytochrome *P*-450 2C11) are positively regulated by the intermittent plasma GH pulses that are a characteristic of adult male rats, whereas others (e.g. cytochrome *P*-450s 3A2 and 2A2) are not obligatorily dependent on GH pulsation, but rather, are negatively regulated by the profile of pituitary-dependent hormones found in adult female rats [34,36]. These two classes of male-specific, GH-regulated liver enzymes are readily distinguished by their responses to hypophysectomy, which markedly decreases expression of enzymes of the former group, but substantially elevates (de-represses) expression of the latter group of enzymes, particularly in adult female rats. We therefore examined the effects of hypophysectomy and hormone replacement on the expression of the sex-dependent liver GSTs (Figure 2). Northern blotting and slot-blot analysis revealed that hypophysectomy elevated GST Ya_1 mRNA to similar levels in adult male and female rats, corresponding to an approx. 3-fold increase in hypophysectomized adult male rats and an approx. 8-fold increase in adult females (Figure 3a; see also Figure 2, lanes 3 and 4). In contrast, while hypophysectomy did not significantly change GST Yb_1 and GST Yb_2 mRNA levels in male rats, it did increase the level of Yb_1 mRNA by approx. 1.5-fold and the level of Yb_2 mRNA by approx. 4-fold in hypophysectomized females (Figure 4). The responses of these three male-dominant GST mRNAs to hypophysectomy are qualitatively similar to those of cytochrome *P*-450s 3A2 and 2A2, i.e. some increase in hypophysectomized males and a more substantial increase in hypophysectomized females (c.f. [36]). This finding indicates that, as in the case of cytochrome *P*-450 forms 3A2 and 2A2, GST Ya_1, Yb_1 and Yb_2 mRNAs are subject to negative regulation by pituitary-dependent hormones.

To test this hypothesis we treated hypophysectomized rats with GH and thyroxine, the two pituitary-dependent factors that play a significant role in the expression of the male-specific liver cytochrome *P*-450s [29,37]. Continuous GH treatment led to a 70–75% decrease in GST Ya_1 mRNA levels in both male and female hypophysectomized rats (Figure 3a). Thyroxine also decreased levels of Ya_1, although less extensively than did GH, while thyroxine in combination with GH was more effective than either hormone alone at decreasing GST Ya_1 expression from the high levels found in hypophysectomized rats to the substantially lower levels present in untreated female controls. GST Yb_1 and GST Yb_2 mRNA levels were also decreased by continuous GH treatment of hypophysectomized rats (Figure 4). By contrast, treatment with GH by intermittent injection, a protocol designed to mimic the pulsatile pattern of plasma GH present in adult male rats [38,39], had no effect on the elevated levels of Ya_1, Yb_1 or Yb_2 mRNA present in hypophysectomized female rats (Figure 2, lanes 5 and 6 compared with lanes 3 and 4). As in the case of GST Ya_1, thyroxine in combination with continuous GH further decreased these mRNAs to levels that were at or below intact female controls.

Hypophysectomy led to a 2-fold increase in GST Yc mRNA levels in male rats, but had only a small positive effect on the already high mRNA levels found in females (Figure 3b). Treatment with GH by continuous infusion had little effect, while thyroxine increased the level of GST Yc mRNA in the hypophysectomized rats by 1.5–2-fold. Thyroxine also increased the levels of GST Yb_1 mRNA (but not the other male-dominant GST mRNAs) in hypophysectomized female rats (Figure 4a). The fact that GST Yc expression in adult female rats is not decreased by hypophysectomy (Figure 3b) demonstrates that this GST mRNA does not exhibit the marked dependence on pituitary

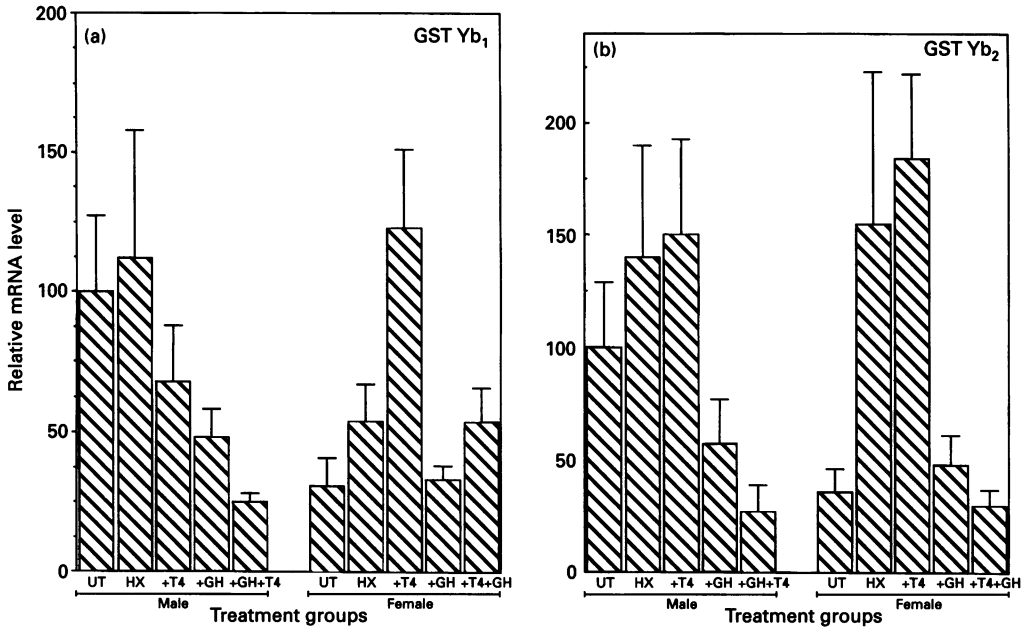

Figure 4 GST Yb_1 (a) and GST Yb_2 mRNA levels (b) in hypophysectomized male and female rats

Relative mRNA levels were determined by slot-blotting analysis of the same series of RNA samples analysed in Figure 3.

hormones that characterizes other female-dominant liver enzymes and mRNAs, such as steroid 5α-reductase and cytochrome *P*-450 2C7 [33]. While the high-level expression of GST Yc mRNA in hypophysectomized rats is analogous to that of the female-dominant liver cytochrome *P*-450 2A1, that enzyme, unlike GST Yc, is negatively regulated by thyroxine [40].

Expression of GST in kidney

GST Ya_1 and Yc mRNAs are expressed in rat kidney at levels that are at least as high as in liver. Expression of both these mRNAs occurs at a somewhat higher level in male as compared with female rat kidney [16]. Treatment of intact male rats with GH, by continuous exposure, did not have any detectable effect, however, on the expression in kidney of either GST Ya_1 or Yc mRNA (results not shown). Thus, the suppressive effects of continuous GH on liver GST Ya_1 mRNA levels (Figure 1) are not achieved in kidney.

DISCUSSION

Hormonal regulation has been reported for several classes of liver drug-metabolizing enzymes, including multiple cytochrome *P*-450s [34,41], sulphotransferases [42] and *N*-acetyltransferase [43]. In the case of the cytochrome *P*-450s, individual enzymes are subject to sex-dependent expression under the influence of pituitary-dependent hormones, in particular GH, and to a lesser extent thyroxine. The present study establishes that several liver cytosolic GST mRNAs are also expressed in a sex-dependent fashion under the influence of GH and thyroxine. Thus, Northern-blot analysis of total liver RNA using probes specific to individual GSTs demonstrated that GST Ya_1, Yb_1 and Yb_2 mRNAs are expressed at 2.5–3-fold higher levels in male as compared with female rat liver, while GST Ya_2 and Yc mRNA levels are 2–3-fold higher in females. These findings are consistent with the finding of Igarashi et al. that liver GST Yb protein is more abundant in male as compared with female rat liver, while GST Yc protein levels are higher in females [23]. In the present study, hypophysectomy essentially eliminated the sex-specific differences in liver GST mRNAs, and GH- and thyroxine-replacement experiments provided evidence for important roles for these two pituitary-dependent hormones in determining the adult pattern of liver GST expression. As is the case for the GH-responsive liver cytochrome *P*-450s [34], we found the effects of GH on GST mRNA expression to be dependent on the temporal pattern of hormone administration. Thus, continuous GH infusion (mimicking the adult female rat plasma GH profile), but not intermittent GH treatment (mimicking the pulsatile GH pattern characteristics of adult male rats) suppressed the expression of each of the male-dominant GST mRNAs. This indicates that the sex-specific difference in circulating GH profiles [44] is probably a major determinant of the sexual dimorphism of rat liver GST mRNA expression reported in the present study. The cellular and molecular mechanisms whereby these two distinct secretory patterns give rise to unique patterns of liver gene expression are likely to be complex and could involve effects of GH on gene transcription, as occurs in the case of liver cytochrome *P*-450s [45,46].

In addition to these effects of GH, we observed an important role for thyroid hormone in the regulation of GST mRNA expression, with thyroxine suppressing the level of GST Ya_1 mRNA and elevating the level of GST Yc mRNA in hypophysectomized rats. By contrast, thyroxine had no effect on Yb_2 mRNA levels, while its effects on Yb_1 differed between males and females. These effects of thyroxine on GST Ya_1 and Yc (Alpha class) mRNAs are analogous to the feminizing effects that thyroid hormone can have on liver cytochrome *P*-450 expression [33,37]. Since thyroxine elicited these effects in hypophysectomized rats, they do not result from the positive effects that thyroid hormone has on pituitary GH secretion. Rather, they suggest that thyroid hormone may have a direct effect on liver GST gene expression. Indirect effects of thyroid hormone could

also be occurring, as indicated by our finding that thyroxine enhanced the GH suppression of GST Yb_2 mRNA levels in hypophysectomized rats, even though thyroxine alone had no effect on this mRNA. The requirement of thyroid hormone to maintain normal hepatic GH receptor levels [47] could provide an explanation for this result.

Our conclusions in this study are largely consistent with those of Staffas et al. in a report that appeared after completion of this present study [48]. Those investigators described the use of h.p.l.c. analysis of affinity-purified rat liver GST protein mixtures to characterize the expression of GST subunits 1, 3 and 4 (GSTs Ya, Yb_1 and Yb_2 respectively) as male dominant in adult rat liver. Hypophysectomy generally increased enzyme expression and continuous GH treatment decreased enzyme expression, in general agreement with the mRNA analyses described in the present study. One apparent difference between the two reports is that GST Ya_1 and Ya_2 mRNAs were individually characterized using gene-specific oligonucleotide probes and were thereby found to be regulated in opposite fashions in the present study, whereas the data reported for GST Ya protein in the study by Staffas et al. [48] reflect the summation of the two closely migrating subunits 1 peaks, which respectively correspond to GSTs Ya_1 and Ya_2 [49]. In addition, GST subunit 2 protein (GST Yc) was found in [48] to be relatively unaffected by the hormonal factors examined, in contrast with the present characterization of GST Yc mRNA as female dominant. It is conceivable that this could reflect a differential regulation of this GST protein as compared with its mRNA. However, the earlier report that GST Yc protein levels are higher in female than in male rat liver [23], and our observation that the anti-cancer drug cisplatin induces both GST Yc mRNA and protein by processes that probably involve alterations in endogenous hormonal regulators of these GSTs [16], suggest that this may not be the case.

We previously reported [16] that treatment of adult male rats with the anti-cancer drug cisplatin results in the suppression of GST Ya_1 and the elevation of GST Yc mRNA and protein in liver in a manner that is similar to the effects that cisplatin has on several hormone-regulated liver cytochrome *P*-450s [27,28]. The present demonstration that GST Ya_1 and Yc mRNAs are indeed expressed in a sexually dimorphic fashion, in turn, suggests that these effects of cisplatin derive from its depletion of circulating testosterone [27,50,51], which is expected to have a substantial impact on the plasma GH profiles of affected rats [52]. In view of the important role that GSTs play in metabolism, leading to inactivation of a number of therapeutically useful alkylating-agent anti-cancer drugs [12], the effects of cisplatin on these sex-dependent GST mRNAs could lead to clinically significant drug interactions in the case of chemotherapeutic regimens involving cisplatin in combination with other alkylating agents. Further study will be required to establish whether human liver GSTs, which undergo distinct developmental changes in liver and other tissues [53], are also subject to sex-dependent expression and hormone regulation. If this is found to be the case, it will be of interest to determine whether cisplatin or other anti-cancer drugs alter the normal patterns of GST enzyme expression in cancer patients in a manner that impacts on anti-cancer drug activity or host toxicity. Gender-related differences in the expression of class Alpha, Mu and Pi GSTs in human colon tissue have recently been reported [54], but the underlying hormone regulatory mechanisms, and the possible importance of this gender dependence for processes such as carcinogen susceptibility and drug sensitivity, have yet to be established.

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REFERENCES

1 Mannervik, B. and Danielson, U. H. (1988) CRC Crit. Rev. Biochem. **23**, 283–337
2 Pickett, C. B. and Lu, A. Y. (1989) Annu. Rev. Biochem. **58**, 743–764
3 Mannervik, B., Alin, P., Guthenberg, C., Jensson, H., Tahir, M. K., Warholm, M. and Jornvall, H. (1985) Proc. Natl. Acad. Sci. U.S.A. **82**, 7202–7206
4 Meyer, D. J., Coles, B., Pemble, S. E., Gilmore, K. S., Fraser, G. M. and Ketterer, B. (1991) Biochem. J. **274**, 409–414
5 Pemble, S. E. and Taylor, J. B. (1992) Biochem. J. **287**, 957–963
6 Morgenstern, R. and DePierre, J. W. (1988) in Glutathione Conjugation, Mechanisms and Biological Significance (Sies, H. and Ketterer, B., ed.), pp. 157–174, Academic Press, New York
7 Bolton, M. G., Colvin, O. M. and Hilton, J. (1991) Cancer Res. **51**, 2410–2415
8 Ciaccio, P. J., Tew, K. D. and LaCreta, F. P. (1990) Cancer Commun. **2**, 279–285
9 Smith, M. T., Evans, C. G., Doane, S. P., Castro, V. M., Tahir, M. K. and Mannervik, B. (1989) Cancer Res. **49**, 2621–2625
10 Tew, K. D., Bomber, A. M. and Hoffman, S. J. (1988) Cancer Res. **48**, 3622–3625
11 Ford, J. M., Hait, W. N., Matlin, S. A. and Benz, C. C. (1991) Cancer Lett. **56**, 85–94
12 Waxman, D. J. (1990) Cancer Res. **50**, 6449–6454
13 Morrow, C. S. and Cowan, K. H. (1990) Cancer Cells **2**, 15–22
14 Pickett, C. B., Telakowski-Hopkins, C. A., Ding, G. J. F., Argenbright, L. and Lu, A. Y. H. (1984) J. Biol. Chem. **249**, 5182–5188
15 Pearson, W. R., Reinhart, J., Sisk, S. C., Anderson, K. S. and Adler, P. N. (1988) J. Biol. Chem. **263**, 13324–13332
16 Waxman, D. J., Sundseth, S. S., Srivastava, P. K. and Lapenson, D. P. (1992) Cancer Res. **52**, 5797–5802
17 Fujita, S., Kitagawa, H., Ishizawa, H., Suzuki, T. and Kitani, K. (1985) Biochem. Pharmacol. **34**, 3891–3894
18 Chengelis, C. P. (1988) Xenobiotica **18**, 1225–1237
19 McCusker, F. M. G., Boyce, S. J. and Mantle, T. J. (1989) Biochem. J. **262**, 463–467
20 Tee, L. B. G., Gilmore, K. S., Meyer, D. J., Ketterer, B., Vandenberghe, Y. and Yeoh, G. C. T. (1992) Biochem. J. **282**, 209–218
21 Abramovitz, M. and Listowsky, I. (1988) Xenobiotica **18**, 1249–1254
22 Lamartiniere, C. A. (1981) Biochem. J. **198**, 211–217
23 Igarashi, T., Irokawa, N., Ono, S., Ohmori, S., Ueno, K., Kitawaga, H. and Satoh, T. (1987) Xenobiotica **17**, 127–137
24 Butera, L. (1988) Ph.D. Thesis, Yeshiva University, New York
25 Igarashi, T. and Satoh, T. (1989) Drug Metab. Drug Interact. **7**, 191–212
26 Mankowitz, L., Castro, V. M., Mannervik, B., Rydstrom, J. and DePierre, J. W. (1990) Biochem. J. **265**, 147–154
27 LeBlanc, G. A. and Waxman, D. J. (1988) J. Biol. Chem. **263**, 15732–15739
28 LeBlanc, G. A., Sundseth, S. S., Weber, G. F. and Waxman, D. J. (1992) Cancer Res. **52**, 540–547
29 Ram, P. A. and Waxman, D. J. (1991) Mol. Endocrinol. **5**, 13–20
30 Waxman, D. J., Pampori, N. A., Ram, P. A., Agrawal, A. K. and Shapiro, B. H. (1991) Proc. Natl. Acad. Sci. U.S.A. **88**, 6868–6872
31 Waxman, D. J., Morrissey, J. J. and LeBlanc, G. A. (1989) Endocrinology **124**, 2954–2966
32 Waxman, D. J. (1991) Methods Enzymol. **206**, 249–267
33 Ram, P. A. and Waxman, D. J. (1990) J. Biol. Chem. **265**, 19223–19229
34 Waxman, D. J. (1992) J. Ster. Biochem. Mol. Biol. **43**, 1055–1072
35 Sundseth, S. S. and Waxman, D. J. (1992) J. Biol. Chem. **267**, 3915–3921
36 Waxman, D. J., LeBlanc, G. A., Morrissey, J. J., Staunton, J. and Lapenson, D. P. (1988) J. Biol. Chem. **263**, 11396–11406
37 Waxman, D. J., Ram, P. A., Notani, G., LeBlanc, G. A., Alberta, J. A., Morrissey, J. J. and Sundseth, S. S. (1990) Mol. Endocrinol. **4**, 447–454
38 Kato, R., Yamazoe, Y., Shimada, M., Murayama, M. and Kamataki, T. (1986) J. Biochem. (Tokyo) **100**, 895–902
39 Morgan, E. T., MacGeoch, C. and Gustafsson, J.-A. (1985) J. Biol. Chem. **260**, 11895–11898
40 Yamazoe, Y., Ling, X., Murayama, N., Gong, D., Nagata, K. and Kato, R. (1990) J. Biochem. (Tokyo) **108**, 599–603
41 Zaphiropoulos, P. G., Mode, A., Norstedt, G. and Gustafsson, J. A. (1989) Trends Pharmacol. Sci. **10**, 149–153
42 Yamazoe, Y., Gong, D., Murayama, N., Abu, Z. M. and Kato, R. (1989) Mol. Pharmacol. **35**, 707–712
43 Sabry, I. and Reiter, R. J. (1988) Experientia **44**, 509–511
44 Jansson, J.-O., Ekberg, S. and Isaksson, O. (1985) Endocrine Rev. **6**, 128–150
45 Sundseth, S. S., Alberta, J. A. and Waxman, D. J. (1992) J. Biol. Chem. **267**, 3907–3914
46 Legraverend, C., Mode, A., Westin, S., Strom, A., Eguchi, H., Zaphiropoulus, P. G. and Gustafsson, J.-A. (1992) Mol. Endocrinol. **6**, 259–266
47 Hochberg, Z., Bick, T. and Harel, Z. (1990) Endocrinology **126**, 325–329

48 Staffas, L., Mankowitz, L., Soderstrom, M., Blanck, A., Porsch-Hallstrom, I., Sundberg, C., Mannervik, B., Olin, B., Rydstrom, J. and DePierre, J. W. (1992) Biochem. J. **286**, 65–72
49 Hayes, J. D., Kerr, L. A., Harrison, D. J., Cronshaw, A. D., Ross, A. G. and Neal, G. E. (1990) Biochem. J. **268**, 295–302
50 Maines, M. D. and Mayer, R. D. (1985) J. Biol. Chem. **260**, 6063–6068
51 Maines, M. D., Sluss, P. M. and Iscan, M. (1990) Endocrinology **126**, 2398–2406
52 Jansson, J. O. and Frohman, L. A. (1987) Endocrinology **120**, 1551–1557
53 Faulder, C. G., Hirrell, P. A., Hume, R. and Strange, R. C. (1987) Biochem. J. **241**, 221–228
54 Singhal, S. S., Saxena, M., Awasthi, S., Ahmad, H., Sharma, R. and Awasthi, Y. C. (1992) Biochim. Biophys. Acta **1171**, 19–26

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