Biochem. J. (1992) 286, 65–72 (Printed in Great Britain)

Further characterization of hormonal regulation of glutathione transferase in rat liver and adrenal glands

Sex differences and demonstration that growth hormone regulates the hepatic levels

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Immunoblot experiments and reverse-phase h.p.l.c. were used to study the levels of glutathione transferase subunits 1, 2, 3, 4, 6, 7 and 8 in the liver and adrenal of intact and hypophysectomized male and female Sprague–Dawley rats. A sexual dimorphism in the levels of several of these isoenzymes and in their responses to hypophysectomy was demonstrated. In the liver of sham-operated females and males there are differences in glutathione transferase activities and isoenzyme pattern. H.p.l.c. analysis showed higher levels of subunits 1, 3 and 4 in male rats compared with females. In contrast with the pronounced sex differences in sham-operated rats, the isoenzyme patterns of hypophysectomized males and females were very similar. In the adrenal glands, however, a sexual dimorphism became apparent only after hypophysectomy, when the level of subunit 4 was increased 14-fold in the female, whereas the corresponding increase in the male rat was only 2.7-fold. The hepatic pattern of glutathione transferase subunits could be altered by continuous infusion of growth hormone to both sham-operated and hypophysectomized rats of both sexes. This treatment feminized the isoenzyme pattern in sham-operated males and a similar effect was obtained upon treating hypophysectomized rats with thyroxine, cortisone acetate and a continuous infusion of growth hormone.

INTRODUCTION

The glutathione transferases (GSTs) are a family of isoenzymes involved in the detoxication of xenobiotics and endogenous substances [1,2]. Numerous exogenous substrates, including ethacrynic acid, *trans*-stilbene oxide and 1-chloro-2,4-dinitrobenzene (CDNB) are known, and examples of endogenous substrates are products of lipid peroxidation, such as 4-hydroxyalkenals and cholesterol epoxides [1]. These enzymes may also have a function as binding and carrier proteins [1,3] and/or be involved in other physiological functions.

GSTs are dimers with somewhat different, but overlapping, substrate specificities and are located in different amounts and with different isoenzyme patterns in every aerobic organism and organ investigated to date [1,3]. GSTs conjugate GSH to various electrophiles, often produced in reactions catalysed by mono-oxygenases such as the cytochrome *P*-450 system. In the rat, cytosolic GSTs are divided into four classes, Alpha, Mu, Pi and Theta, on the basis of primary structure, immunological properties and substrate specificity [4–8]. At present 13 subunits are known [9]. Hepatic GSTs, like most other xenobiotic-metabolizing enzymes, are induced by, e.g., phenobarbital and 3-methylcholanthrene [10,11]. However, no short-term post-translational regulation by, e.g., phosphorylation, has yet been found for the cytosolic GSTs.

Little is known about the endogenous regulation of the GSTs. It has, however, been reported that the activities of GSTs vary during development in the rat and mouse [12,13] and that these enzymes are regulated by sex hormones [13,14]. A sexual dimorphism in the isoenzyme pattern of cytosolic GSTs [15] and in activities towards several substrates such as CDNB and 1,2-dichloro-4-nitrobenzene (DCNB) [16] has been observed in rat liver. A regulatory mechanism involving the pituitary has also been demonstrated both in the liver and the adrenal by ourselves [17] and by others [16]. We found increased levels of several GSTs in both these organs after hypophysectomy. The largest effects were obtained with the class-Mu enzymes, especially subunit 4, where 14- and 3-fold increases were observed in the female adrenal and liver respectively. The elevated level of subunit 4 in the adrenals of hypophysectomized female rats is largely suppressed by administration of adrenocorticotropic hormone (ACTH) [17].

The activities of another enzyme system involved in the metabolism of xenobiotics, i.e., cytochrome *P*-450, also demonstrates a sexual dimorphism. Several sex-specific hepatic cytochrome *P*-450 isoenzymes have been identified and shown to be regulated in the adult rat by the pattern of growth hormone (GH) secretion [18,19], for which the liver is an important target organ. The secretion of GH is pulsatile in both male and female rats, but the amplitudes of the pulses are higher in males than in females, whereas the basal level of GH is higher in females than in males [20]. The female secretory pattern can be mimicked by continuous administration of GH to rats. By measuring the microsomal metabolism of 4-androstene-3,17-dione, several sex-differentiated reactions can be determined that reflect the endocrine status of the animal [18,19]. The level of the female-predominant 5α-reductase increases after continuous GH administration to male or hypophysectomized rats, whereas the

Abbreviations used: GST, glutathione *S*-transferase; DCNB, 1,2-dichloro-4-nitrobenzene; CDNB, 1-chloro-2,4-dinitrobenzene; ACTH, adrenocorticotropic hormone; GH, growth hormone; PBS, phosphate-buffered saline (for composition, see the text).

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levels of the male-predominant 6β-, 16α- and 16β-hydroxylases decrease in response to the same treatment.

The aim of our present study was to investigate further the sexual dimorphism of GSTs in rat liver and adrenal and to determine whether these differences, as well as the changes observed after hypophysectomy, are regulated by GH.

EXPERIMENTAL

Material

GSH, CDNB, L-thyroxine and cortisone acetate were purchased from the Sigma Chemical Co. (St. Louis, MO, U.S.A.). Cellulose nitrate membrane filters (Schleicher und Schüll, Dassel, Germany) and ^{125}I-labelled Protein A (100 μCi/ml) (The Radiochemical Centre, Amersham, Bucks., U.K.) were obtained from the sources indicated.

trans-[^{3}H]Stilbene oxide (2 Ci/mmol) was synthesized by Dr. Åke Pilotti, Department of Organic Chemistry, University of Stockholm, Stockholm, Sweden, according to a published procedure [21]. The compound was purified by t.l.c. on 0.2 mm-thick silica-gel plates (Merck, Darmstadt, Germany) with hexane/ethyl acetate (19:1, v/v) as the eluant, scraped off the plate and recovered by two extractions with ethanol. The final purity was > 99%.

Human GH (genotropin) was kindly given by Kabi AB (Stockholm, Sweden). Osmotic minipumps used for subcutaneous implantation were bought from ALAB (Sollentuna, Sweden).

All other chemicals were of reagent grade and purchased from common commercial sources.

Methods

Treatment of animals and preparation of cytosolic fractions. Age-matched male and female Sprague–Dawley rats, hypophysectomized or sham-operated at 6 weeks of age, were purchased from Møllegaards Avlslaboratorium (Skensved, Denmark). The animals had free access to tap water and a diet (R-3) containing 5% fat, 24% protein and 49% carbohydrate (Ewos AB, Södertälje, Sweden). They were maintained under a light/dark cycle of 24 h (lights on from 06.00 h to 18.00 h) and a temperature of 21 °C.

Treatment of the animals was started 4 weeks after surgery. The animals used for studies on hormonal regulation of the hepatic GSTs were divided into six sexually segregated groups as shown in Table 1. GH (50 m-i.u./h and kg body wt.) was administered via minipumps (Alzet model 2001) implanted subcutaneously under the neck. L-Thyroxine (10 μg/day per kg body wt.) and cortisone acetate (500 μg/day per kg body wt.) were administered by daily subcutaneous injections [18,19]. GH treatments lasted 1 week and injection of L-thyroxine and cortisone acetate was performed for a total of 9 days, commencing 2 days before beginning GH treatment. Two separate experiments were performed, one involving groups 1, 2, 3 and 4 and the other groups 1, 2, 5 and 6 (see Table 1). The results of these experiments are presented separately.

Table 1. Treatment of the animals in each group

For details concerning dosage, route and period of administration, see the text. Each group contained three rats. Abbreviations used: T_4, thyroxine; cort., cortisone acetate; ♀, female; ♂, male.

Group	Sex	Sham-operated	Hypophysectomized	T_4 + cort.	GH
1A	♀	+	−	−	−
1B	♂	+	−	−	−
2A	♀	−	+	−	−
2B	♂	−	+	−	−
3A	♀	+	−	−	+
3B	♂	+	−	−	+
4A	♀	−	+	−	+
4B	♂	−	+	−	+
5A	♀	−	+	+	−
5B	♂	−	+	+	−
6A	♀	−	+	+	+
6B	♂	−	+	+	+

After killing the animals by decapitation, their livers and adrenals were immediately removed and placed into ice-cold 0.25 M-sucrose, washed, and the livers homogenized with a Potter–Elvehjem homogenizer using four up-and-down-strokes at 440 rev./min. This homogenate was centrifuged at 900 *g* for 10 min, and the resulting supernatant then centrifuged at 10000 *g* for 10 min. The supernatant thus obtained was centrifuged at 105000 *g* for 60 min and the resulting supernatant (cytosol) stored at −20 °C.

In the case of the adrenals, organs from the animals in a given experimental group were pooled and homogenized in 0.25 M-sucrose/20 mM-Tris/HCl, pH 7.4. Adrenal homogenates were then subfractionated in the same manner as the liver homogenates.

Enzyme and protein assays. GST activity was measured spectrophotometrically with CDNB as substrate, as described by Habig *et al.* [22].

GST 4–4 was measured with *trans*-stilbene oxide as a selective substrate, according to a published procedure [21]. 100 μM-*trans*-[^{3}H]Stilbene oxide was incubated at 37 °C for 15–30 min with the cytosolic adrenal fractions (100–150 μg of protein). The reaction was terminated by extraction with hexan-1-ol, and aliquots of the aqueous phase were then subjected to liquid-scintillation counting. The time and protein amounts used are within the linear range of the reaction and the substrate concentrations of 100 μM-*trans*-stilbene oxide and 5 mM-GSH were chosen to approach saturation [17].

Androstenedione metabolism was determined as described previously by Blanck *et al.* [23].

Protein was determined as described by Lowry *et al.* [24].

Antibodies. Rat GST isoenzyme 1–1, 2–2, 3–3, 4–4 and 8–8 and the human transferase π were purified and antibodies raised in rabbits as described previously by Mannervik *et al.* [4].

SDS/PAGE and immunoblot analysis. Electrophoresis was performed on 15% (w/v) SDS/polyacrylamide gels by the method of Laemmli [25]. The separated proteins were transferred electrophoretically to 0.2 μm-pore-size nitrocellulose membrane filters [26]. The nitrocellulose sheets were then treated essentially as described by Berzins and co-workers [27]. The sheets were blocked with 3% (w/v) BSA for at least 2 h and then cut into strips. Each strip was incubated separately for approx. 12 h with specific antibodies directed against GST subunits 1, 2, 3, 4 or 8. Antibodies against transferase π demonstrate complete cross-reactivity with rat subunit 7 and were thus used for detection of this protein [4].

The washing buffer used in the following procedure contained 0.2% (w/v) BSA, 0.1% Triton X-100 and 0.02% (w/v) SDS in phosphate-buffered saline (140 mM-NaCl/3.7 mM-KH_2PO_4/11.3 mM-Na_2HPO_4, pH 7.4) (PBS) and the incubation buffer was the same as the washing buffer, except that it contained 1% BSA. After the incubation with antibodies, the nitrocellulose strips were washed four times for 15 min each in washing buffer and subsequently incubated for 1 h with ^{125}I-labelled protein A

(0.1 μCi/ml) in incubation buffer, washed for 4 × 15 min in washing buffer, dried and the immunocomplexes revealed by autoradiography.

H.p.l.c. analysis. The cytosols were first passed through a Sephadex G-25 column to remove endogenous GSH, and the cytosolic GSTs were then purified by affinity chromatography on *S*-hexyl-GSH–Sepharose as described by Mannervik & Guthenberg [28]. The elution buffer was 10 mM-Tris-HCl, pH 8.0 (buffer A). The eluate from the G-25 column was applied to the affinity column and washed with buffer A + 0.2 M-NaCl (buffer B) until no protein could be detected in the eluate. Finally the GSTs were eluted with 5 mM-*S*-hexyl-GSH in buffer B. The protein concentration was determined in this case by the modified Lowry procedure described by Peterson [29].

H.p.l.c. analysis was performed using a reverse-phase column (Dynamax 300 A, C4-83-503-C5; Rainin Instruments, Woburn, MA, U.S.A.) on a Gilson h.p.l.c. system. The method is a modified version of the one described by Ostlund Farrants *et al.* [30]. The gradients were run with 0.1% trifluoroacetic acid in water (A) and acetonitrile (B) as follows: for liver samples, either (1) 5 min with 40% B, a linear gradient increasing to 60% B during the course of 35 min, 10 min with 60% B and return to 40% B in 7 min or (2) 7 min with 36% B, a linear gradient increasing to 44% B during a period of 12 min, then 44% B for 3 min, followed by a linear gradient increasing to 52% B in the course of 12 min and another increase to 60% B in 3 min, 6 min with 60% B and then, finally, a return to 36% B in 2 min. The adrenal GSTs were eluted with the following gradient: 1 min with 42% B, 30 min with a linear gradient from 42 to 53.5% B, 10 min with a linear gradient of 53.5 to 60% B, 5 min with 60% B and 3 min with a linear gradient from 60 to 42% B. For reasons which remain unclear, different columns require different gradients for optimal resolution in our hands. GST subunits were detected at 214 nm.

RESULTS

Demonstration that hypophysectomy and hormone treatment were effective

Hypophysectomized animals, whether they received thyroxine + cortisone acetate or not, gained no weight during the treatment period, since no GH could be secreted in these rats. Both sham-operated and hypophysectomized animals receiving GH gained between 10 and 20 g during 1 week's treatment. These differences in weight gain confirmed that hypophysectomy was complete and that GH administration had been successful. In some of the samples, control of the minipumps was performed by measuring the activities of the sex-specific and cytochrome *P*-450-mediated reactions towards androstenedione, i.e., 6β-, 16β- and 16α-hydroxylases, as well as the 5α-reductase activity, known to be regulated by the serum level of GH (see the Introduction). The male-specific 6β-, 16β- and 16α-hydroxylases decreased after continuous infusion of GH in both hypophysectomized and sham-operated animals, whereas the activity of the female-specific 5α-reductase increased after the same treatment. Mean enzyme activities towards androstenedione under various conditions are shown in Table 2.

Choice of time point after hypophysectomy for performance of the studies

We had reason to believe from reports in the literature concerning changes in adrenal cytochrome *P*-450-catalysed activity after hypophysectomy [31] that a period of 4 weeks after hypophysectomy would be sufficient to obtain a maximal or near-maximal increase in the amount and activity of GST. This had, however, to be verified in our case. *trans*-Stilbene oxide is a selective substrate for GST 4–4, which is one of the isoenzymes whose levels increase in both liver and adrenal after hypophysectomy. Figs. 1(*a*) and 1(*b*) show the increase in cytosolic activity towards this substrate in adrenal and liver during the first weeks after hypophysectomy and demonstrate

Table 2. Microsomal metabolism of 4-androstenedione in livers from intact and hormonally manipulated rats of both sexes

Each activity is an average calculated from three animals in each group. Analysis of statistical significance was performed using the Student's *t* test. Levels of significance were obtained by comparison of each value with the control value of the same enzyme and sex. Statistical significance: * $P < 0.01$; ** $P < 0.005$; *** $P < 0.001$. Abbreviations: Sham, sham-operated; hypox, hypophysectomized; ♀, female; ♂, male.

		Enzyme activity (nmol/min per mg of protein)				
		Hydroxylations				
Sex	Treatment	7α	6β	16β	16α	5α-Reductase
♀	Sham	0.64	0.19	0.07	0.06	13.00
	Hypox	0.57	0.55*	0.12**	0.36**	0.99*
	Sham + GH	0.62	0.24	0.05	0.04	17.57
	Hypox + GH	0.57	0.15	0.09	0.10	4.80
♂	Sham	0.36	0.74	0.12	1.20	2.03
	Hypox	0.44	0.76	0.12	0.45**	0.61*
	Sham + GH	0.31	0.14**	0.06*	0.14***	11.14**
	Hypox + GH	0.55*	0.19**	0.09**	0.11***	5.95*

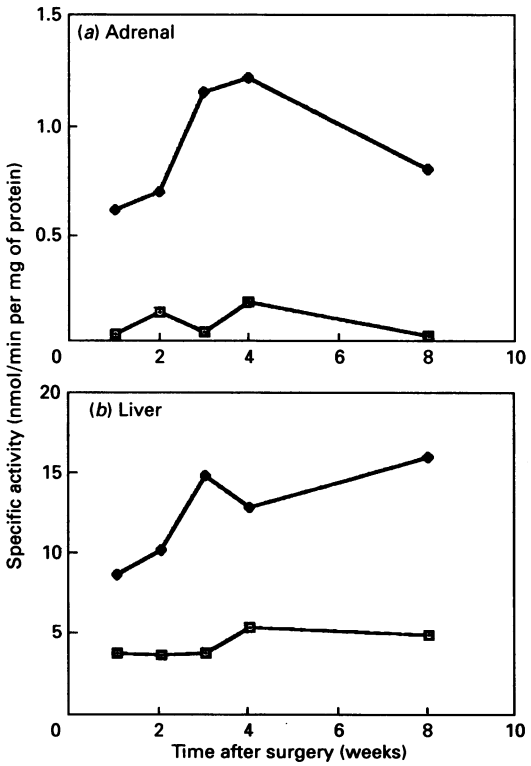

Fig. 1. Cytosolic GST activities towards *trans*-stilbene oxide in female rat adrenal (*a*) and liver (*b*) at various times after hypophysectomy

The activities were measured using the radiometric distribution assay described in the Materials and methods section. ⊡, Sham-operated; ◆, hypophysectomized.

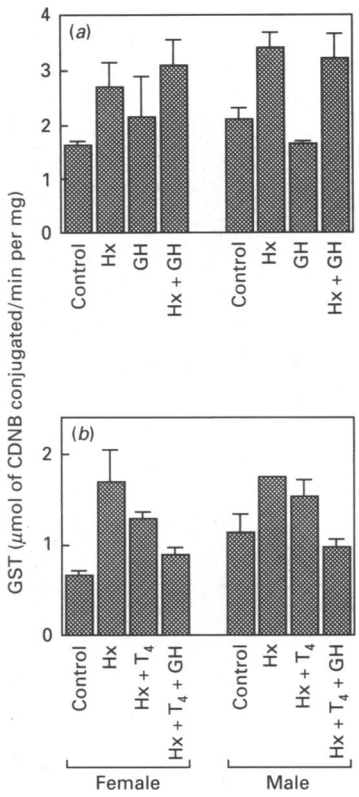

Fig. 2. GST activity towards CDNB in female and male rat liver cytosols after different treatments

(*a*) and (*b*) represent two different experimental set-ups, as described in the Materials and methods section. The activity was measured spectrophotometrically as described by Habig *et al.* [22]. Each bar represents the mean value for three samples. Abbreviations: Hx, hypophysectomized; GH, administered human GH; T_4, thyroxine + cortisone acetate.

that, 4 weeks after hypophysectomy, the amount and activity of GST are apparently maximal or near-maximal.

Effects of hypophysectomy and hormone treatment on GST activities towards CDNB

The activities towards CDNB in the liver cytosols are depicted in Figs. 2(*a*) and 2(*b*). The difference in activities between females and males is less evident in the first experiment (Fig. 2*a*) than in the second (Fig. 2*b*), but is still significant. The expected increase in activity after hypophysectomy is seen in both sexes. This increase is 2-fold in females and 1.6-fold in males. Continuous administration of GH to sham-operated males brings the activities to the same levels as in control females, whereas administration of GH alone to hypophysectomized animals (groups 4A and B) does not affect the activity towards CDNB compared with hypophysectomized controls (see Fig. 2*a*).

Fig. 2(*b*) shows that the activities towards CDNB are not altered significantly by treatment of hypophysectomized animals with thyroxine and cortisone acetate, but that these hormones in combination with GH lower the activities to almost the same level as that observed in the female control group. It is noteworthy that the activities in hypophysectomized males receiving thyroxine, cortisone acetate and GH, as well as the activities in sham-operated males receiving GH, are significantly lower than those of the sham-operated control males.

In the adrenal, there were no differences between hypophysectomized and sham-operated animals with respect to GST activity towards CDNB [17].

Immunochemical analysis of the pattern of GST subunits in adrenal cytosol before and after hypophysectomy

The subunit pattern of soluble GST isoenzymes in the adrenal cytosol was investigated by immunoblot analysis. By this technique it is possible to compare the levels of the same subunit under different conditions. However, the method is not strictly quantitative, and different subunits cannot be compared, since

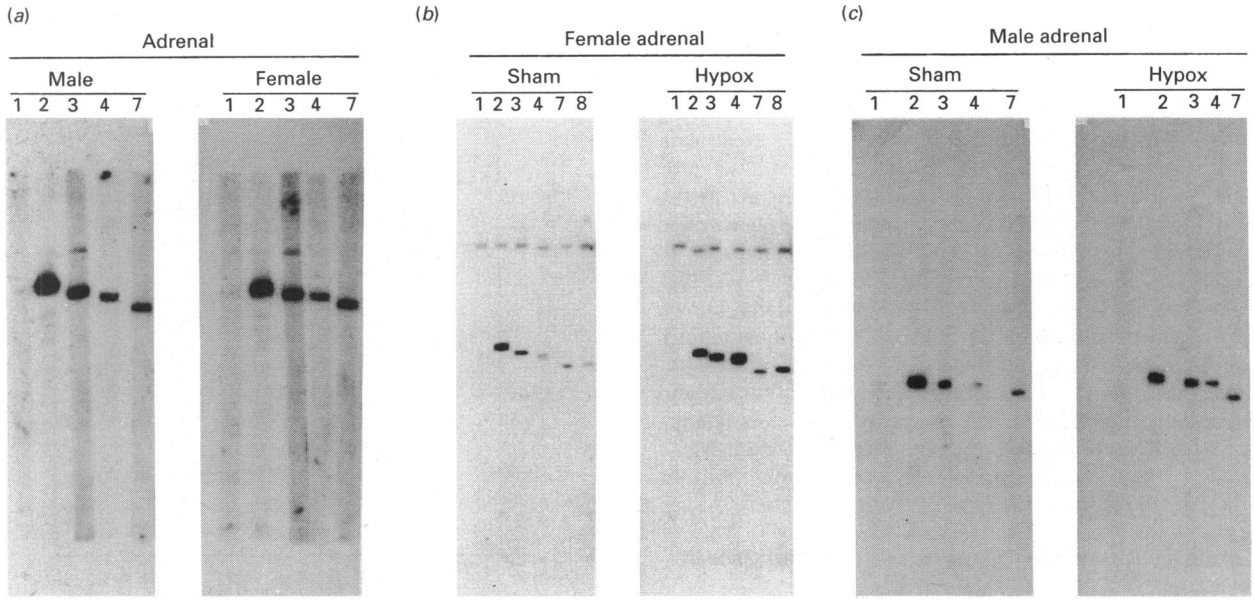

Fig. 3. Immunoblot analysis of GST subunits in adrenal cytosols of control male and female (*a*), sham-operated (Sham) and hypophysectomized (Hypox) female (*b*) and sham-operated and hypophysectomized male rats (*c*)

Samples (150 μg of cytosolic protein in each case) were subjected to SDS/PAGE, transferred electrophoretically to nitrocellulose paper and treated with specific antibodies as described in the Materials and methods section. Antibodies towards subunit 8 were not available at the time when immunoblots (*a*) and (*c*) were performed. Numbers along top of the figure indicate GST subunits.

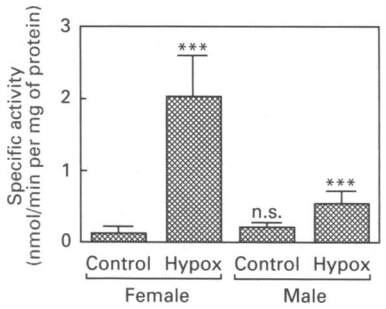

Fig. 4. Activity of GST towards *trans*-stilbene oxide in adrenal cytosol from control and hypophysectomized female and male rats

The activity was measured using the radiometric distribution assay described in the Materials and methods section. Analysis of statistical significance was performed using Student's *t* test. The values represent the means ± S.D. for experiments in each of which the adrenals from five to 20 animals were pooled. In the case of male rats, six such experiments were performed, and in the case of the females, three experiments. Levels of statistical significance (***$P < 0.001$) were obtained by comparison of hypophysectomized (Hypox) rats with control rats of the same sex and n.s. (no significance) by comparison of control female with control male rats.

the extent of the staining depends on the status of the antibody solution. As the immunoblots in Fig. 3(*a*) show, there are only slight differences between male and female rats. Neither sex demonstrated subunit 1 and male adrenal appeared to contain somewhat more of subunit 2.

At 4 weeks after hypophysectomy, the levels of several of the subunits increased in both sexes, but to different extents, as demonstrated in Figs. 3(*b*) and 3(*c*). In the female rat the levels of subunits 3, 4, and 8 increased markedly, as reported previously [17], whereas in the male rat the level of subunit 4, but not of subunit 3, increased significantly after hypophysectomy. Antibodies towards subunit 8 were not available to us at the time of analysis of the male subunit pattern.

Another remarkable sex difference was the extent of the elevation of the level of subunit 4. In order to quantify the change in the adrenal level of subunit 4, we measured its activity with *trans*-stilbene oxide, which is a selective substrate for the isoenzyme 4–4. The 4–4 enzyme demonstrates an activity which is at least 20-fold higher than those of other isoenzymes under the assay conditions employed [1]. Fig. 4 shows that, in the female adrenal, the activity of GST 4–4 increases from 0.14 to 2.0 nmol/min per mg of protein (14-fold), whereas the increase in male adrenal is not as striking, i.e., from 0.2 to 0.54 nmol/min per mg of protein (i.e. a 2-fold increase). The difference in activities between sham-operated male and female rats is not significant.

H.p.l.c. analysis of GST subunits in liver and adrenal cytosol of male and female rats: effects of sex, hypophysectomy and hormone treatment

Another method for characterizing the GST subunit pattern is separation by reverse h.p.l.c., which allows comparison of the amounts of the different subunit proteins. Chromatograms from adrenal cytosols from sham-operated and hypophysectomized female rats are shown in Figs. 5(*a*) and 5(*b*), respectively. Subunits 3, 2 and 6 are well separated from each other, but it was not possible to satisfactorily separate subunits 4 and 7. It is, however, possible to see the large increase in subunit 4 after hypophysectomy and also the increase in subunit 3. The pattern for normal rat adrenal (Fig. 5*a*) resembles that obtained by immunoblotting, with a dominance of subunit 2 and lack of subunit 1.

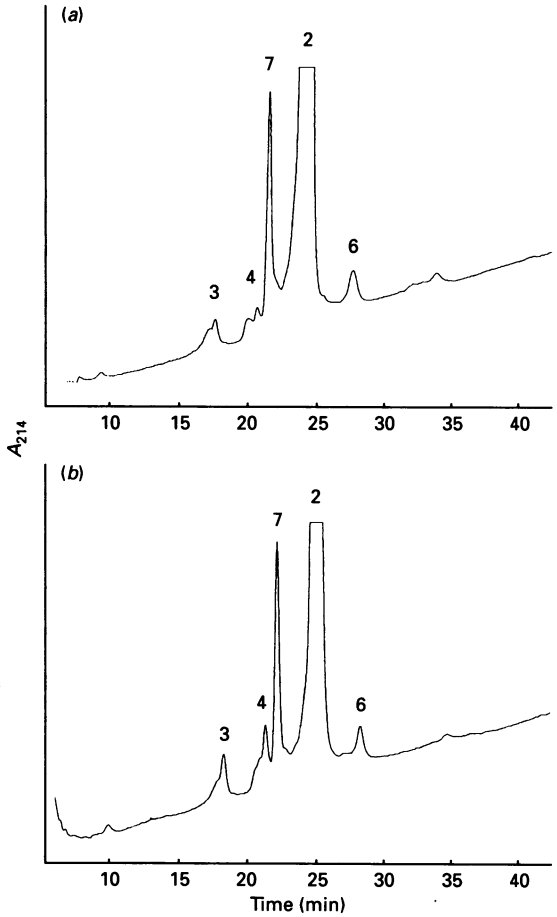

Fig. 5. H.p.l.c. pattern of glutathione transferase subunits in adrenal cytosols of sham-operated (*a*) and hypophysectomized (*b*) female rats

The cytosols were purified by affinity chromatography on *S*-hexyl-GSH–Sepharose, as described in the Materials and methods section before analysis by reverse-phase h.p.l.c. The subunits were eluted with a gradient of 0.1% trifluoroacetic acid in water and acetonitrile and detected at 214 nm. Numbers indicate GST subunits.

From the liver cytosols we could separate subunits 1, 2, 3, 4 and 6 and the areas under the peaks were integrated. Subunit 8 is eluted shortly after subunit 1, but is present in such small amounts in rat liver that it could not be detected. Subunit 1 gave rise to two peaks, as has also been observed by others [7,30].

In Figs. 6(*a*) and 6(*b*), h.p.l.c. chromatograms of GSTs from the liver cytosols of sham-operated female and male rats are shown. In males, subunit 4 and 3 were dominant, followed by subunit 2, but in females there were high levels of subunit 2 and less of subunit 3 and 4, as reported by Igarashi *et al.* [32]. Figs. 6(*c*) and 6(*d*) depict chromatograms from hypophysectomized female and male rats and show that the sex differences seen between sham-operated animals are partly eliminated by hypophysectomy; subunit 4 became by far the major subunit in both sexes and there were about equal amounts of subunits 3 and 2. Males in groups 3 and 6 have subunit patterns similar to those of control females, with levels of subunit 3 lower than those of both subunits 4 and 2 and with low levels of subunit 6 as well (Fig. 7*a*, 7*b* and 7*d*). Treatment with thyroxine + cortisone acetate was not sufficient to feminize the subunit patterns in hypophysectomized animals (Fig. 7*c*).

Tables 3(*a*) and 3(*b*) document the variations of each subunit of GST after each treatment. Administration of GH alone to hypophysectomized animals did not alter the isoenzyme pattern

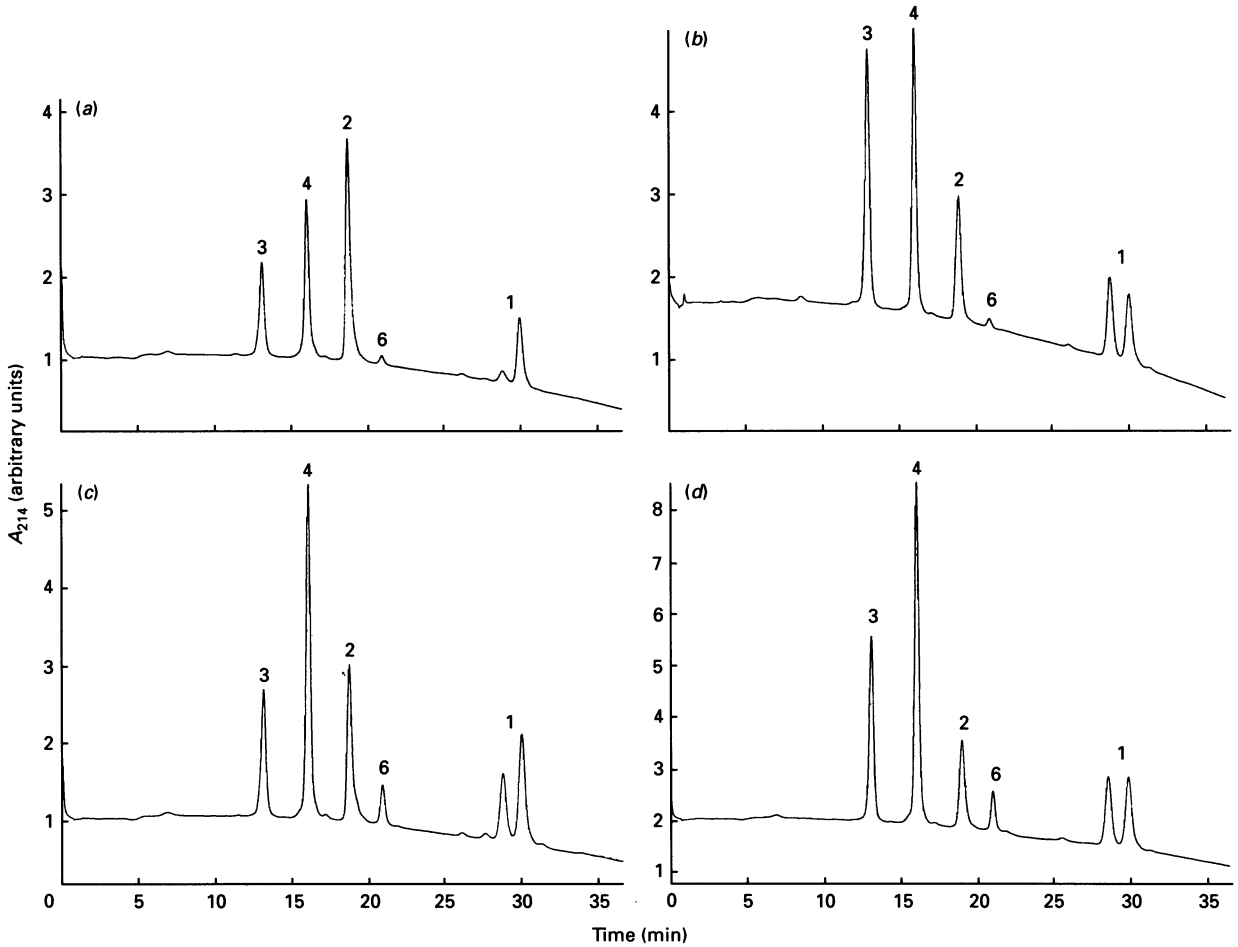

Fig. 6. H.p.l.c. pattern of GST subunits in hepatic cytosols from sham-operated female (*a*), sham-operated male (*b*), hypophysectomized female (*c*) and hypophysectomized male (*d*) rats

The cytosols were passed through a Sephadex G-25 column and then purified by affinity chromatography on *S*-hexyl-GSH–Sepharose as described in the Materials and methods section before analysis by reverse-phase h.p.l.c. The subunits were eluted with a gradient of 0.1% trifluoroacetic acid in water and acetonitrile and detected at 214 nm. Numbers indicate GST subunits.

Table 3. Variations in each GST subunit after treatment with GH alone (*a*) or with GH, thyroxine + cortisone acetate (*b*)

The peak areas from the h.p.l.c. chromatograms were integrated and divided by the area of the peak for subunit 2, which was used as an internal standard, since it is relatively unaffected by the hormonal changes examined. The values are means ± S.D. for three different animals. Analysis of statistical significance was performed using Student's *t* test. Levels of significance indicated by * were obtained by comparison of each value with the control value of the same subunit and sex. * $P < 0.05$; ** $P < 0.01$, *** $P < 0.001$. Letters indicate levels of significance on comparison with the female control value. [a] $P < 0.05$; [b] $P < 0.01$; [c] $P < 0.001$. Abbreviations used: Sham, sham-operated; hypox, hypophysectomized; T_4, thyroxine + cortisone acetate

	Sex	Treatment	GST subunit...	Relative peak area 1	3	4	6
(*a*)	♀	Sham		0.38 ± 0.04	0.42 ± 0.04	0.70 ± 0.04	0.03 ± 0.01
		Hypox		0.99 ± 0.24*	0.86 ± 0.10**	1.88 ± 0.28**	0.17 ± 0.07*
		Sham + GH		0.73 ± 0.09**	0.28 ± 0.03**	0.47 ± 0.05**	0.02 ± 0.01
		Hypox + GH		0.87 ± 0.62	0.78 ± 0.16*	1.49 ± 0.24**	0.12 ± 0.02**
	♂	Sham		0.94 ± 0.38	1.65 ± 0.34[b]	1.72 ± 0.44[a]	0.06 ± 0.005[b]
		Hypox		1.50 ± 0.80	1.28 ± 0.37[a]	2.81 ± 0.27*[c]	0.28 ± 0.05**[b]
		Sham + GH		0.42 ± 0.08	0.76 ± 0.33*	0.99 ± 0.42	0.04 ± 0.01*
		Hypox + GH		1.32 ± 0.42[a]	0.75 ± 0.10[b]	1.49 ± 0.21[b]	0.17 ± 0.06*[b]
(*b*)	♀	Sham		0.53 ± 0.07	0.22 ± 0.04	0.55 ± 0.11	0.02 ± 0.01
		Hypox		2.24 ± 0.38**	0.56 ± 0.21*	2.40 ± 0.12***	0.23 ± 0.02***
		Hypox + T_4		1.05 ± 0.21*	0.45 ± 0.13*	1.62 ± 0.12***	0.14 ± 0.04**
		Hypox + T_4 + GH		0.76 ± 0.46	0.19 ± 0.10	0.94 ± 0.09***	0.05 ± 0.03
	♂	Sham		1.15 ± 0.65	0.56 ± 0.26	1.99 ± 0.42[b]	0.05 ± 0.02
		Hypox		1.74 ± 0.95	0.73 ± 0.25	2.54 ± 0.10[c]	0.25 ± 0.04**[c]
		Hypox + T_4		0.68 ± 0.13	0.74 ± 0.32	1.82 ± 0.42[b]	0.17 ± 0.04**[b]
		Hypox + T_4 + GH		0.57 ± 0.25	0.34 ± 0.07	1.23 ± 0.26[a]	0.10 ± 0.02*[b]

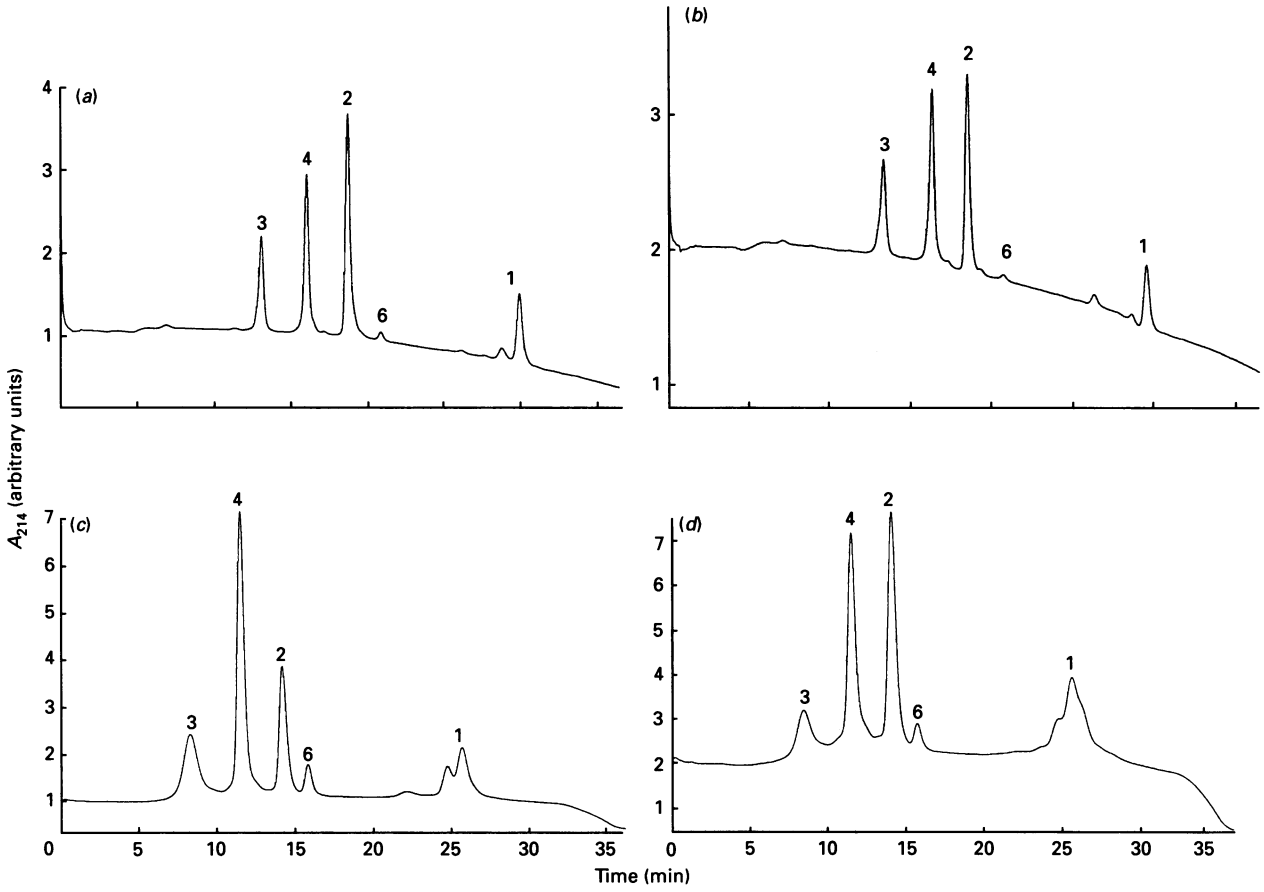

Fig. 7. H.p.l.c. pattern of GST subunits of hepatic cytosols from female rat (*a*), sham-operated male rat receiving GH (*b*), hypophysectomized male rat receiving thyroxine + cortisone acetate (*c*) and hypophysectomized male rat receiving thyroxine + cortisone acetate + GH (*d*)

The cytosols were passed through a Sephadex G-25 column and then purified by affinity chromatography on *S*-hexyl GSH–Sepharose as described under the Materials and methods section before analysis by reverse-phase h.p.l.c. The subunits were eluted with a gradient of 0.1 % trifluoroacetic acid in water and acetonitrile and detected at 214 nm. Numbers indicate GST subunits.

(Table 3*a*). The most effective down-regulation of all the GST subunits in hypophysectomized animals was obtained by GH, thyroxine and cortisone acetate (Table 3*b*). All the class-Mu GSTs were affected by the treatments to which the animals in groups 2, 3, 5 and 6 were subjected; the level of subunit 6 increased 4–10-fold after hypophysectomy in both females and males and decreased almost to the control levels. The levels of subunit 3 increased about 2-fold in females after hypophysectomy and was down-regulated to female control levels in both sexes by treatment with GH together with thyroxine and cortisone acetate. Subunit 4 was regulated in the same manner, showing approx. 4- and 2-fold increases after hypophysectomy in females and males respectively, and being down-regulated to twice the female control level. In males there was a small increase in subunit 2 after hypophysectomy that cannot, however, be considered significant.

There was no consequent variation in the levels of subunit 1. In sham-operated females, GH infusion increased the level of this subunit, whereas this same treatment decreased the levels in sham-operated male rats. However, comparison of the levels of subunit 1 in groups 2 and 6 showed a down-regulation of subunit 1 by continuous administration of GH to hypophysectomized animals of both sexes. Administration of thyroxine and cortisone acetate to hypophysectomized animals affects the level of subunit 1 and somewhat subunit 4, leaving the others on the same levels as in the hypophysectomized animals receiving no other treatment (group 2).

Finally, it should also be mentioned that although the levels of the different subunits of GST in sham-operated rats were considered to be the most appropriate control values for hypophysectomized rats, these values were, in fact, the same as in untreated rats (results not shown).

DISCUSSION

Earlier studies made in our laboratory and others have demonstrated a pituitary-dependent regulation of GSTs in the rat liver and adrenal. Several of the isoenzymes increase in amount and activity after hypophysectomy. Common to both organs is the observation that primarily the class-Mu enzymes are affected, i.e., those isoenzymes which demonstrate high activities towards epoxides [1]. In the liver the levels of subunit 1, 3, 4 and 6 increased after hypophysectomy, whereas in the adrenal subunits 3, 4 and 8 were affected.

A sexual dimorphism in the hepatic GSTs was previously reported for control rats [32]. The present results demonstrate a sexual dimorphism in the response of hepatic and adrenal GST levels to removal of the pituitary. In contrast with the liver, a sexual dimorphism in the adrenal becomes apparent only after hypophysectomy. The most striking sex difference was seen with the subunit 4, the level of which increased 14-fold in the female rat, but only 2.7-fold in the male rat after hypophysectomy. Apparently, the female adrenal GST 4–4 is more down-regulated by ACTH than is the male isoenzyme under normal physiological

conditions. The mechanism responsible for this striking sexual difference is still not known. One possibility is that the level of subunit 4 in the rat adrenal might also be under simultaneous control by hormones other than ACTH, e.g., sex steroids.

In the liver, the sexual dimorphism is larger in control than in hypophysectomized rats, which indicates that a pituitary hormone is responsible for this sex difference. Indeed, we have found that the sexual difference in the pattern of GH secretion is involved in this regulation; a high basal level of GH yields the feminine isoenzyme pattern. This is also the case for the regulation of the activities of several sex-specific isoenzymes of cytochrome *P*-450, as reported by Mode and co-workers [18,19].

In the present study we have shown that in order to obtain a female GST isoenzyme pattern in the liver of male control rats, continuous infusion of GH is sufficient, whereas simultaneous administration of thyroxine, cortisone acetate and GH is required to obtain feminization of the isoenzyme pattern in hypophysectomized rats. Results published by Mode *et al.* [18] show that administration of GH alone can affect the pattern of cytochrome *P*-450 in hypophysectomized rats, although thyroxine and cortisol/cortisone acetate are needed to obtain complete feminization.

The pituitary secretes many hormones, of which thyroid-stimulating hormone is one. Thyroid-stimulating hormone, in turn, stimulates the secretion of thyroxine and tri-iodothyronine from the thyroid gland, and these secretions are thus inhibited by hypophysectomy. Upon administration of thyroxine to hypophysectomized animals, the levels of both this hormone and tri-iodothyronine are restored. Hypophysectomy also inhibits steroid synthesis in the adrenal, by eliminating the secretion of ACTH. Cortisone acetate is a derivative of cortisol, which is synthesized from cholesterol, and it is easily taken up into cells. Thyroxine and cortisol/cortisone acetate have a general stimulating effect on various cell reactions and are thus a fundamental requirement for normal metabolism. Consequently, the absence of these substances in hypophysectomized animals may have profound effects.

Our studies here show that the class-Mu transferases, i.e., GST isoenzymes composed of subunits 3, 4 and 6, are the most sensitive to GH, thyroxine and cortisone acetate and are clearly suppressed by these factors. The most dramatic percentage increase in amount of enzyme after hypophysectomy was observed for subunit 6, although subunit 4, which demonstrated the largest absolute increase, became the dominant subunit after hypophysectomy and returned to control levels after administration of GH. Subunit 3 is regulated in the same manner, even though the changes in amounts in this case are not as striking as for the other class-Mu enzymes.

The mechanism(s) underlying hormonal regulation of the amounts and activities of GSTs in rat liver and adrenal is still not known, but our preliminary results indicate that this regulation is at least partially pretranslational in both organs. The physiological function(s) of hormonal regulation of GSTs by ACTH and GH is also unknown. The hormonal status of a hypophysectomized animal is of little relevance to the natural physiology of a prepubertal or adult animal. In the fetus, however, levels of ACTH vary during development [33]. The regulation of the activities of hepatic and adrenal GSTs observed in the present study might thus be of importance during fetal development.

This work was supported by grants from the Swedish Cancer Society and the Knut and Alice Wallenberg Foundation.

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Received 18 November 1991/10 February 1992; accepted 18 February 1992