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DIFFERENTIAL INDUCTION OF RAT LIVER MICROSOMAL UDP-GLUCURONOSYLTRANSFERASE ACTIVITIES BY VARIOUS INDUCING AGENTS

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Abstract—The selectivity of various inducers of UDP-glucuronosyltransferase was investigated in rat liver microsomes and compared with their effect on monooxygenase reactions. (1) Similar to 3-methylcholanthrene β-naphthoflavone selectively stimulated the glucuronidation of 1-naphthol and 4-methylumbelliferone (GT_1 substrates). (2) In contrast, DDT preferentially enhanced the glucuronidation of morphine, 4-hydroxybiphenyl (GT_2 substrates) and bilirubin, similar to phenobarbital. (3) Clofibric acid and bezafibrate selectively enhanced bilirubin glucuronidation without affecting GT_1 and GT_2 reactions. (4) Similar to ethoxyquin and Aroclor 1254, trans-stilbene oxide enhanced both GT_1 and GT_2 activities but not bilirubin glucuronidation. (5) In contrast to 3-methylcholanthrene-type inducers which induce both cytochrome $P\text{-}450_{MC}$ and GT_1, probably through a common receptor protein, ethoxyquin and trans-stilbene oxide markedly induced GT_1 reactions without affecting benzo[a]pyrene monooxygenase.

It is well established that inducing agents of the 3-methylcholanthrene- (MC)‡ or phenobarbital-type [3] cause a selective increase of distinct forms of microsomal cytochrome P-450 and thus stimulate different monooxygenase reactions [2, 4]. Conjugation with glucuronic acid catalysed by UDP-glucuronosyltransferase (GT) is quantitatively the most important phase II reaction [5, 6]. Similar to cytochrome P-450 GT probably consists of a family of closely related enzyme forms with differing substrate specificity and inducibility [7, 8]. Two enzyme forms which are selectively induced by MC or phenobarbital in rat liver, tentatively designated GT_1 and GT_2, respectively, have recently been separated and purified [9]. These enzyme forms can also be distinguished by their perinatal development [10] and tissue distribution [11]. There is accumulating evidence that bilirubin [12] and oestrone [13] are substrates of yet other enzymes forms of GT.

Induction studies with aryl hydrocarbon 'responsive' and 'nonresponsive' mice [14] and with hepatoma cell cultures [15] have led to the concept that certain forms of cytochrome P-450 and GT are cooperatively induced by MC-type inducing agents.

In the present report the selectivity of various inducers of GT was investigated with regard to multiple enzyme forms and compared with their effects on monooxygenase reactions. GT_1 was assayed with 1-naphthol and 4-methylumbelliferone as substrates [8, 9], GT_2 with morphine and 4-hydroxybiphenyl [9]. A comparison of the inducibility of phase I and II reactions may be particularly interesting in light of the current concept that a balance between activating and inactivating reactions is decisive for the accumulation of reactive metabolites within cells.

MATERIALS AND METHODS

Chemicals

1-[1-^{14}C]Naphthol, [*N*-methyl-^{14}C]morphine hydrochloride, [4-^{14}C]oestrone and [G-^{3}H]-benzo[a]pyrene were from Amersham Buchler, Braunschweig, West Germany. Unlabelled chemicals were obtained from the following sources: Aroclor 1254 from Monsanto Chemical Co., St. Louis, MO; Brij 58 (a condensate of hexadecyl alcohol with 20 moles ethylene oxide/mole) from Atlas, Essen, West Germany; clofibric acid (sodium salt) from ICI-Pharma, Plankstadt, West Germany; bezafibrate from Boehringer, Mannheim, West Germany; trans-stilbene oxide from EGA-Chemie, Steinheim, West Germany (it was twice recrystallized from methanol before use, melting point 69–70°); 3-methylcholanthrene from Ferak, West Berlin and 4-methylumbelliferyl-β,D-glucuronide from Serva, Heidelberg, West Germany. 7-Ethoxycoumarin was a kind gift from Prof. V. Ullrich, Homburg/Saar, West Germany.

Treatment of animals and preparation of liver microsomes

Male Wistar rats (180–240 g) were fed a commercial diet (Altromin, Lage, Lippe, West Germany). Inducing agents were dissolved in 0.5 ml olive oil and given once i.p. unless specified. Doses: β-

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‡ Abbreviations and definitions: GT, UDP-glucuronosyltransferase (EC 2.4.1.17); DDT, 1,1,1-trichloro-2,2-bis(*p*-chlorophenyl)ethane; MC, 3-methylcholanthrene. Cytochrome $P\text{-}450_{MC}$ is used operationally as the MC-inducible form of cytochrome P-450 most closely related to arylhydrocarbon hydroxylase. It may be identical with rat liver microsomal cytochrome $P\text{-}450_c$ [1] and may be related to mouse liver cytochrome P_1-450 as defined by Nebert [2] despite its different spectral properties.

naphthoflavone (100 mg/kg); DDT (160 mg/kg); Aroclor 1254 (500 mg/kg); trans-stilbene oxide (50–400 mg/kg, once daily for 4 days). Clofibric acid, sodium salt (300 mg/kg, dissolved in H_2O) or bezafibrate (300 mg/kg, suspended in H_2O and subsequently dissolved by addition of 1 N NaOH, pH was adjusted between 8 and 9) was given once daily s.c. under the back skin for 7 days.

Microsomes were prepared as previously described [7]. Microsomal preparations were diluted to 10 mg protein/ml with 0.25 M sucrose containing 5 mM Tris–HCl buffer, pH 7.4, and stored at −20° for up to 4 weeks. No decrease of GT activities was observed during this time. Monooxygenase activities were determined within one week after preparation of microsomes. Protein content was determined according to the method of Lowry *et al.* [16] using bovine serum albumin as protein standard.

Assays of UDP-glucuronosyltransferase

Enzyme activities towards various substrates were assayed by the following aglycone concentrations and by methods already described (unless specified): 0.5 mM 1-naphthol [17]; 0.5 mM 4-methylumbelliferone (see below); 1.5 mM morphine [18]; 0.5 mM 4-hydroxybiphenyl [9] (see below); 0.2 mM bilirubin [19]; and 0.1 mM oestrone [20]. With 4-hydroxybiphenyl as substrate, perchloric acid (0.5 M) instead of trichloroacetic acid was used to stop the reaction. By this modification the fluorescence of the glucuronide is enhanced 3-fold without affecting background fluorescence. For reasons of standardization and comparison the assays were performed at 37° in the presence of 0.1 M Tris–HCl (pH 7.4) and 5 mM $MgCl_2$. Microsomes were fully activated by addition of Brij 58 (0.05%, w/v). Bilirubin-GT was fully activated by addition of 0.15% digitonin. Reactions were started by addition of 3 mM UDP-glucuronic acid. Assays were performed under conditions leading to linear reaction rates with time and protein concentration. Fluorescence assays were carried out using a Perkin Elmer 650-10 S fluorescence spectrophotometer. Fluorescence intensity was calibrated with quinine sulfate.

UDP-glucuronosyltransferase activity with 4-methylumbelliferone as substrate

4-Methylumbelliferone (0.5 mM final concentration) was dissolved in dimethylsulfoxide and added to the standard incubation mixture (0.5 ml) specified above. The reaction was started by addition of 3 mM UDP-glucuronic acid and stopped by addition of 0.5 ml 0.5 M perchloric acid. Excessive substrate was extracted with 2 ml chloroform. After centrifugation a 0.5 ml aliquot of the water phase containing the glucuronide was mixed with 0.5 ml 1.6 M glycine/NaOH, pH 10.3. Fluorescence was measured at 365 nm with excitation at 315 nm. Calibration was performed with 4-methylumbelliferyl-β,D-glucuronide. Traces of the unconjugated substrate, showing maximal fluorescence at 445 nm with excitation at 365 nm, did not interfere with the fluorescence of the glucuronide. The reaction was linear up to 30 min and 0.5 mg/ml microsomal protein. Less than 100 pmole of 4-methylumbelliferyl glucuronide per assay were detectable under our conditions.

Monooxygenase assays

Benzo[a]pyrene monooxygenase activity was assayed using two methods: (a) total metabolites were determined by the method of dePierre *et al.* [21] as modified in [22]; (b) the formation of fluorescent phenols by arylhydrocarbon hydroxylase was determined by the method of Nebert and Gelboin [23]. 7-Ethoxycoumarin-*O*-dealkylase was assayed using the method of Ullrich and Weber [24] as modified [25]. Aminopyrine-*N*-demethylase was determined as described [26, 27]. Standard incubation mixtures contained 0.1 M Tris–HCl (pH 7.4), 0.5 mM NADP, 5 mM $MgCl_2$, 5 mM sodium isocit-

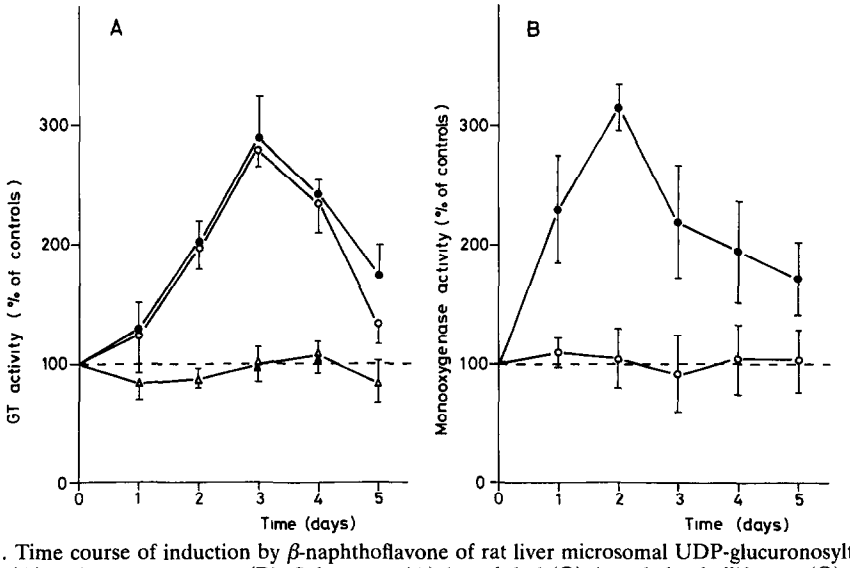

Fig. 1. Time course of induction by β-naphthoflavone of rat liver microsomal UDP-glucuronosyltransferase (A) and monooxygenase (B). Substrates: (A) 1-naphthol (●) 4-methylumbelliferone (○), morphine (▲), 4-hydroxybiphenyl (△); (B) benzo[a]pyrene (●), aminopyrine (○). β-Naphthoflavone (100 mg/kg) was administered once i.p. Enzyme activities of controls are given in Table 1. Values represent the mean ± S.D. of 4 experiments.

Table 1. Effects of DDT or Aroclor 1254 administration on rat liver microsomal UDP-glucuronosyltransferase (A) and monooxygenase (B) activities*

Substrate	Enzyme activity (nmole/min per mg protein)		
	Controls	DDT	Aroclor 1254
(A) 1. 1-Naphthol	67.2 ± 6.9	80.2 ± 5.5 (1.2)	432 ± 44 (6.4)
4-Methylumbelliferone	68.3 ± 2.8	100 ± 7 (1.5)	446 ± 49 (6.5)
2. Morphine	10.7 ± 2.7	24.6 ± 4.4 (2.3)	27.4 ± 5.1 (2.6)
4-Hydroxybiphenyl	25.2 ± 4.4	68.7 ± 6.3 (2.7)	67.7 ± 9.3 (2.7)
3. Bilirubin	1.4 ± 0.3	2.5 ± 0.5 (1.8)	1.0 ± 0.2 (0.7)
4. Oestrone	0.9 ± 0.1	0.9 ± 0.1 (1.0)	1.6 ± 0.1 (1.8)
(B) Benzo[a]pyrene	2.8 ± 0.5	3.8 ± 0.4 (1.4)	10.0 ± 1.4 (3.6)
Aminopyrine	8.9 ± 0.6	15.1 ± 1.6 (1.7)	17.8 ± 1.0 (2.0)
7-Ethoxycoumarin	0.6 ± 0.1	2.1 ± 0.3 (3.5)	10.3 ± 1.9 (17.2)

* Values are the mean ± S.D. of 4 experiments. Numbers in parentheses represent the fold increase over controls.

rate, and 0.2 IU isocitrate dehydrogenase. In blanks the NADPH regenerating system was omitted. Cytochrome P-450 was determined by the method of Omura and Sato [28].

Statistical evaluation of the results was done using Student's *t*-test.

RESULTS

Treatment with β-naphthoflavone. GT activities towards 1-naphthol and 4-methylumbelliferone were enhanced nearly 3-fold after three days (Fig. 1A). In contrast the glucuronidation of 4-hydroxybiphenyl and morphine or of bilirubin and oestrone (not shown) was not influenced. Benzo[a]pyrene monooxygenase activity was also stimulated 3-fold, whereas aminopyrine *N*-demethylase remained unaffected (Fig. 1B). No further stimulation of GT and monooxygenase activities was observed when β-naphthoflavone (100 mg/kg) was injected daily for three days or when a single dose of 200 mg/kg was applied. Benzo[a]pyrene monooxygenase activity reached its maximum earlier than GT probably due to the faster turnover of cytochrome P-450$_{MC}$. The rapid decline of both GT and monooxygenase activities may be ascribed to the rapid metabolism of β-naphthoflavone [29].

Treatment with DDT. In agreement with previous studies of 4-nitrophenol glucuronidation [30] GT activities (1-naphthol and 4-hydroxybiphenyl as substrates) reached a broad maximum of induction after 10–14 days following a single dose of DDT (not shown). Delayed induction of monooxygenase [30] and GT activities by DDT is probably due to slow release of DDT from lipophilic storage sites, e.g. fat tissue. In contrast to β-naphthoflavone DDT preferentially induced the glucuronidation of 4-hydroxybiphenyl and morphine 2.7- and 2.3-fold, respectively. The glucuronidation of 1-naphthol and 4-methylumbelliferone was enhanced only 1.2- and 1.5-fold, respectively (Table 1). Bilirubin glucuronidation was stimulated nearly 2-fold whereas the specific activity of oestrone-GT was not altered by DDT. As anticipated the monooxygenase activities were also stimulated.

Treatment with clofibric acid and bezafibrate. Clofibric acid or bezafibrate selectively enhanced the glucuronidation of bilirubin (Table 2). Effects of administration of clofibrate, the ethyl ester of clofibric acid, on bilirubin-GT activity have been noted previously [31]. Control values of GT activities listed in Table 2 were lower than in Table 1 probably because these animals were from a different breeder. Concentration of cytochrome P-450 was slightly

Table 2. Effects of clofibric acid or bezafibrate administration on rat liver microsomal UDP-glucuronosyltransferase (A) and monooxygenase (B) activities*

Substrate	Enzyme activity (nmole/min per mg protein)		
	Controls	Clofibric acid	Bezafibrate
(A) 1. 1-Naphthol	40.4 ± 3.9	35.4 ± 4.1 (0.9)	28.3 ± 2.5 (0.7)
4-Methylumbelliferone	54.6 ± 7.0	47.4 ± 10.9 (0.8)	41.6 ± 4.3 (0.8)
2. Morphine	6.6 ± 1.2	7.4 ± 1.1 (1.1)	4.2 ± 0.8 (0.6)
4-Hydroxybiphenyl	19.8 ± 2.1	24.3 ± 2.3 (1.2)	17.9 ± 2.8 (0.9)
3. Bilirubin	0.9 ± 0.2	1.8 ± 0.4 (2.0)†	1.9 ± 0.3 (2.1)†
4. Oestrone	0.7 ± 0.1	0.7 ± 0.1 (1.0)	0.7 ± 0.04 (1.0)
(B) Benzo[a]pyrene	2.8 ± 0.7	2.5 ± 0.3 (0.9)	2.6 ± 0.4 (0.9)
Aminopyrine	8.5 ± 1.0	9.6 ± 1.1 (1.1)	7.9 ± 0.9 (0.9)
7-Ethoxycoumarin	0.7 ± 0.1	0.7 ± 0.1 (1.0)	0.7 ± 0.2 (1.0)
Cytochrome P-450‡	0.78 ± 0.05	1.04 ± 0.06 (1.3)†	0.89 ± 0.12 (1.1)

* Values are the mean ± S.D. of 4 experiments. Numbers in parentheses represent the fold increase over controls.

† Significantly increased over controls ($P < 0.005$).

‡ nmole/mg protein.

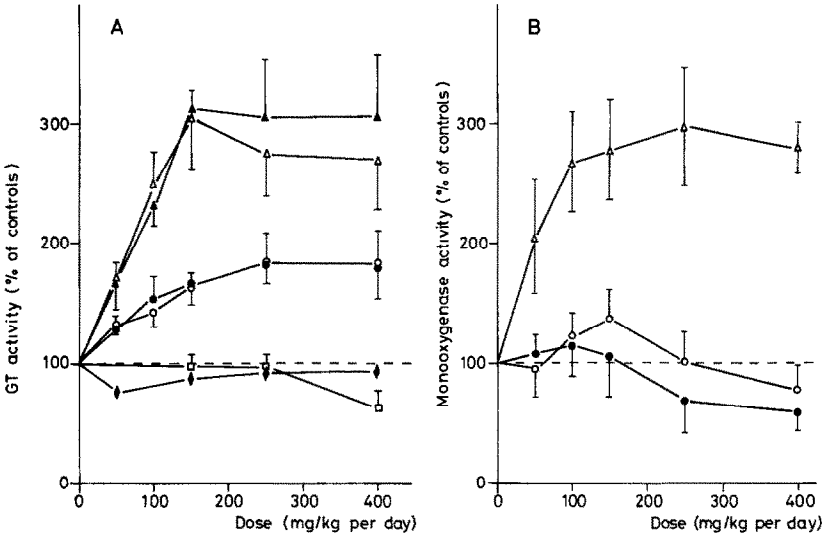

Fig. 2. Dose-dependence of induction by trans-stilbene oxide of rat liver microsomal UDP-glucuronosyltransferase (A) and monooxygenase (B). Substrates: (A) bilirubin (□), oestrone (◆); (B) 7-ethoxycoumarin (△). Other substrates and symbols are the same as in Fig. 1. Trans-stilbene oxide was administered once i.p. daily for 4 days, and the animals were killed 24 hr after the last treatment. Enzyme activities of controls are given in Table 1. Values represent the mean ± S.D. of 4 experiments.

enhanced by clofibric acid. However monooxygenase activities assayed were not affected. In particular 7-ethoxycoumarin-*O*-dealkylase was not induced in contrast to the effects of all other inducers investigated.

Treatment with trans-stilbene oxide. Glucuronidation of 4-hydroxybiphenyl and morphine could be enhanced up to 3-fold by administration of trans-stilbene oxide. The conjugation of 1-naphthol and 4-methylumbelliferone was stimulated nearly 2-fold (Fig. 2A). In contrast the glucuronidation of bilirubin or oestrone was not altered by trans-stilbene oxide. Benzo[a]pyrene monooxygenase and aminopyrine-*N*-demethylase activities were barely enhanced or even decreased by trans-stilbene oxide whereas ethoxycoumarin-*O*-dealkylase was increased up to 3-fold under these conditions (Fig. 2B).

Treatment with Aroclor 1254. The effects of Aroclor 1254 were studied under treatment conditions recommended by Ames *et al.* for the salmonella microsome mutagenicity test [32]. GT_1 activities were stimulated more than 6-fold, i.e. higher than by β-naphthoflavone. GT_2 activities were stimulated 2.3-fold. In contrast to the effects of other inducers oestrone glucuronidation was enhanced 1.8-fold whereas bilirubin-GT was not stimulated but even decreased. In agreement with the proposed 'mixed type' inducing properties of Aroclor 1254 [33] monooxygenase activities were strongly induced under these conditions.

DISCUSSION

Differential induction of GT activities

The effects of various inducing agents on rat liver GT are summarized in Table 3. It is obvious that typical inducers of the MC- or phenobarbital-type [3] differentially stimulate GT reactions. β-Naphthoflavone, like MC, selectively stimulates GT_1 activities, illustrated by 1-naphthol-GT. Comparable results have been described for TCDD (2,3,7,8-tetrachlorodibenzo-*p*-dioxin), the strongest inducer of this type [34]. DDT like phenobarbital preferentially stimulates GT_2 activities, illustrated by morphine-GT, and the glucuronidation of bilirubin.

It is recognized that, in addition to its inducibility by MC, GT_1 is also inducible by phenobarbital. Inducibility of 4-methylumbelliferone-GT by phenobarbital has been clearly shown in rat hepatoma cell cultures at high inducer concentrations, not tolerated by rats *in vivo* [15]. The effects of phenobarbital are somewhat underestimated in our model in which the marked proliferation of endoplasmic reticulum membranes by phenobarbital is not taken into account [35]. Phenobarbital and MC probably induce GT_1 by different mechanisms. This is supported by the additive effects of these inducers on GT_1 activity [15, 36]. It may also be the reason for the high induction factor for GT_1 observed in our studies with Aroclor 1254 (a mixture of polychlorinated biphenyls with phenobarbital- and MC-type inducer properties [33]).

The hypolipidemic drugs clofibric acid and congeners differ from agents of the MC- or phenobarbital-type, e.g. by the fact that bilirubin glucuronidation is selectively enhanced whereas GT_1 and GT_2 activities remain unaffected. A different regulation of bilirubin-GT and GT_2 activities is also seen in our studies with ethoxyquin, trans-stilbene oxide and Aroclor 1254. Both GT_1 and GT_2 activities were stimulated whereas bilirubin-GT was not affected or even decreased.

The specific activity of oestrone-GT was not altered by the inducers listed in Table 3, except Aroclor 1254. These data confirm previous observations indicating that the glucuronidation of oestrone is relatively resistant to inducing agents [9, 34, 37–40]. Similarly the specific activity of

Table 3. Summary of differential effects of various inducing agents on rat liver microsomal UDP-glucuronosyltransferase and monooxygenase activities

Inducer	UDP-glucuronosyltransferase activity				Monooxygenase activity
	1-Naphthol	Morphine	Bilirubin	Oestrone	Benzo[a]pyrene
	(fold increase over controls)*				
(a) 3-Methylcholanthrene†	3.2	1.3	0.8	1.2	4.4 (4.3)‡
β-Naphthoflavone	2.9	1.0	1.0	1.0	3.1
(b) Phenobarbital†	1.3	4.1	1.9	1.0	1.5 (1.3)‡
DDT	1.2	2.3	1.8	1.0	1.4
(c) Clofibric acid	0.9	1.1	2.0	1.0	0.9
Bezafibrate	0.7	0.6	2.1	1.0	0.9
(d) Ethoxyquin†	3.0	4.2	1.1	1.2	1.0
Trans-stilbene oxide	1.8	3.0	1.0	1.0	0.8
(e) Aroclor 1254	6.4	2.7	0.7	1.8	3.6

* Control activities are listed in Tables 1 and 2.
† Data taken from [40].
‡ Data in parentheses were obtained using the fluorimetric assay. Control activity: 0.7 ± 0.1 nmole/min per mg protein.

phenolphthalein-GT is not altered by MC or phenobarbital under our conditions (unpublished). Hence there may be at least one form of GT which is not readily inducible.

The possibility was considered that high levels of inducer and/or its metabolites interfered with microsomal GT assays, e.g. when high doses of trans-stilbene oxide were administered. High concentrations of trans-stilbene oxide (1 mM) weakly inhibited 1-naphthol-GT and 4-hydroxybiphenyl-GT activity, 26 and 44%, respectively. Inhibition would lead to a dose-dependent decrease of enzyme activity. It may be the reason for decreased enzyme activities, below the enzyme level of untreated controls, seen at the highest dose of trans-stilbene oxide (Fig. 2). However differential induction was obvious at much lower doses where it is unlikely that interfering levels of inducer accumulate in microsomes. In addition, low doses of Aroclor 1254 (100 mg/kg) differentially induced GT activities similar to high doses (Table 1) except for GT_1 activities which were 30% lower and for bilirubin-GT activity which was closer to the level of untreated controls.

In conclusion the model of short-term treatment of male rats with various inducing agents allows one to distinguish four groups of substrates, characterized by the substrates listed in Table 3. Whether these groups of substrates are conjugated by different enzyme forms remains to be established. However recent results on enzyme separation and purification support this view [9, 12, 13]. Moreover, studies with inhibitors of protein synthesis [15, 41], or the incorporation of [^{14}C]leucine into purified GT_1 [42] and quantitative determination of the enzyme protein with antibodies to GT_1 [43] substantiate the hypothesis that the alteration of GT activity by inducing agents truly reflects enzyme induction, i.e. increased synthesis of different enzyme proteins.

Comparison between the induction of GT_1 and benzo[a]pyrene monooxygenase

Recent studies with 'responsive' and 'nonresponsive' inbred strains of mice suggest that the inducibility by MC of both benzo[a]pyrene monooxygenase and 4-methylumbelliferone-GT are regulated by a single genetic locus called the Ah locus [14]. The coordinate induction is probably mediated through a common receptor protein for MC-type inducers [14, 15]. Coordinate induction of these enzymes is surprising since other drug metabolizing enzymes such as epoxide hydrolase [44] and glutathione *S*-transferase [45] are not linked with the Ah-locus. Studies with the prototypes of responsive (C57BL/6) and nonresponsive (DBA/2) mice indicate distinct GT_1 and GT_2 activities in mice with similar substrate specificity, inducibility and tissue distribution as in rats [46]. Parallel induction of rat liver GT_1 and benzo[a]pyrene monooxygenase activities (Table 3) is in support of a coordinate induction of these metabolically related enzymes. However, there is also evidence for independent control of both enzymes [6]. This is clearly indicated by the studies with ethoxyquin and trans-stilbene oxide. GT_1 is clearly induced under conditions when benzo[a]pyrene monooxygenase is unaltered. On the basis of its inducing properties towards several drug metabolizing enzymes trans-stilbene oxide has been classified as a new type of inducer, distinct from MC- or phenobarbital-type inducers [47–49]. Our studies on GT induction support this view. Similar to the effects of phenobarbital GT_2 activities are more strongly induced than GT_1 activities by trans-stilbene oxide. However bilirubin GT is not affected by this inducer. In contrast to MC, benzo[a]pyrene monooxygenase is not enhanced by trans-stilbene oxide [47] and by ethoxyquin [50]. The lack of induction of benzo[a]pyrene monooxygenase [50, 51] and the stimulation of various phase II enzymes [40, 51–53] may contribute to the protective action of ethoxyquin and other antioxidants against chemical carcinogens [54].

The significance of coordinate induction of cytochrome $P\text{-}450_{MC}$ and GT_1 remains to be elucidated. It may greatly facilitate the inactivation and elimination of polycyclic aromatic compounds from the organism. This is supported by the following observations: (a) GT_1 efficiently conjugates benzo[a]pyrene phenols [9, 11, 55], quinols [56, 57] and to a lesser extent dihydrodiols [11, 55]; (b) benzo[a]pyrene monooxygenase activity is markedly

stimulated by concomitant glucuronidation especially with microsomes from MC-treated rats [58, 59]; (c) when cofactors of glucuronidation are added to liver homogenates along with the NADPH regenerating system in the Ames test, benzo[a]pyrene mutagenicity is markedly decreased. Both the activation of benzo[a]pyrene to mutagenic metabolites and the inactivation of benzo[a]pyrene metabolites by glucuronidation is much more pronounced with liver homogenates from MC-treated rats than with those from phenobarbital-treated animals or untreated controls [60]. On the other hand it is recognized that glucuronidation also enhances benzo[a]pyrene mutagenicity in the Ames test at very high benzo[a]pyrene concentrations [61] and it increases benzo[a]pyrene diol-epoxide modification of DNA under certain conditions. The significance of MC-inducibility of drug metabolizing enzymes by the Ah locus is still uncertain. Under many experimental conditions MC-inducibility enhances the toxicity of polycyclic aromatic compounds [2]. However, when these compounds are administered via the gastrointestinal tract, which may have been the most common exposure during evolution, MC-inducibility leads to decreased toxicity [2, 3] probably due to efficient inactivation by presystemic metabolism in the gut and the liver.

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