Increase in the amount of glutathione transferase 4-4 in the rat adrenal gland after hypophysectomy and down-regulation by subsequent treatment with adrenocorticotrophic hormone

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The effect of hypophysectomy and subsequent treatment with adrenocorticotrophic hormone (adrenocorticotropin, ACTH) on the isoenzymes of glutathione transferase in the rat adrenal gland was investigated. A large increase (approx. 11-fold) in the level of transferase subunit 4 was observed in hypophysectomized animals by immunoblotting. When the activity of glutathione transferase 4-4 was measured in adrenal cytosol using *trans*-stilbene oxide as a selective substrate, a 15-fold increase was noted. Lack of the pituitary hormone ACTH is apparently related to this increase, since treatment of hypophysectomized animals with ACTH for 2 weeks partially down-regulated subunit 4. Glutathione transferase subunits 3 and 8 in the adrenal were also increased in amount by hypophysectomy, but not at all to the same extent. The activity of glutathione transferase 4-4 was elevated also in the liver and ovary (5 and 1.5 times respectively) after hypophysectomy. These elevated enzyme levels were, however, not affected by ACTH treatment. This down-regulation of glutathione transferases in the rat adrenal by ACTH may be related to the fact that, under normal conditions, this organ is highly susceptible to the toxic effects of various polycyclic hydrocarbons, whereas under circumstances where there is no ACTH production, as in hypophysectomized rats, the adrenal is resistant to these same hydrocarbons.

INTRODUCTION

The adrenal gland contains relatively high levels of various cytochrome *P*-450-dependent hydroxylases. The main function of these enzymes, both in the mitochondria and endoplasmic reticulum of the adrenal cortex, is linked to the synthesis of the various steroid hormones secreted by this organ [1]. The levels of these steroid hydroxylases are regulated by adrenocorticotrophic hormone (adrenocorticotropin, ACTH) released by the pituitary. This induction of steroid hydroxylases, resulting from the binding of ACTH to its plasma-membrane receptor, is mediated by increased levels of cyclic AMP and subsequently activated protein kinases [2,3].

It has been demonstrated that, in addition to steroid hydroxylases, the adrenal contains cytochrome *P*-450-dependent activities metabolizing xenobiotics [4,5]. The levels of these activities are also regulated by ACTH, but not by classical inducers of the cytochrome *P*-450 system, such as 3-methylcholanthrene. For example, adrenal benzo[*a*]pyrene mono-oxygenase activity is decreased dramatically in hypophysectomized rats, but is unaffected by treatment with 3-methylcholanthrene [6].

7,12-Dimethylbenz[*a*]anthracene (DMBA) is another polycyclic aromatic hydrocarbon known to be metabolized by rat adrenal cytochrome *P*-450 systems

[4]. Moreover, this hydrocarbon, which is a very potent skin and mammary carcinogen, has a strong necrotic effect on the two inner zones of the rat adrenal cortex [7,8]. The 7-hydroxymethyl derivative of DMBA (7-OHM-12-MBA), produced by liver cytochrome(s) *P*-450, but not by the adrenal enzymes [9], is thought to be directly responsible for this adrenocorticolysis [7,10]. In contrast with its parent compound, DMBA, metabolic activation of 7-OHM-12-MBA in the adrenal by monooxygenation is apparently not a prerequisite for toxicity. Such necrosis does not occur in the adrenals of rats having no ACTH production, i.e. infant or hypophysectomized rats. If ACTH is administered to such animals, they regain their adrenal sensitivity to DMBA [7]. Similar results have been obtained with cultures of rat adrenal cells [10]. The mechanism of the dependency of this toxicity on ACTH has not yet been clarified.

The general protective action of glutathione transferases in xenobiotic metabolism is well known. These enzymes catalyse the conjugation of glutathione with highly reactive electrophilic molecules, such as epoxides, and some of them also demonstrate peroxidase activity towards organic peroxides [11,12]. Such molecules, which can be deleterious to the cell, may be produced during the detoxication of xenobiotics.

In the adrenal gland, where glutathione transferases are present at relatively high levels [13,14], the isoenzyme

Abbreviations used: ACTH, adrenocorticotrophic hormone (adrenocorticotropin); DMBA, 7,12-dimethylbenz[*a*]anthracene; 7-OHM-12-MBA, its 7-hydroxymethyl derivative; PAGE, polyacrylamide-gel electrophoresis; PBS, phosphate-buffered saline (composition and pH given in the text); CDNB, 1-chloro-2,4-dinitrobenzene.

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pattern is unique, as it is in all other tissues investigated to date [11,12]. In the adrenal, glutathione transferases may influence the accumulation and secretion of steroid hormones, in addition to detoxifying reactive electrophiles [15]. An endogenous age-dependent regulation of glutathione transferases in adrenals, as well as in other organs, has been noted [16,17]. It has also been reported that testosterone is involved in the regulation of the hepatic class-Pi glutathione transferase in mice [18].

As is the case with cytochrome *P*-450-dependent steroid hydroxylases, adrenal glutathione transferases are not inducible by common inducers of xenobiotic-metabolizing enzymes as they are in other organs, e.g., the liver [9]. Since ACTH regulates the level of several enzymes in the adrenal cortex and is required for the susceptibility of this organ to the toxic effects of certain xenobiotics, we considered it of value to investigate further the influence of this hormone on detoxifying enzymes. Thus, in the present study, we have investigated the effect of hypophysectomy and subsequent ACTH administration on soluble isoenzymes of glutathione transferase in the adrenal gland, as well as in the liver and ovary of the female rat.

MATERIALS AND METHODS

Materials

trans-[³H]Stilbene oxide (2 Ci/mmol) was synthesized by Dr. Åke Pilotti, Department of Organic Chemistry, University of Stockholm, according to a published procedure [19]. The compound was purified by t.l.c. on 0.2 mm-thick-silica-gel plates (Merck, Darmstadt, Germany) with hexane/ethyl acetate (95:5, v/v) as the eluent, scraped off the plate and recovered by two extractions with ethanol. As judged by repeated t.l.c. chromatography, the purity was > 99%.

Cellulose nitrate membrane filters (Schleicher and Schuell, Dassel, Germany), ¹²⁵I-labelled Protein A (100 µCi/ml) (The Radiochemical Centre, Amersham, Bucks., U.K.) and Cibacron Blue (Sigma Chemical Co., St. Louis, MO, U.S.A.) were all purchased from the sources indicated. ACTH was kindly donated by Ciba-Geigy (Basel, Switzerland). All other chemicals used were of reagent grade and purchased from common commercial sources.

Methods

Animals, treatment and preparation of cytosolic fractions. Female Strague-Dawley rats (200 g), either hypophysectomized or sham-operated, were purchased from Moellegaards Avelslaboratorium (Skensved, Denmark). Treatment of animals (5–20 in each group) was commenced 2 weeks after surgery. ACTH (8 i.u. in 0.5 ml of acid/albumin/saline [i.e. 7.2 mM-HCl, 0.14 M-NaCl and bovine albumin (1 mg/ml)] or the vehicle alone was administered subcutaneously to the rats twice daily for 14 days. These animals had free access to a commercial rat diet [R-3 from Ewos AB (Södertälje, Sweden), containing 5% (w/w) fat, 24% protein and 49% carbohydrate].

Animals were killed between 08:00 and 10:00 h. Adrenals, livers and ovaries were removed after decapitation and either subfractionated immediately or stored at –20 °C for several days before subfractionation. Adrenals and ovaries from each group of animals

were pooled separately, homogenized and subfractionated in 0.25 M-sucrose/20 mM-Tris/HCl, pH 7.4. Individual livers were subfractionated in 0.25 M-sucrose. The homogenates (10–20%, w/v) were first centrifuged at 900 *g* for 10 min and the resulting supernatants were re-centrifuged at 10000 *g* for 10 min. The cytosolic fractions were obtained by centrifugation of the supernatants thus obtained for 1 h at 105000 *g*. The mean weights of the different organs under the different conditions employed are shown in Table 1.

Protein was determined as described by Lowry *et al.* [19], with bovine serum albumin as standard.

Enzyme assay. Glutathione transferase activity towards *trans*-stilbene oxide was measured as described by Gill *et al.* [20]. *trans*-[³H]Stilbene oxide (100 µM in 0.1 M-potassium phosphate, pH 6.5) was incubated at 37 °C for 15–30 min with the cytosolic fractions from the different organs (1–150 µg of protein). The reaction was terminated by extraction with hexan-1-ol, and aliquots of the aqueous phase were quantified by liquid-scintillation counting.

In the kinetic experiments K_m and V_{max} values were determined graphically from Lineweaver-Burk plots.

Glutathione transferase activity towards 1-chloro-2,4-dinitrobenzene (CDNB) was determined spectrophotometrically as described by Habig *et al.* [21].

Antibodies. Rat glutathione transferase isoenzymes 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 by Mannervik *et al.* [22], and antibodies against glutathione reductase were raised in rabbits as described by Carlberg *et al.* [23].

SDS/polyacrylamide-gel electrophoresis (SDS/PAGE) and immunoblot analysis. SDS/15%-(w/v)-PAGE was performed by the method of Laemmli [24]. The separated

Table 1. Mean weights of the different organs in sham-operated (Sham), hypophysectomized (Hypox), hypophysectomized + ACTH-treated (Hypox + ACTH) and sham-operated + ACTH-treated (Sham + ACTH) rats

The weights shown for the adrenal and ovary are for each individual organ. The values represent the means for three different experiments $\pm$ S.D. for sham-operated and hypophysectomized rats, two different experiments $\pm$ S.D. for hypophysectomized + ACTH-treated rats and one experiment for sham-operated + ACTH-treated rats. In each experiment adrenals and ovaries from 5 to 20 rats were pooled separately.

Organ	Conditions	Mean weight (mg/100 g body wt.)
Adrenal	Sham	10.6 $\pm$ 1.0
	Hypox	4.4 $\pm$ 0.4
	Hypox + ACTH	5.6 $\pm$ 0.2
	Sham + ACTH	11.5
Ovary	Sham	30.5 $\pm$ 5.4
	Hypox	21.0 $\pm$ 0.6
	Hypox + ACTH	18.7 $\pm$ 3.5
	Sham + ACTH	28.4
Liver	Sham	(4.6 $\pm$ 0.78) $\times 10^3$
	Hypox	(4.6 $\pm$ 0.24) $\times 10^3$
	Hypox + ACTH	(3.9 $\pm$ 0.54) $\times 10^3$
	Sham + ACTH	4.0 $\times 10^3$

proteins were transferred electrophoretically on to 0.2 μm -pore-size nitrocellulose membrane filters [25]. The nitrocellulose sheets were then treated essentially as described by Berzins and co-workers [26]. The sheets were blocked with 3% (w/v) bovine serum albumin for at least 2 h, washed four times for 15 min and cut into strips. Each strip was incubated separately for approx. 12 h with specific antibodies directed against glutathione transferase 1-1, 2-2, 3-3, 4-4, 8-8 or glutathione reductase. Antibodies against the human π form demonstrate complete cross-reactivity with rat subunit 7 and were used for detection of this subunit.

The nitrocellulose strips were subsequently incubated for 1 h with ^{125}I -labelled Protein A (0.1 $\mu\text{Ci}/\text{ml}$) in incubation buffer A [1% (w/v) bovine serum albumin, 0.1% (v/v) 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) containing 0.02% (w/v) NaN_3], washed four times for 15 min in incubation buffer A containing 0.2% (w/v) bovine serum albumin and dried, and the immunocomplexes were revealed by autoradiography.

Quantification of the staining on autoradiograms was performed by densitometry.

RESULTS

Isoenzymes of cytosolic glutathione transferase in untreated mature female rats

Upon SDS/PAGE, proteins dissociate into their

subunits. By subsequent immunoblotting, the various subunits of the dimeric cytosolic glutathione transferases in the adrenal could be revealed. Sham-operated rats, which were considered to be the most appropriate control for hypophysectomized animals, demonstrated the same subunit pattern and levels of enzyme activities as untreated rats (results not shown).

Fig. 1 shows the isoenzyme pattern of cytosolic adrenal glutathione transferases in sham-operated rats. Subunit 2 was the most abundant subunit, whereas subunit 1 seemed to be absent or below the level of detection using this method. This pattern is in agreement with previous reports [11,14]. Subunits 4, 7 and 8 were present in relatively low amounts, whereas subunit 3 was intermediate in concentration.

Effects of hypophysectomy and subsequent treatment with ACTH

The most striking difference observed between sham-operated and hypophysectomized rats was the large increase in the amount of subunit 4 obtained in the adrenal after hypophysectomy. Judging from densitometric measurements of the autoradiogram, the elevation was 11-fold (Fig. 2). This increase was partially reversed when the hypophysectomized rats were treated with ACTH (8 i.u., twice daily for 2 weeks). In addition, the concentrations of subunits 3 and 8 were apparently affected by hypophysectomy. Whether the increases in the concentration of these subunits were also partially reversed by ACTH or not is difficult to judge from the

Fig. 1. Immunoblot analysis of glutathione transferase subunits in cytosol fractions from rat adrenals

Samples (150 μg of cytosolic protein was used in each case) were subjected to SDS/PAGE, transferred electrophoretically to nitrocellulose paper and treated with specific antibodies towards subunits 1, 2, 3, 4, 7 and 8 and glutathione reductase (GR) (as indicated). Cytosol from sham-operated (Sham), hypophysectomized (Hypox) and hypophysectomized rats subsequently treated with ACTH (Hypox + ACTH) were compared. The weak band seen near the top of the nitrocellulose strips in some experiments was probably an artefact caused by albumin.

Fig 2. Quantification of glutathione transferase subunits 1, 2, 3, 4, 7 and 8 and glutathione reductase (GR) (as indicated) in cytosol from rat adrenals

Densitometric measurements of an autoradiogram from the immunoblot analysis was performed. Cytosol from sham-operated (Sham), hypophysectomized (Hypox) and hypophysectomized rats subsequently treated with ACTH (Hypox + ACTH) were compared.

autoradiographs. Subunits 1, 2 and 7 and glutathione reductase seemed to be unaffected by hypophysectomy.

Quantification of effects using a diagnostic substrate and inhibitor

In order to quantify the increase in subunit 4 on the basis of its enzymic activity, *trans*-stilbene oxide was chosen as a diagnostic substrate [12]. Glutathione transferase 4-4 has at least a 20-fold higher activity with *trans*-stilbene oxide than any other rat isoenzyme investigated, which means that, under the conditions used here, the activity measured was catalysed primarily by this isoenzyme.

In order to determine optimal conditions, *trans*-stilbene oxide conjugation was measured as a function of time, protein amount and substrate concentration at a constant glutathione concentration (5 mM). In Fig. 3 the activities obtained with cytosol from hypophysectomized rats are shown. The velocity was found to be approximately constant for 30 min, and a *trans*-stilbene oxide concentration of 100 μ M was chosen to approach saturation. Similar results were obtained under all conditions for all three organs examined. The K_m and V_{max} values in the case of the adrenal cytosol activity were calculated to be 36 μ M and 1.4 $\text{nmol} \cdot \text{min}^{-1} \cdot \text{mg}^{-1}$ respectively (Fig. 3).

Fig 3. Glutathione transferase activity towards *trans*-stilbene oxide in rat adrenal cytosol

Activity is shown as a function of protein amount (30 min, 50 μ M-*trans*-stilbene oxide) (a), time (100 μ M-*trans*-stilbene oxide, 50 μ g of protein) (b) and substrate concentration (30 min, 50 μ g of protein) (c). A Lineweaver-Burk plot is also shown (d). The vertical bars indicate the S.D..

Table 2. Activity towards *trans*-stilbene oxide of glutathione transferase in rat adrenal cytosol from sham-operated (Sham), hypophysectomized (Hypox), hypophysectomized + ACTH-treated (Hypox + ACTH) and sham-operated + ACTH-treated (Sham + ACTH) animals

The activity towards *trans*-[³H]stilbene oxide was measured with the distribution assay described under 'Methods', under the optimal conditions determined in the experiment illustrated in Fig. 3. The values represent the means for three different experiments $\pm$ S.D. for hypophysectomized rats, two different experiments $\pm$ S.D. for sham-operated rats and for hypophysectomized + ACTH-treated rats, and one experiment for sham-operated + ACTH-treated rats. In each experiment, adrenals and ovaries from five to 20 rats were pooled separately.

Organ	Conditions	Specific activity (nmol $\cdot$ min ⁻¹ $\cdot$ mg ⁻¹)	Increase (fold)
Adrenal	Sham	0.13 $\pm$ 0.1	1.0
	Hypox	2.0 $\pm$ 0.6	15
	Hypox + ACTH	0.53 $\pm$ 0.0	4.0
	Sham + ACTH	0.12	0.9
Liver	Sham	7.5 $\pm$ 0.7	1.0
	Hypox	38.3 $\pm$ 3.7	5.1
	Hypox + ACTH	38.9 $\pm$ 6.9	5.2
	Sham + ACTH	8.1	1.1
Ovary	Sham	3.8 $\pm$ 0.1	1.0
	Hypox	6.0 $\pm$ 0.3	1.6
	Hypox + ACTH	5.3 $\pm$ 0.9	1.4
	Sham + ACTH	4.0	1.0

Table 3. Activity towards CDNB of glutathione transferases in rat cytosol from rat adrenal, liver and ovary

Rats were sham-operated (Sham), hypophysectomized (Hypox), hypophysectomized + ACTH-treated (Hypox + ACTH) and sham-operated + ACTH-treated (Sham + ACTH). 5 μ M-Cibacron Blue was used as an inhibitor. The values represent the means for two different experiments $\pm$ S.D., except for 'Sham + ACTH', where one experiment was done. In each experiment, the adrenals and ovaries from five to 20 rats were pooled separately.

Organ	Inhibitor ... Conditions	None		5 μ M-Cibacron Blue	
		Specific activity (μ mol $\cdot$ min ⁻¹ $\cdot$ mg ⁻¹)	Activity (% of control)	Specific activity (μ mol $\cdot$ min ⁻¹ $\cdot$ mg ⁻¹)	Activity (% of control)
Adrenal	Sham	0.52 $\pm$ 0.13	100	0.37 $\pm$ 0.13	100
	Hypox	0.44 $\pm$ 0.07	84	0.34 $\pm$ 0.14	92
	Hypox + ACTH	0.40 $\pm$ 0.15	77	0.25 $\pm$ 0.06	68
	Sham + ACTH	0.41	79	0.23	62
Liver	Sham	1.1 $\pm$ 0.4	100	0.48 $\pm$ 0.16	100
	Hypox	2.0 $\pm$ 1.4	180	0.47 $\pm$ 0.24	98
	Hypox + ACTH	2.4 $\pm$ 1.6	220	0.68 $\pm$ 0.23	140
	Sham + ACTH	1.4	130	0.64	130
Ovary	Sham	0.23 $\pm$ 0.02	100	0.10 $\pm$ 0.02	100
	Hypox	0.26 $\pm$ 0.02	110	0.11 $\pm$ 0.01	110
	Hypox + ACTH	0.22 $\pm$ 0.07	96	0.12 $\pm$ 0.05	120
	Sham + ACTH	0.27	110	0.13	130

The amount of protein and the time of incubation used in this assay were varied within the linear range for the different organs and after different treatments, depending on the level of activity.

Table 2 presents cytosolic adrenal glutathione transferase activities towards *trans*-stilbene oxide for the various groups of rats. In hypophysectomized rats this activity was increased about 15-fold in the adrenals as compared with sham-operated rats. In the hypophysectomized animals subsequently treated with ACTH the activity was about 4-fold that of sham-operated animals, but significantly down-regulated as compared with hypophysectomized rats. ACTH thus partially reversed

the increase in enzyme level, but was without effect on sham-operated rats.

When CDNB was used as substrate (Table 3), no significant differences in cytosolic adrenal glutathione transferase activity in the different groups of animals were observed. The explanation for this finding is probably the high K_m value of glutathione transferase 4-4 for CDNB as compared with other isoenzymes [27]. Glutathione transferase subunit 2, which, judging from immunoblotting, was unaffected by hypophysectomy, has a high affinity for CDNB and is also the most abundant subunit in adrenal cytosol. At an appropriate concentration Cibacron Blue inhibits the activities of the

Fig 4. Immunoblot analysis of glutathione transferase subunits in cytosol from (a) rat liver and (b) ovary

Samples (150 μ g of cytosolic protein was used in each case) were subjected to SDS/PAGE, transferred electrophoretically to nitrocellulose paper and treated with specific antibodies towards subunits 1, 2, 3, 4, 7 and 8 and glutathione reductase (GR) (as indicated). Cytosol from sham-operated (Sham), hypophysectomized (Hypox) and hypophysectomized rats subsequently treated with ACTH (Hypox + ACTH) were compared. The weak band seen near the top of the nitrocellulose strips in some experiments was probably an artefact caused by albumin.

different glutathione transferases towards CNDB to different extents, since this inhibitor binds to the different subunits with different affinities [27]. A concentration of 5 μ M was chosen here to inhibit all subunits except 2

(Table 3). There was no increase in the non-inhibitable rate of CDNB conjugation in hypophysectomized rats compared with sham-operated rats, which is in agreement with the immunoblot analysis of subunit 2. A small

decrease in hypophysectomized + ACTH-treated rats and sham operated + ACTH-treated rats can, however, not be excluded.

Effects on the liver and ovary

For comparison, the liver and ovary were also investigated. Fig. 4 shows the patterns of glutathione transferase subunits in the cytosol of these two organs. In liver there was an increase in the levels of subunits 3 and 4 in hypophysectomized animals, as seen in the adrenal, although the extents of the hepatic increases were smaller. Subunit 8, however, seemed unaffected in this organ, which was also the case for subunits 1, 2 and 7 and glutathione reductase. The hepatic activities towards *trans*-stilbene oxide were 7.5 and 38.3 nmol·min⁻¹·mg of protein⁻¹ for sham-operated and hypophysectomized rats respectively (Table 2), an increase of about 5-fold. A 2-fold increase in the hepatic activity towards CDNB in hypophysectomized as compared with sham-operated rats was noted, which most probably reflects the high content of glutathione transferase 4-4 in rat liver (see Fig. 4). A difference between the adrenal and liver was that the increase in subunits 3 and 4 in the liver of hypophysectomized rats could not be down-regulated by ACTH (Fig. 4 and Table 2). This was not unexpected, since liver is not a major target organ for ACTH.

In rat ovary no differences were observed upon immunoblotting (Fig. 4), but a slight increase in cytosolic glutathione transferase activity towards *trans*-stilbene oxide in hypophysectomized (6.0 nmol·min⁻¹·mg of protein⁻¹) as compared with sham-operated rats (3.8 nmol·min⁻¹·mg of protein⁻¹) was observed (Table 2). ACTH had no effects on the ovarian glutathione transferase levels.

DISCUSSION

The aim of the present investigation was to elucidate the possible effects of ACTH on the soluble glutathione transferases in the rat adrenal gland. Glutathione transferases are either homo- or hetero-dimers, consisting of subunits within one of the three classes Alpha, Mu and Pi [22]. the following combinations have been identified in the rat adrenal: Alpha: 1-1, 1-2, 2-2 and 8-8; Mu: 3-3, 3-4, 4-4 and 6-6; Pi: 7-7 and 5-5 (not yet classified) [the present investigation; 11].

A large increase in subunit 4, as measured both by immunoblotting and by activity towards *trans*-stilbene oxide, was observed in adrenals from hypophysectomized rats as compared with sham-operated animals. This increase reflects an elevation in glutathione transferase 3-4 and/or 4-4. Since subunit 3 did not increase to the same extent as subunit 4, we conclude that the increased level of subunit 4 reflects primarily an elevation of glutathione transferase 4-4. Part of the elevated level of subunit 3 indicated by immunoblotting might also be the result of immunological cross-reactivity between the closely related subunits 3 and 4 [22].

The fact that ACTH down-regulates subunit 4 indicates that this pituitary hormone is responsible for the different enzyme levels observed under the different conditions examined. The presence of ACTH thus represses the synthesis of this protein, whereas it induces the steroid hydroxylases (see the Introduction). This change in glutathione transferase subunit 4 might partially explain the resistance of the adrenal to necrosis

induced by DMBA and 7-OHM-DMBA when the pituitary has been removed, since under these conditions an increased level of protective detoxifying enzymes are present.

Also of interest in this respect is the fact that the class Mu enzymes are highly active towards epoxides [28], which are reactive compounds often produced during xenobiotic metabolism or, as in the case of cholesterol epoxide [29], produced from endogeneous substrates. Glutathione transferase 4-4 has also been shown to have high activity towards peroxidized DNA [30]. One reason for the high level of glutathione transferases in the adrenal gland might thus be to protect the cells from injury caused by oxygen toxicity. It has been suggested that the adrenal gland is exposed to relatively high concentrations of reactive oxygen species as a result, among other things, of its high content of cytochromes P-450 [31].

Other glutathione transferase subunits in the rat adrenal, such as 3 and 8, were also increased by hypophysectomy, but not to the same extent, whereas still others, i.e. 1, 2 and 7, were unaffected. Transferase isoenzymes are thus, as shown previously, regulated independently [32].

In the liver as well, the largest increase after hypophysectomy was seen with subunit 4. However, this increase could not be down-regulated by ACTH. Growth hormone may be the pituitary hormone responsible for the regulation of hepatic subunit 4. As in the adrenal, subunit 3 also increased in the liver, but subunit 8 was unaffected by hypophysectomy.

The ovarian glutathione transferases were not affected to the same extent as the liver and adrenal enzymes, if at all. There may have been a small increase in the amount of subunit 4, whereas subunit 1 might have decreased as a consequence of hypophysectomy. These tentative results need, however, to be further investigated before any firm conclusions can be drawn.

Whether the mechanism by which ACTH regulates adrenal levels of cytosolic glutathione transferases is of the same nature as the regulation of adrenal cytochromes P-450, that is, involving increased cyclic AMP levels and activated protein kinases, or whether the regulation is in this case indirect, for example, mediated by an increased level of steroid hormones, requires further investigation. The importance of this regulation in steroid and xenobiotic metabolism and toxicity/genotoxicity remains to be elucidated.

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