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DEFICIENT INDUCTION OF SULFOBROMOPHTHALEIN CONJUGATING ACTIVITY BY PHENOBARBITAL IN HAMSTER LIVER

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Abstract—Administration of phenobarbital, a known inducer of glutathione *S*-transferase activity in rat liver, failed to stimulate sulfobromophthalein (BSP) conjugation by liver cytosol in hamsters. The latter displayed poor ability to conjugate this substrate, despite very high glutathione-conjugating activity with the broad-spectrum substrate 1-chloro-2,4-dinitrobenzene (CDNB). Of the six substrates tested, in this species, 1,2-epoxy-3-(4-nitrophenoxy)propane (ENPP) was the only one whose conjugation was greatly enhanced by phenobarbital (+172%). Nevertheless, hamsters proved as responsive to phenobarbital induction as rats, since it increased their relative liver weight and microsomal enzyme activity. The deficient induction of liver BSP-conjugating activity observed with phenobarbital is consistent with the finding that it did not affect the hepatic transport of this substrate in hamsters.

Phenobarbital is a potent inducer of liver enzymes involved in the biotransformation of drugs and of several physiological compounds. Its administration to rats raises the concentration of cytochrome P-450 in the liver, as well as the activity of related monooxygenases [1, 2], UDP-glucuronosyltransferase [3], epoxide hydrolase [4] and several glutathione *S*-transferases [5]. This enzyme stimulation enhances excretion by the liver of compounds such as sulfobromophthalein (BSP) [6], bilirubin [7] and drugs mainly excreted in bile [8]. Nevertheless, no such enhancement was observed in a study in the hamster [9], and despite rises in liver weight and the concentration of liver cytochrome P-450, phenobarbital administration failed to increase bile flow and BSP Tm. Biliary excretion of BSP is dependent on its conjugation with glutathione, since a glutathione derivative is preferentially excreted by the liver in several species [10, 11]. As induction [12] or reduction [13] of the BSP-conjugating capacity of the liver either augments or reduces biliary excretion of this dye, we compared the glutathione *S*-transferase activity (EC 2.5.1.18) of hamster liver with that of rat liver, and examined the effect of phenobarbital on this activity. Various parameters of the xenobiotic-metabolizing enzyme system were measured as indices of this enzyme's induction produced by phenobarbital.

MATERIALS AND METHODS

Chemicals. Bilirubin, BSP, cytochrome *c* type III, 5-5′-dithiobis-(2-nitrobenzoic acid), 1-chloro-2,4-dinitrobenzene (CDNB), cumene hydroperoxide (CH), 1,2-epoxy-3-(4-nitrophenoxy)propane (ENPP), ethacrynic acid (EA), glutathione, glutathione reductase type III, 4-nitrobenzyl chloride (4-NBC), 4-nitrophenol (4-NP) and uridine diphosphoglucuronic acid (UDPGA) were purchased from Sigma Chemical Co, St. Louis, U.S.A. 1,2-dichloro-4-nitrobenzene (DCNB) was obtained from Aldrich Chemical Co, Belgium; NADPH was from Boehringer Mannheim, F.R.G.

Animals. Male golden Syrian hamsters weighing 100–110 g (Fichot, Ormesson, France) and male Sprague–Dawley rats weighing 200–220 g (Charles River, France), were fed with appropriate UAR standard chow, and injected once daily i.p. with either 100 mg·kg body wt^{-1} sodium phenobarbital or 1 ml·kg body wt^{-1} saline for either 4 or 10 days. Animals were killed 24 hr after the last drug dose.

Glutathione-conjugating activity in vitro. Livers were removed, weighed, perfused with chilled saline and homogenized in two volumes of ice-cold buffer containing 0.001 M EDTA, 0.003 M 2-mercaptoethanol, 0.03 M sodium phosphate and 0.25 M sucrose, pH 7.4. The homogenate was centrifuged at 10,000 *g* for 10 min, and the 10,000 *g* supernatant, at 100,000 *g* for 60 min. The resulting supernatant and pellet were the sources of cytosolic and microsomal enzymes respectively. The protein concentration in the cytosolic and microsomal fractions was determined by the method of Lowry *et al.* [14].

Cytosolic glutathione *S*-transferase activity (subsequently referred to as glutathione *S*-transferase) was measured with the following six substrates: 1 mM CDNB, 1 mM DCNB, 0.5 mM 4-NBC, 0.5 mM ENPP or 0.2 mM EA in each case according to the method of Habig [15], and with 0.22 mM BSP, according to Goldstein and Combes [16]. Cytosolic

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Table 1. Effect of phenobarbital (PB) on the activity of liver cytosol glutathione S-transferase

Groups	Cytosolic proteins (mg·g liver^{-1})	Glutathione S-transferase activity (nmol·min^{-1}·mg protein^{-1}) BSP*	CDNB†	DCNB‡	4 NBC§	EA‖	ENPP¶
Hamsters							
Control	61.0 ± 1.9	1.2 ± 0.1	5520 ± 600	18·5 ± 0·6	432 ± 24	34.8 ± 1.4	93 ± 4
PB-treated	56.0 ± 2.0	1.3 ± 0.1	5060 ± 400	18·6 ± 0.6	517 ± 17	39.6 ± 1.6	253 ± 12
P	N.S.	N.S.	N.S.	N.S.	<0.01	<0.05	<0.001
Rats							
Control	59.5 ± 1.0	45.0 ± 0.1	1830 ± 40	73 ± 3	278 ± 9	29.5 ± 1.3	109 ± 5
PB-treated	66.1 ± 2.4	71.2 ± 5.0	2920 ± 150	98 ± 6	391 ± 24	35.1 ± 0.7	148 ± 4
P	<0.05	<0.001	<0.001	<0.01	<0.001	<0.01	<0.001

Phenobarbital (100 mg·kg body wt^{-1}) was administered i.p. daily, for 4 days and animals were killed 24 hr after receiving the last dose. Results are means ± SEM for 8 animals. * Sulfobromophthalein; † 1-chloro-2,4-dinitrobenzene; ‡ 1,2-dichloro-4-nitrobenzene; § 4-nitrobenzyl chloride; ‖ ethacrynic acid; ¶ 1,2-epoxy-3-(4-nitrophenoxy)propane.

glutathione peroxidase activity was determined with 1.2 mM CH by the method of Paglia and Valentine [17]. In a preliminary study, we verified that conditions for optimal enzyme activity were identical in both hamsters and rats for all the substrates used. Liver glutathione was estimated as described by Ellman [18].

Microsomal drug-metabolizing enzymes. The concentrations of cytochrome P-450 and cytochrome *b* 5 were measured according to Omura and Sato [19]. NADPH cytochrome *c* reductase activity was measured by the method of Mazel [20]. UDP-glucuronosyltransferase activity was determined with 4-NP [21] and bilirubin [22] on digitonin-activated microsomes as previously described [23].

The Student *t*-test was used for statistical analysis of results.

RESULTS

As shown in Table 1, the rate of BSP-glutathione synthesis by liver cytosol was found to be much lower in hamsters than in rats. In contrast, hamsters exhibited very high glutathione-conjugating activity with CDNB. CDNB is a substrate for all rat liver glutathione *S*-transferases. However, with DCNB as substrate, this activity was lower in hamster than in rat. We therefore studied glutathione-conjugating activity with three other substrates, 4-NBC, EA and ENPP, and found that it was similar in hamsters and rats.

In hamsters, administration of phenobarbital failed to increase BSP conjugation with glutathione even when it was given for 10 days instead of 4 (data not shown). The same absence of effect was observed for the conjugation of CDNB and DCNB. 4-NBC and EA conjugation were slightly enhanced, by 20 and 14% respectively. In contrast, ENPP conjugation rose drastically, by 172%. In rats, the conjugation of all these substrates was induced and the cytosolic protein concentration increased. CH glutathione peroxidase activity was not modified by phenobarbital in rats and only increased slightly in hamsters whereas the concentration of reduced glutathione, the co-substrate of glutathione *S*-transferase, was enhanced to the same extent in both species (Table 2). Kinetic studies of hamster liver ENPP conjugation with glutathione showed that the

Table 2. Effect of phenobarbital (PB) on the activity of liver cytosol glutathione peroxidase and the concentration in reduced glutathione

Groups	CH-glutathione* peroxidase activity (nmol·min^{-1}·mg protein^{-1})	Reduced glutathione (μmol·g liver^{-1})
Hamsters		
Control	466 ± 20	5.5 ± 0.2
PB-treated	538 ± 22	7.1 ± 0.3
P	<0.05	<0.001
Rats		
Control	133 ± 5	5.7 ± 0.2
PB-treated	138 ± 6	7.3 ± 0.4
P	N.S.	<0.01

Phenobarbital (100 mg·kg body wt^{-1}) was administered i.p. daily, for 4 days and animals were killed 24 hr after receiving the last dose. Results are means ± SEM for 8 animals.

* Cumene hydroperoxide glutathione peroxidase activity was expressed in nmol NADPH oxidized·min^{-1}·mg protein^{-1}.

Table 3. Effect of phenobarbital (PB) on glutathione conjugation of ENPP in hamster liver

Hamsters	Glutathione		ENPP	
	V_{max}	K_m	V_{max}	K_m
Control	154 ± 18	2.58 ± 0.19	295 ± 49	1.00 ± 0.06
PB-treated	328 ± 34	2.65 ± 0.10	609 ± 57	0.96 ± 0.07
P	<0.01	N.S.	<0.01	N.S.

Cytosol was isolated from hamsters killed 24 hr after the last of 4 daily i.p. doses of 100 mg·kg body wt^{-1} phenobarbital. V_{max} are expressed in nmol·min^{-1}·mg cytosolic protein^{-1} and K_m, in mM.

Values are means ± SEM for 8 animals.

increase in this enzyme's conjugating activity was mainly due to an increase in the apparent V_{max} for both ENPP and glutathione. No difference in K_m values was observed (Table 3).

As expected, administration of phenobarbital to hamsters and rats increased relative liver weight and the microsomal protein concentration. The microsomal concentrations of cytochrome P-450 and *b* 5 rose significantly to about the same levels in hamsters and rats. NADPH-cytochrome *c* reductase, 4-NP and bilirubin glucuronosyltransferase activities were also significantly and similarly enhanced in both species (Table 4).

DISCUSSION

The results of the present study show that despite very high liver glutathione-conjugating activity, the capacity of hamsters to conjugate BSP is poor compared to that of rats. Several glutathione *S*-transferases have been isolated from rat liver and can be distinguished by their substrate specificity [24]. This is why, in hamster liver, we studied cytosolic glutathione *S*-transferase activity with several substrates. We also studied CH glutathione peroxidase activity, as it was reported to be a property of glutathione *S*-transferases [25]. With the broad-spectrum substrate CDNB, hamster liver cytosol displayed specific glutathione *S*-transferase activity that was three times higher than that of rats, as previously reported [26]. With DCNB, this activity in the hamster was 25% of that of the rat, and with BSP, 2%. With the other substrates, glutathione *S*-transferase activity was very similar in both species.

The very low BSP conjugating activity in hamster is consistent with its lower hepatic transport of BSP compared to that of the rat [9]. Similar species variations in the metabolism and excretion of BSP were previously noted by Klaassen and Plaa for rats, rabbits and dogs [27]. Administration of phenobarbital has been observed to increase hepatic transport of BSP in rats [6, 28] but not in hamsters [9]. Although Klaassen and Plaa reported that the increased BSP excretion in the bile of phenobarbital-treated rats was not due to enhanced BSP conjugation but rather to an increase in bile flow [6], we wondered whether phenobarbital would induce BSP conjugation in hamsters. However, no induction of BSP conjugating activity was observed in these animals, even when phenobarbital was administered for 10 days instead of 4. The ability of hamsters to conjugate other substrates with glutathione remained essentially unchanged except for ENPP, whose conjugation was greatly enhanced. The increase in V_{max} in response to phenobarbital treatment was not accompanied by any change in the K_m value. It is noteworthy that ENPP conjugating activity increased much more in hamsters than in rats. The degree of induction of rat liver glutathione *S*-transferases was comparable to that reported by Kaplowitz [5]. In both hamster and rat, we observed a rise in the liver glutathione concentration, one of the known effects of phenobarbital in the rat [29]. Since ENPP conjugating activity was stimulated by phenobarbital, hamsters

Table 4. Effect of phenobarbital (PB) on liver weight and microsomal drug-metabolizing enzymes

Groups	Liver wt /body wt (%)	Microsomal proteins (mg·g liver^{-1})	Cytochromes		NADPH cytochrome *c* reductase	UDP-Glucuronosyltransferase	
			P-450 (nmol·mg prot^{-1})	*b* 5 (nmol·mg prot^{-1})		4-NP* (nmol·min^{-1}·mg prot^{-1})	Bilirubin (nmol·min^{-1}·mg prot^{-1})
Hamsters							
Control	4.70 ± 0.11	20.5 ± 0.9	1.19 ± 0.07	0.31 ± 0.01	202 ± 8	33.5 ± 0.8	0.78 ± 0.04
PB-treated	5.73 ± 0.12	27.3 ± 1.1	2.24 ± 0.10	0.44 ± 0.02	324 ± 13	51.8 ± 3.2	1.32 ± 0.09
P	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
Rats							
Control	4.69 ± 0.10	22.2 ± 0.7	1.11 ± 0.04	0.54 ± 0.02	129 ± 4	45.3 ± 2.8	0.99 ± 0.03
PB-treated	5.73 ± 0.10	29.2 ± 0.7	2.55 ± 0.05	0.65 ± 0.02	215 ± 6	61.0 ± 3.2	1.67 ± 0.08
P	<0.001	<0.001	<0.001	<0.001	<0.001	<0.01	<0.001

Phenobarbital (100 mg·kg body wt^{-1}) was administered i.p. daily, for 4 days and animals were killed 24 hr after receiving the last dose. Results are means ± SEM for 8 animals. * 4-Nitrophenol.

appeared to be responsive to this inducer. Indeed, all the components of the microsomal metabolism were stimulated similarly in both hamsters and rats.

The present data suggest that in the hamster, separate mechanisms control the respective activities of microsomal and cytosolic enzymes and also of the different glutathione *S*-transferases. Such separate regulation has been shown in the rat. Thus, in this species, the activity induced by phenobarbital differed for each glutathione *S*-transferase in accordance with its subunit composition, since this drug enhanced the concentration of mRNAs specific for subunit Ya but not of those specific for subunit Yc [30]. Although here we did not isolate or characterize the different glutathione *S*-transferases in hamster liver, it is likely that several isoforms are present but are not identical with those of rats, as shown by the poor ability of the hamster to conjugate BSP and by the selective induction of ENPP conjugation by phenobarbital in this species. The deficient induction of liver BSP-conjugating activity by this drug might explain the previous observation that it had no effect on the hepatic transport of BSP in hamsters [9].

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