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EFFECTS OF MICROSOMAL ENZYME INDUCERS ON GLUTATHIONE *S*-TRANSFERASE ISOENZYMES IN LIVERS OF RATS AND HAMSTERS

ARMELLE FOLIOT* and PHILIPPE BEAUNE

INSERM U 75, Université René Descartes, CHU Necker, 156 rue de Vaugirard 75730, Paris, France

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Abstract—The effects of microsomal enzyme inducers on glutathione *S*-transferase (GST) isoenzymes were studied in livers of rats and hamsters using three hypolipidemic drugs of the peroxisome proliferator type and the two model substances phenobarbital (PB) and 3-methylcholanthrene (MC). The effects were investigated by immunoblot analysis of the various GST subunits using polyclonal antibodies directed to rat subunits 1–4. In untreated animals the subunit composition was different, with hamsters having a much higher content of class mu isoenzymes. Administration of all three hypolipidemic drugs reduced the protein concentration of both alpha and mu class GSTs in rats but reduced only class mu subunits in hamsters. This reduction was in good agreement with the decreased activity observed with the broad-spectrum substrate 1-chloro-2,4-dinitrobenzene (CDNB) in both species. As expected, PB and MC increased GST activity together with the concentration of subunits 1 and 3 in rats. In hamsters, PB significantly increased subunit 1 and slightly reduced subunits 3 and 4, although this decrease was not significant. Total GST, measured with CDNB, was reduced by 17%. In contrast, MC slightly decreased subunit 1 and markedly raised subunits 3 and 4, resulting in a net increase in total GST activity. All drugs increased relative liver weight, microsomal protein concentration and total P450 in both species; in contrast, total cytosolic proteins were raised by all drugs in rats but not in hamsters, except for MC. The results obtained in these two species show that GST activity is not always increased by microsomal enzyme inducers. The response may depend in part on isoenzyme profile, and varies with the subunit considered.

Key words: glutathione *S*-transferase; inducers; liver; rat; hamster; *in vivo*

Many drugs and xenobiotics are microsomal enzyme inducers [1], especially in the liver, the main organ involved in their metabolism and excretion. Among these are fibrates, widely used as hypolipidemic agents. When administered to rats, these drugs produce: PB† and MC as model substances; hepatomegaly; proliferation of the endoplasmic reticulum; an increase in the hepatic content of proteins and P450; and an elevation in the activity of monooxygenases, epoxide hydrolases and glucuronosyltransferases [2–6]. In contrast with PB and MC, as previously shown [7–9], fibrates decrease the activity of cytosolic GSTs (EC 2.1.5.18), although total cytosolic proteins are increased [10, 11]. The mechanism of this fibrate effect has not yet been established. It could result from either a decrease in GST proteins, a relative increase in other cytosolic proteins or even a direct interaction of drugs with these enzymes.

GSTs play an important role in drug biotransformation and the metabolism of xenobiotics, both as enzymes and as binding proteins [12]. As enzymes they conjugate, with glutathione, a large number of electrophilic compounds, such as peroxides, epoxides, alkyl- and arylhalides; as binding proteins they bind covalently to strong electrophiles and non-covalently to a number of non-substrate ligands, such as bilirubin. In mammals, these enzymes, which are homo- and heterodimers of different subunits, belong to four classes (alpha, mu, pi and theta) according to their gene and aminoacid sequences, enzymatic properties and immunological cross-reactivity [13–15]. Present in all organs studied, they are abundant primarily in liver cells [16]. In rat liver the major subunits are 1, 2 and 3, 4 which belong to the alpha and mu classes, respectively [17]. Some isoenzymes, especially those belonging to alpha class exhibit Se-independent glutathione peroxidase activity which plays an inhibitory role in overall lipid peroxidation, preventing membrane damage [18, 19]. Since lipid peroxidation has been proposed as a cause of carcinogenesis induced by peroxisome proliferators, such as fibrates [20], changes not only in GST activity but also in the expression of the various isoenzymes may be of importance in this process. This group has previously shown that reduction in GSH conjugating activity by fibrates leads to a reduction in excretion of compounds conjugated with glutathione in rats [7, 10], and that the deficient induction of this activity by PB was

* Corresponding author. Tel. 40 61 56 30; FAX 40 61 55 82.

† Abbreviations: PB, phenobarbital; MC, 3-methylcholonthrene; GST, glutathione *S*-transferase; CDNB, 1-chloro-2,4-dinitrobenzene; DCNB, 1,2-dichloro-4-nitrobenzene; EA, ethacrynic acid; ENPP, 1,2-epoxy-3-(*p*-nitro-phenoxy) propane; TPBO, *trans*-4-phenylbut-3-en-2-one; BSP, bromosulphophthalein; CuOOH, cumene hydroperoxide.

responsible for its absence of effect on the hepatic transport of BSP in hamsters [21]. In the latter study, it was clearly shown that hamsters displayed a poor ability to conjugate some substrates, despite very high glutathione-conjugating activity with the broad-spectrum substrate CDNB and that, except for GST activity, they were as responsive to PB induction as rats, since it increased their relative liver weight and microsomal enzyme activity.

The present study was designed: (1) to investigate whether fibrates decrease the activity of cytosolic GSTs by reducing their protein content; and (2) to determine whether the response of GST activity to inducers is dependent on the isoenzyme profile and whether it varies, as for P450s, with the isoenzyme considered. Therefore, the effects of three fibrates and those of the model substances PB and MC on hamster liver GSTs, in comparison with well-characterized rat liver GSTs, were investigated.

MATERIALS AND METHODS

Chemicals. Polybuffers 96 and 74, Pharmalyte (pH 8–10.5) and PBE 94 were obtained from Pharmacia Fine Chemicals (Uppsala, Sweden). Glutathione and glutathione–agarose were purchased from Sigma (St Louis, MO, U.S.A.); the conjugated second antibodies were from Dako (Copenhagen, Denmark). Anti-human GST pi antibody and anti-rat GST 1–4 antibodies were purchased from Bioprep (Stillorgan, Dublin, Ireland). All other chemicals were of the highest quality available from Prolabo (Paris, France) or from Sigma (St Louis, MO, U.S.A.).

Animals and treatment. Male Sprague–Dawley rats weighing 200–220 g (Charles River, France) and male Syrian hamsters weighing 100–110 g (Depré, Saint Doulchard, France) were fed with appropriate UAR standard chow and injected once daily i.p. with either 100 mg/kg body weight sodium phenobarbital, 400 mg/kg body weight clofibrate, 100 mg/kg body weight fenofibrate, 100 mg/kg body weight nafenopin, 20 mg/kg body weight MC or 1 mL/kg body weight vehicle for 4 days. Compounds were injected in saline for PB and in corn oil for the others, with control animals receiving the corresponding vehicle. Because GST activities of these control groups were not significantly different from one another, they were combined. Animals were killed 24 hr after the last drug dose.

Livers were removed, weighed, perfused with chilled saline and cytosolic and microsomal fractions were prepared as described previously [21]. Protein concentration was determined by the method of Lowry *et al.* [22].

Enzyme assays. Measurement of the enzymatic activity of GSTs towards the glutathione conjugation of CDNB, DCNB, EA, ENPP, TPBO, BSP was performed at 37° according to Habig and Jakoby [23], and CuOOH according to Paglia and Valentine [24].

Total microsomal P450 concentration was measured according to Omura and Sato [25].

Partial purification of GST isoenzymes. GST isoenzymes were purified essentially as described by Alin *et al.* [26]. The cytosol fraction (30 mg protein) was loaded onto an affinity column of GSH–agarose (2.5 × 10 cm), equilibrated with 10 mM sodium phosphate, pH 7.0, containing 150 mM KCl and 1 mM EDTA (buffer A). The column was washed with 150 mL of buffer A until no further protein could be detected in the effluent. The bound GSTs were eluted with 50 mM Tris, 150 mM KCl, 10 mM GSH, 0.5 mM dithiothreitol, pH 9.6, containing 10% glycerol (buffer B). The GST-containing fractions were pooled, adjusted to pH < 8.0 by addition of 2 M sodium phosphate, pH 7.0, and concentrated by ultrafiltration (Amicon Diaflo PM-10 filter) to ~6 mL. GST isoenzymes were subsequently separated by chromatofocusing in the range of pH 7.5–10.5 on a PBE 94 column (1 × 30 cm) equilibrated with 25 mM triethylamine–HCl, pH 10.5. The concentrate (containing ~20 mg protein) was adjusted to pH 9.0 with 25 mM triethylamine immediately before chromatofocusing. Elution was first performed with 250 mL of 1:170 dilute Pharmalyte (8–10.5), pH 9.0, and subsequently with a mixture of 1.5 mL Pharmalyte (8–10.5) and 7 mL Polybuffer 96 diluted to 300 mL with deionized water and adjusted to pH 7.0 with 1 M HCl. The flow rate was 25 mL/hr. The effluent was monitored spectrophotometrically at 280 nm and protein-containing fractions were collected in 2.5 mL fractions. GST activity in each fraction was determined using CDNB as substrate. Each peak was concentrated on Diaflo and characterized for enzyme activity. Homogeneity was checked by immunoblotting. All steps were performed at 4°.

Immunoblotting analysis. Equal amounts of liver cytosol fractions from both control and treated animals were separated by sodium dodecyl sulphate (SDS)–polyacrylamide gel electrophoresis on gels of 12.5% acrylamide, according to Laemmli [27]. Proteins were stained with Coomassie brilliant blue R-250 or electrotransferred on nitrocellulose sheets as described by Towbin *et al.* [28]. Immunoblotting analysis was performed using rabbit polyclonal antibodies raised against rat GST subunits 1–4 and swine anti-rabbit-IgG conjugated with horseradish peroxidase as staining antibody. In a preliminary study, it was verified that no antibody showed any cross-reactivity with other classes of GST, despite a slight reactivity with other GSTs of the same class, and that they showed good reactivity against the same GST classes in hamsters. The relative amounts of various GST subunits per mg of cytosol protein were determined by quantitative densitometry of the immunoblots. The level of each subunit in control cytosols was arbitrarily selected as the reference (100%) value for comparison with cytosols of treated animals. A standard curve for each subunit was constructed by electrophoresing serial dilutions of control liver cytosol, blotting, scanning the immunoblots and plotting the integrated area of the densitometry peak vs cytosolic protein concentration. Comparisons of control or treated cytosols were made on 4 μg of cytosolic protein. To verify the quality and reproducibility of individual blots, one cytosol sample was selected as a control and was run on every blot processed. When samples from different blots were compared values were normalized to a common control value.

Table 1. Effects of the various inducers on liver weight and the concentration of cytosolic and microsomal proteins and P450

Groups	Liver weight (% of body weight)	Cytosolic proteins (mg/g liver)	Microsomal proteins (mg/g liver)	P450 (nmol/mg protein)
Rats				
Control	4.43 ± 0.25	55.7 ± 2.0	19.3 ± 1.4	0.72 ± 0.08
PB	6.53 ± 0.37‡	62.4 ± 3.1*	31.1 ± 3.4‡	1.73 ± 0.23‡
MC	5.75 ± 0.23‡	53.8 ± 2.6	24.5 ± 1.4†	1.25 ± 0.08‡
Clofibrate	5.78 ± 0.45‡	57.4 ± 5.1	23.3 ± 1.6*	0.98 ± 0.11‡
Fenofibrate	6.31 ± 0.31‡	57.2 ± 2.6	24.6 ± 1.1†	0.93 ± 0.11†
Nafenopin	6.66 ± 0.40‡	57.4 ± 3.1	24.3 ± 1.4†	0.83 ± 0.17*
Hamsters				
Control	4.04 ± 0.14	57.4 ± 2.2	20.6 ± 1.1	0.85 ± 0.08
PB	5.07 ± 0.17‡	49.2 ± 2.6*	27.5 ± 2.5‡	1.96 ± 0.25‡
MC	5.24 ± 0.22‡	57.2 ± 4.5	26.1 ± 1.4‡	1.71 ± 0.14‡
Clofibrate	4.71 ± 0.19‡	54.5 ± 6.5	25.8 ± 2.0‡	1.18 ± 0.08‡
Fenofibrate	4.87 ± 0.24‡	52.9 ± 5.4	25.9 ± 2.0‡	1.60 ± 0.14‡
Nafenopin	4.91 ± 0.29‡	53.1 ± 4.2	26.3 ± 2.3‡	1.33 ± 0.14‡

Drugs were administered i.p. daily for 4 days and animals were killed 24 hr after receiving the last dose. Results are means ± SD for eight animals.

Significantly different from control animals: *$P < 0.05$; †$P < 0.01$; ‡$P < 0.001$.

Table 2. Effects of three hypolipidemic drugs, PB and MC on the level of rat liver GST subunits 1–4

Treatment	Relative subunit concentration*				Relative GST activity† (% of control)
	1	2	3	4	
PB	1.68 ± 0.21‖	0.91 ± 0.09	1.96 ± 0.29‖	1.13 ± 0.13	168‖
MC	1.64 ± 0.20‖	1.04 ± 0.19	1.31 ± 0.20§	1.36 ± 0.43‡	148§
Clofibrate	0.79 ± 0.12‖	0.91 ± 0.07	0.70 ± 0.09‖	0.72 ± 0.13‖	73§
Fenofibrate	0.60 ± 0.17‖	0.72 ± 0.25§	0.61 ± 0.07‖	0.61 ± 0.09‖	66‖
Nafenopin	0.65 ± 0.09‖	0.67 ± 0.14§	0.57 ± 0.0§	0.56 ± 0.14‖	67‖

The relative concentration of subunits was determined by quantitative densitometry of immunoblots. The subunit concentrations of the control group were arbitrarily given a value of 1.

* Results are means ± SD for eight animals, each analysed in duplicate.

† GST activity averaged 1650 ± 226 nmol of CDNB conjugated/min/mg of cytosolic protein in control animals.

* Significantly different from control animals: ‡$P < 0.05$; §$P < 0.01$; ‖$P < 0.001$.

Statistical analysis. The data were compared by variance analysis. When this analysis indicated a significant difference the means were compared by the Student's *t*-test. Multiple comparisons between several experimental groups and one control group were carried out using Scheffe's test.

RESULTS

Effects on liver weight and the concentration of cytosolic and microsomal proteins and P450

As expected, all drugs increased relative liver weight and the microsomal protein concentration both in hamsters and rats; the microsomal concentration of P450 was also significantly and similarly enhanced. In contrast, total cytosolic proteins expressed per whole liver were increased by all drugs in rats but not in hamsters, except for MC which raised them markedly (Table 1).

Effects on GST activity

In rats, the specific GST activity of the cytosolic fraction of the liver, measured with the broad-spectrum substrate CDNB, was decreased by all three hypolipidemic drugs, as previously described [10]. As expected, it was increased by PB and MC, (Table 2). In hamsters, administration of the three hypolipidemic drugs also decreased this activity, but PB failed to increase it and MC was a good inducer (Table 3).

Separation of hepatic GSTs

GSTs from rat and hamster livers were purified by the use of affinity chromatography. The GST proteins recovered from the affinity column averaged 6.1 ± 0.7% of total cytosolic proteins, with a specific activity towards CDNB of 24 ± 1 μmol/min/mg protein for rats (N = 6) and 12.3 ± 1.1% with a specific activity of 111 ± 19 μmol/min/mg protein for hamsters (N = 6). Recovery of GST activity (85 ± 8%) and relative purification were similar in both species. The separation by chromatofocusing on PBE 94 of the different forms of GST with basic

Table 3. Effects of three hypolipidemic drugs, PB and MC on the level of hamster liver GST subunits 1–4

Treatment	Relative subunit concentration				Relative GST activity† (% of control)
	1	2	3	4	
PB	1.70 ± 0.20‖	1.06 ± 0.27	0.85 ± 0.24	0.82 ± 0.26	83§
MC	0.85 ± 0.10‡	1.17 ± 0.20	1.78 ± 0.19§	1.39 ± 0.16§	171‖
Clofibrate	1.10 ± 0.18	0.98 ± 0.15	0.79 ± 0.15‡	0.77 ± 0.11§	87‖
Fenofibrate	1.11 ± 0.29	0.96 ± 0.10	0.76 ± 0.14§	0.73 ± 0.11‖	82‖
Nafenopin	1.03 ± 0.35	0.92 ± 0.17	0.59 ± 0.12‖	0.66 ± 0.10‖	63‖

The relative concentration of subunits was determined by quantitative densitometry of immunoblots. The subunit concentrations of the control group were arbitrarily given a value of 1.

Results are means ± SD for eight animals, each analysed in duplicate.

† GST activity averaged 6500 ± 450 nmol of CDNB conjugated/min/mg of cytosolic protein in control animals.

* Significantly different from control animals: ‡$P < 0.05$; §$P < 0.01$; ‖$P < 0.001$.

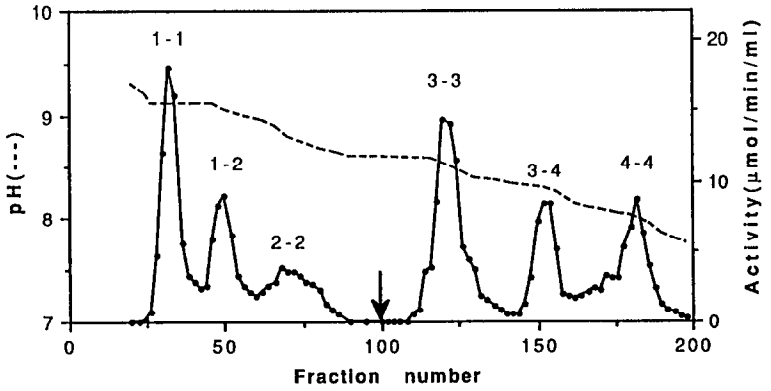

Fig. 1. Chromatofocusing elution profile of the GST isoenzymes of rat liver. The chromatographic conditions are described in Materials and Methods. GST activity was measured with CDNB as substrate. The arrow indicates the change of eluant.

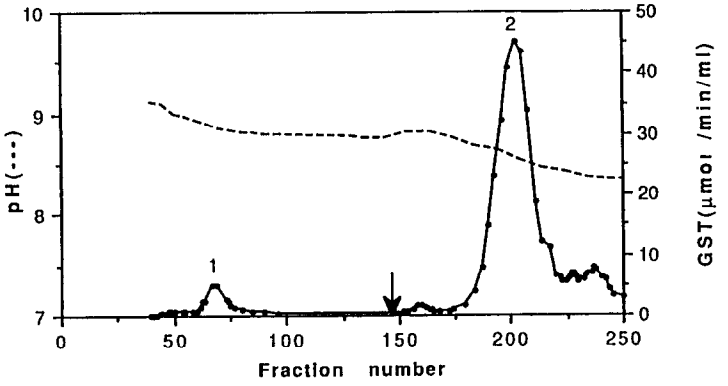

Fig. 2. Chromatofocusing elution profile of the GST isoenzymes of hamster liver. The chromatographic conditions are described in Materials and Methods. GST activity was measured with CDNB as substrate. The arrow indicates the change of eluant.

and neutral pH values is reported in Fig. 1 for rats and in Fig. 2 for hamsters. Six peaks were obtained for rats and two main peaks for hamsters, under the same experimental conditions. As previously reported [26], immunoblotting of the isoenzyme fractions showed that, in rats, iso-enzyme 2–2 was always contaminated with subunit 1. Immunoblotting of the fractions in hamster separation revealed the

Table 4. Substrate specificities of cytosolic hamster liver GST

Substrate	Specific activity	
	Peak 1	*Peak 2*
CDNB	50.20	338.00
DCNB	0.10	0.58
TPBO	0	0.01
EA	1.07	0.11
ENPP	0	1.57
CuOOH	20.00	1.40
BSP	0	0.06
Protein (mg)	1.50	2.98

Number of peaks refers to order of elution from chromatofocusing column. Peak 1 included fractions from 63 to 74; peak 2 fractions from 190 to 213. GST activity is expressed in μmol/min/mg protein except for conjugation of CuOOH expressed in μmol of oxidized NADPH/min/mg protein. Results are means of two separate experiments.

presence of two or three different subunits in each fraction. Peak 1 reacted only with antibodies raised against rat alpha class GSTs 1 and 2, while peak 2 reacted primarily with antibodies raised against rat mu class GSTs 3 and 4 and weakly with those directed to alpha class GSTs. Thus, peak 1 seemed to be composed of two main subunits, while peak 2 appeared to be contaminated by alpha class subunits. Each fraction was assayed for reactivity with antisera for rat alpha and mu GSTs and all isozymes reacted, thus these antisera could be used to determine the content of different subunits in hamster liver. These subunits were arbitrarily termed "1", "2", "3" and "4" to allow comparison with rat liver. No fraction reacted with antiserum for human placental pi class GST, either in rats or hamsters. In addition to CDNB, six other substrates were used to characterize hamster GST activity in the two main peaks separated by chromatofocusing on PBE 94. The GST protein in peak 1 displayed specific activity comparable to that of rat liver for CDNB, and was consistent with alpha class for the other substrates, especially CuOOH. GSTs in peak 2, assigned to mu class from immunoblot analysis, could be differentiated from known rat mu GSTs by their very high activity with CDNB and their low activity towards DCNB, TPBO and BSP. It is noteworthy that the amount of protein in this peak was twice that of peak 1 (Table 4).

Effects on GST isoenzyme profile

As shown in Table 2, administration of all three hypolipidemic drugs to rats reduced the concentration of both alpha and mu class GSTs from 21 to 44%, depending on the subunit considered. This reduction was highly significant and is in good agreement with the decreased activity observed with the broad-spectrum substrate CDNB. PB and MC, two good inducers of GST activity in rats, increased the concentration of subunits 1 and 3; subunit 2 was not modified and subunit 4 was slightly increased by MC. In hamsters, administration of the same hypolipidemic drugs left the level of alpha class subunits unchanged, but reduced mu class enzymes to the same extent as in rats. PB significantly increased subunit "1" and slightly reduced mu class subunits, although this decrease was not significant. In contrast, MC moderately decreased subunit "1" and increased both subunits "3" and "4" to a greater extent than in rats (Table 3). In both species, no pi class GST was detected in any of the cases, in treated or untreated animals.

DISCUSSION

The results of this study indicate that administration of various microsomal enzyme inducers leads to markedly different effects on liver cytosolic GSTs in hamsters and rats. PB and MC were, as previously shown [29–31], good inducers of this activity in rats. Immunoblot analysis of rat cytosols treated with either PB or MC were in good agreement with previously published data concerning effects on subunit composition [32, 33]. Subunits 1 and 3 were primarily elevated and appeared to be responsible for the total elevation in GST transferase activity. Administration of clofibrate, fenofibrate or nafenopin, three hypolipidemic drugs belonging to peroxisome proliferators, decreased total GST activity, as reported previously [10], along with increasing total cytosolic proteins. Such a decrease in GST activity could result from a lower concentration of GST protein or might be related to a direct interaction with the enzyme. Although any inhibition of *in vitro* GST activity could not be shown (personal observation) by using clofibrate or nafenopin at a concentration of 3 mM, Awasthi *et al.* [8] using ciprofibrate, another hypolipidemic agent and peroxisome proliferator, observed irreversible inhibition of this activity. The latter observation is not consistent with the fact that, *in vivo*, decreased activity was apparent only after two daily doses of clofibrate and was resolved only 5 days after arrest of treatment [7]. In the present study, all subunits decreased after treatment with all three hypolipidemic drugs as shown by immunoblotting. Since these drugs produced a sharp increase in total cytosolic proteins, it may be argued that the decrease is only apparent due to a possible marked increase in other cytosolic proteins, such as FABPs [34–36] or bifunctional enoyl CoA hydratase [37]. On the contrary, results of immunohistochemical staining for GST pi on hepatocellular carcinomas produced by administering rats either diethylnitrosamine associated with clofibrate or diethylnitrosamine alone indicated that less GST pi was detected when associated with clofibrate, although the total number of lesions was significantly greater [38]. On the other hand, Schramm *et al.* [39] have reported that treating rats with perfluorodecanoic acid, a structural analogue of naturally occurring fatty acids and a potent peroxisome proliferator, decreased the levels of various GST activities as well as GST proteins synthesized *in vitro* by translating isolated hepatic mRNAs. This decrease in the amount of *in vitro* translated GST proteins was not apparent 1 day after treatment with perfluorodecanoic acid, and only became patent 3 days later, although a strong inhibitory effect of this compound on GST

activity *in vitro* was clearly noted. These results suggest that the decrease in the various GST enzyme activities was caused mainly by repression rather than by inhibition of GSTs *in vivo*. Nevertheless, this peroxisome proliferator did not produce the same effect in mouse liver [40]. Indeed, this inhibitory effect of peroxisome proliferators might occur only in rat, and thus be species-related. But in another study [41], it was observed that the administration of clofibrate or nafenopin did not raise the concentration of liver cytosolic proteins in hamsters, although it did decrease total GST activity. The present data confirm this preliminary observation: in hamsters none of the three hypolipidemic drugs had any influence on hepatic cytosolic protein concentration, but did reduce total GST activity. This reduction was linked to a preferential decrease in the subunits recognized by antibodies directed to mu class GSTs of rat liver. The subunits recognized by antibodies directed to alpha class were unmodified. Since the mu class in hamster liver represented two thirds of the GST protein recovered after partial purification, and the bulk of total GST activity (the specific activity of these subunits towards CDNB was approximately six times higher than that of alpha class, as shown in Table 4), this decrease is consistent with the reduction in total GST activity observed with CDNB as substrate. Thus, the response of hamster liver GSTs appeared to be different from that of rat liver. This difference was much more evident when the effects of PB on this activity were considered. Subunit "1" was markedly raised while subunits "3" and "4" were slightly reduced. Although this reduction was not significant, decreased GST activity was observed. This differential response to PB at the enzyme activity level was previously observed by this group and others [21, 42], and has been confirmed by immunoblotting analysis of the various GSTs. In contrast, MC, which, as expected, proved to be a good inducer of total GST activity [41, 43], increased subunits "3" and "4", but somewhat decreased subunit "1". It is noteworthy that all drugs tested in this study increased relative liver weight and microsomal protein concentration, as well as total P450, in both species, and may thus be considered microsomal enzyme inducers.

To check the quality of antibodies directed to rat GSTs and to validate their use in this study, partial purification of hepatic GSTs both in rats and hamsters was performed. Partial purification confirmed this groups previous observation [21] that, in hamsters, several isoforms were present but were not identical to those of rats. Hamster GSTs present in peak 2 and reacting with antibodies directed to rat liver mu class GSTs exhibited more pronounced differences—very high activity with CDNB, weak activity with DCNB and very weak activity with TPBO and BSP—consistent with activities determined using the cytosolic fraction [21]. These results are in good agreement with those of Bogaards *et al.* [44], who purified and characterized two alpha and two mu class GSTs from hamster liver. Although GST isoenzymes were separated by a more reliable and efficient procedure than used here each class contained a major and minor isoenzyme, with the major mu class isoenzyme contaminated by an alpha class isoenzyme, as in this experiment. Chromatofocusing pHs of the main isoenzymes were similar to those of the two main peaks in this work. However, specific activities of hamster GST isoenzymes towards CDNB and other substrates were surprisingly low compared to these results (Table 4) or to those published concerning purified rat liver GSTs [28, 45] and partially purified hamster liver GSTs [46–48]. In addition, the amount of GST protein recovered from the affinity column was 2-fold higher in hamsters than in rats. This higher concentration and the higher specific activity of mu class isoenzymes are in agreement with the very strong cytosolic activity towards CDNB observed in hamsters compared to other species [21, 42, 49].

This comparative study of the effects of some microsomal enzyme inducers on cytosolic GST isoenzymes in liver of rats and hamsters has enabled it to be shown that: (1) hypolipidemic drugs of the peroxisome proliferator type reduce GST activity *in vivo* in both species, presumably by decreasing the amounts of GST proteins; (2) PB induces GST activity only in rats; in hamsters, despite an inducive effect on subunit "1", the repressive effect predominates, since it decreases mu class GSTs, the major isoenzymes in this species; (3) MC is a good inducer of GST activity in both species, although in hamsters it slightly decreases subunit "1". In conclusion, the differential behaviour of hypolipidemic drugs compared with PB and MC indicates that not all microsomal enzyme inducers enhance GST activity.

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