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METABOLISM OF NAPHTHALENE TO NAPHTHALENE DIHYDRODIOL GLUCURONIDE IN ISOLATED HEPATOCYTES AND IN LIVER MICROSOMES

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Abstract—The functional linkage between UDP-glucuronyltransferase (GT) and the monooxygenase–epoxide hydratase system was investigated in studies on the glucuronidation of naphthalene dihydrodiol, which was formed by epoxide hydratase during the metabolism of naphthalene. (1) Naphthalene metabolism was compared in isolated hepatocytes and in liver microsomes incubated with an NADPH regenerating system and UDP-glucuronic acid. Naphthalene dihydrodiol glucuronide was a major metabolite in isolated hepatocytes. In the liver microsomal system free dihydrodiol by far exceeded its glucuronide unless the positive allosteric effector of GT, UDP-*N*-acetylglucosamine, was added. (2) Treatment of rats with phenobarbital or 3-methylcholanthrene, although markedly enhancing the formation of naphthalene dihydrodiol, did not stimulate liver microsomal GT (naphthalene dihydrodiol as substrate). The results suggest that activation of GT by UDP-*N*-acetylglucosamine is an important factor in the coupling of glucuronidation to functionally linked microsomal enzyme reactions.

Glucuronide conjugation catalyzed by microsomal GT (EC 2.4.1.17) is frequently the final step of metabolic pathways converting lipid-soluble compounds into polar products which can be effectively eliminated from the body [1, 2]. In this report a possible linkage between GT and the functionally linked microsomal monooxygenase–epoxide hydratase system was investigated by studying the formation of naphthalene dihydrodiol from naphthalene and its subsequent glucuronidation.

Pathways of naphthalene metabolism (Fig. 1) have been extensively studied *in vivo* [3, 4] and in liver microsomes [5, 6]. Naphthalene is first oxidized to naphthalene 1,2-oxide by a cytochrome P-450 dependent monooxygenase. The reactive epoxide is detoxified by several pathways, particularly by microsomal epoxide hydratase and by conjugation with glutathione. Non-enzymatically the epoxide rearranges to the corresponding phenols, primarily to 1-naphthol [5]. Evidence for a coupling between the monooxygenase system and epoxide hydratase has been described [6]. The product of the epoxide hydratase reaction, naphthalene dihydrodiol, is still of potential hazard since it can be converted to 1,2-dihydroxynaphthalene and 1,2-naphthoquinone. It has been demonstrated that naphthalene dihydrodiol released from the liver during naphthalene metabolism can be converted in the eye to 1,2-naphthoquinone which causes toxic reactions such as cataracts [7, 8]. The conversion of naphthalene dihydrodiol to the toxic *o*-quinone can be prevented by conjugation of the dihydrodiol or 1,2-dihydroxynaphthalene. Hence a linkage between GT and the monooxygenase–epoxide hydratase system would be advantageous to prevent the formation of reactive *o*-quinones.

Abbreviations: UDP-glucuronyltransferase (GT), UDP-*N*-acetylglucosamine (UDPGlcNAc), trans-1,2-dihydroxy-1,2-dihydronaphthalene (naphthalene dihydrodiol).

Naphthalene metabolism was studied in isolated hepatocytes in order to evaluate the quantitative significance of various metabolic pathways, in particular to study naphthalene dihydrodiol glucuronidation. A comparative study on naphthalene metabolism to naphthalene dihydrodiol glucuronide was then performed in liver microsomes under various conditions in an attempt to delineate some factors which affect the reaction sequence in the intact cell. Since it is known that phenobarbital or 3-methylcholanthrene markedly stimulate the conversion of naphthalene into naphthalene dihydrodiol [6] the effect of these inducing agents on GT (naphthalene dihydrodiol as

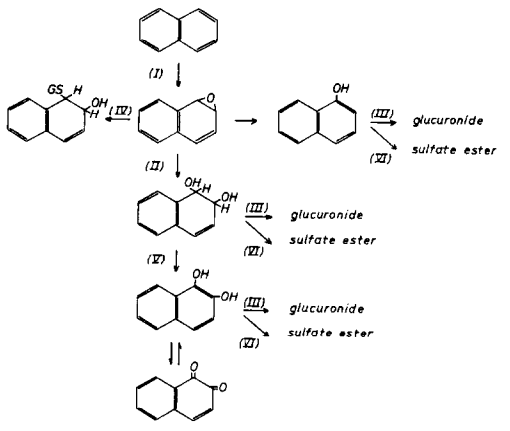

Fig. 1. Metabolism of naphthalene in hepatocytes (adapted from [3, 4]). Enzymes: (I), monooxygenase; (II), epoxide hydratase; (III), UDP-glucuronyltransferase; (IV), glutathione-*S*-transferase; (V), dihydrodiol dehydrogenase; (VI), sulfotransferase. The scheme is not intended to exclude other pathways leading to the same terminal metabolites, e.g. phenol formation by direct oxidation or *o*-hydroxylation of phenols. Enzymes I, II, III are located solely in microsomal membranes. GS = glutathionyl.

substrate) was also studied to find out whether the three functionally linked microsomal enzymes (monooxygenase, epoxide hydratase, GT) are simultaneously induced. Part of this work has been presented in preliminary form [9].

MATERIALS AND METHODS

Chemicals were obtained from the following sources: [1-^{14}C]naphthalene (5.1 mCi/m-mole) and 1-[1-^{14}C]naphthol (20.8 mCi/m-mole) from Radiochemical Center, Amersham; UDP-glucuronic acid, disodium salt (78% purity), arylsulfatase, collagenase (grade II) and isocitrate dehydrogenase from Boehringer, Mannheim; 3-methylcholanthrene from Sigma, St. Louis; β-glucuronidase (sulfatase-free) from Serva, Heidelberg; 1,2-naphthoquinone from Fluka AG., Buchs and carbonylcyanide-*m*-chlorophenylhydrazone from Calbiochem, Los Angeles.

Naphthalene dihydrodiol was synthesized from 1,2-naphthoquinone by reduction with $LiAlH_4$ according to Booth *et al.* [10]. The identity and purity of the compound was verified by its melting point (103°), by chromatography on Silica gel plates in benzene–chloroform–ethyl acetate (1:1:1) (5) and by quantitative conversion of non-fluorescent naphthalene dihydrodiol into fluorescent 1-naphthol. This was performed by heating an aqueous solution of naphthalene dihydrodiol with 1 N HCl for 20 min at 100° according to Sims [11]. After cooling 1.5 N NaOH was added and 1-naphthol was determined fluorometrically [12]. ^{14}C-Naphthalene dihydrodiol was prepared enzymatically from [1-^{14}C]naphthalene as described under naphthalene metabolism using microsomes from phenobarbital-treated rats but omitting UDP-glucuronic acid. After 60 min incubation, unreacted naphthalene and 1-naphthol was extracted once with an equal volume of benzene. From the remaining water phase pure [^{14}C]naphthalene dihydrodiol (>99%) was extracted with ether.

Male Sprague–Dawley rats (about 200 g) were fed *ad lib.* a standard diet containing 20 per cent protein (Altromin, Lage–Lippe, Germany). In phenobarbital treatment, rats received intraperitoneal injections of 100 mg/kg phenobarbital for three days and were sacrificed on the fourth day. Treatment with 3-methylcholanthrene: the animals were injected once intraperitoneally with 40 mg/kg 3-methylcholanthrene dissolved in olive oil. Hepatectomy was performed on the fourth day. Liver microsomes were prepared as previously described [13]. Microsomal protein was determined by the method of Lowry *et al.* [14] using bovine serum albumin as standard.

Isolation and incubation of hepatocytes, viability criteria. Hepatocytes were isolated according to the procedure of Berry and Friend [15] as modified by Baur *et al.* [16] and by Wagle and Ingebretsen [17]. During hepatectomy the liver was perfused for 5 min with Ca^{2+}-free Hanks' medium at 37° via the vena portae. After preparation of the liver, perfusion was continued with a recirculating Eagle's minimal essential medium (MEM) containing 0.03% collagenase. After 30–40 min the liver was disintegrating. Subsequently the liver was transferred to a round-bottomed flask and enzyme treatment was continued for 10 min under slow rotation. After the washing procedure the cells were suspended in MEM containing 2% bovine serum albumin and stored at 0°C. To quickly check the quality of cell preparations trypan blue staining was used. Equal volumes of cell suspensions and trypan blue (0.5%, dissolved in MEM) were mixed. Susceptibility of the cells to staining ranged from 5–10 per cent. Oxygen consumption was measured with a Clark oxygen electrode at pH 7.4 and 37° [18]. Stimulation of respiration by succinate, as an indicator of the integrity of the plasma membrane, was below 20 per cent. The respiratory control ratio which was measured as the ratio of respiration with and without the addition of 2 μM carbonylcyanide-*m*-chlorophenylhydrazone was above 1.6 within 2 hr at 37° [16].

For incubations aliquots of the cell suspension were diluted with MEM to a final concentration of 0.15 ml packed cells/ml. After 5 min preincubation at 37° in a Dubnoff shaker the reaction was started by the addition of substrate. During incubation the cells were gently bubbled with 95% O_2 and 5% CO_2.

One ml packed cells (determined in a Van Allen hemocytometer) corresponded to 127×10^6 hepatocytes or 176 mg cell protein.

Glucuronidation of 1-naphthol in hepatocytes. One-half μmole 1-[1-^{14}C]naphthol (5 nCi) dissolved in dimethylsulfoxide (0.25%, v/v, final concentration) was incubated with 0.15 ml packed cells suspended in 0.85 ml MEM. Naphthol is rapidly absorbed by hepatocytes and concentrated in biological membranes. For comparison the ratio naphthol/cells was kept similar to that used in the liver perfusion system (35 μmole 1-naphthol/10 g liver tissue [13]). Naphthol glucuronide formation was determined with a rapid radiochemical assay as described [13].

Naphthalene metabolism in hepatocytes and liver microsomes. (a) *Isolated hepatocytes.* 0.1 μmole [1-^{14}C]naphthalene (0.5 μCi) dissolved in 5 μl ethanol was added to 0.15 ml packed hepatocytes suspended in 0.85 ml MEM, and incubated for 40 min at 37°. Oxygen consumption of the hepatocytes was only reduced by about 5 per cent in the presence of naphthalene. The reaction was stopped by cooling the tubes in ice. Unconjugated metabolites were then extracted four times with 4 ml ether (fraction I). The remaining aqueous phase was adjusted to pH 4.8 by the addition of 0.2 ml 1 M acetate buffer pH 4.8, and incubated overnight at 37° with 2.5 mg sulfatase-free β-glucuronidase. Hydrolysis products were then extracted with ether (fraction II). After removal of contaminating ether at reduced pressure the ensuing water phase was incubated with 25 μg arylsulfatase for 12 hr. Subsequently the fraction of hydrolyzed sulfate esters was extracted with ether as described above (fraction III). When the extraction of fraction I was repeated after an overnight incubation at 37° in the presence of saccharic acid-1,4-lactone to inhibit β-glucuronidase [19] the radioactivity in the ether extract was only 12 per cent of fraction II (This is within the standard deviation given in Table 1). Similarly after an additional enzyme treatment (fractions II and III) the extracted radioactivity was below 10 per cent of the first extraction indicating that the extraction procedure was virtually complete. Glutathione conjugates were analyzed in the remaining water phase by adsorption to charcoal and subsequent elution with

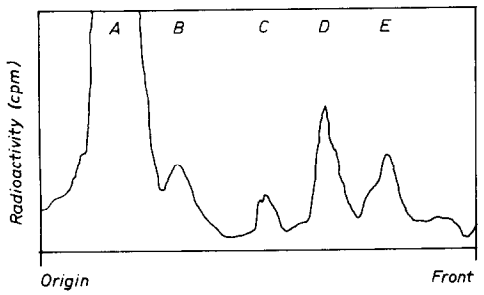

Fig. 2. Thin-layer chromatography of naphthalene metabolites. Radioactive metabolites of the glucuronide fraction (II) after hydrolysis with β-glucuronidase are shown. They were chromatographed on Silica gel plates in benzene–chloroform–ethyl acetate (1:1:1). (A) Naphthalene dihydrodiol; (B) unknown metabolite; (C) 1,2-dihydroxynaphthalene; (D) 1,2-naphthoquinone; (E) 1-naphthol.

methanol–benzene–aqueous ammonia as described by Boyland *et al.* [4]. Glutathione conjugates were chromatographed on Silica gel plates in butanol–acetic acid–water (2:1:1). Three about equal radioactive peaks were found which were ninhydrin positive and strongly absorbing u.v. light. Based on their relative mobilities [4] they were tentatively ascribed to *S*-(1,2-dihydro-2-hydroxy-1-naphthyl)-glutathione (R_f = 0.41); *S*-(1,2-dihydro-2-hydroxy-1-naphthyl)-cysteinylglycine (R_f = 0.59); and *S*-(1,2-dihydro-2-hydroxy-1-naphthyl)-cysteine (R_f = 0.70).

Aliquots of the ether extracts and the remaining water phase were counted for total radioactivity. Ether extracts were evaporated under reduced pressure. The remaining residue was then dissolved in ethanol, chromatographed on Silica gel plates in benzene–chloroform–ethyl acetate (1:1:1) and scanned for radioactivity. Naphthalene in fraction I was completely evaporated by leaving the plates overnight under the hood. Radioactive peaks corresponding with naphthalene dihydrodiol, 1,2-dihydroxynaphthalene, 1,2-naphthoquinone and 1-naphthol were clearly separated and identified by cochromatography with standards (Fig. 2). 1,2-Dihydroxynaphthalene was prepared by reducing 1,2-naphthoquinone with 1 mM ascorbic acid [7]. During chromatography 1,2-dihydroxynaphthalene was partially oxidized to 1,2-naphthoquinone. 1,2-Naphthoquinone in fraction II (Fig. 2) can only arise from 1,2-dihydroxynaphthalene. These two compounds were therefore determined collectively. Peaks were scraped off the plates and the radioactivity was determined.

(b) *Liver microsomes.* Microsomal suspensions were incubated with 1 μmole NADP, 8 μmole isocitrate-Na, 10 μl isocitrate dehydrogenase, 3 μmole UDP-glucuronic acid and 0.1 μmole [1-^{14}C]naphthalene (0.5 μCi). Unconjugated metabolites and glucuronides were analyzed as described above for isolated hepatocytes.

Assays of UDP-glucuronyltransferase. Assays were performed at 37° in a total volume of 1 ml containing 100 mM Tris–HCl (pH 7.4), 5 mM $MgCl_2$, acceptor substrates and microsomal protein as indicated. The enzyme reaction was started by the addition of UDP-glucuronic acid (3 mM, final concentration). In controls UDP-glucuronic acid was omitted.

(a) *1-Naphthol glucuronidation* [13]. One-half mM 1-naphthol dissolved in dimethylsulfoxide (0.25%, v/v, final concentration) was incubated with 0.5 or 1 mg microsomal protein and 0.04 μCi 1-[1-^{14}C]naphthol. After 0.5–2 min incubation the reaction was stopped by addition of 1 ml 0.6 M glycine–0.4 M trichloroacetic acid buffer pH 2.2. Following centrifugation at 3000 *g* for 5 min the supernatant was extracted with 8 ml chloroform to remove the unreacted naphthol. The radioactivity of the aqueous phase was determined in Brays scintillation fluid [20]. Zero time blanks were subtracted. The rate of naphthol glucuronidation was calculated from the total radioactivity of the original naphthol solution.

(b) *Glucuronidation of naphthalene dihydrodiol.* Five mM [^{14}C]naphthalene dihydrodiol (0.02 μCi) was incubated for 10 min with 2 mg microsomal protein. The reaction was stopped by cooling the tubes in ice. The mixture was then extracted four times with 4 ml ether to remove unreacted dihydrodiol. The radioactivity in the aqueous phase was counted in Bray's scintillation fluid [20]. Zero time blanks were subtracted. Up to 10 min and 2 mg protein the reaction was linear with time and protein concentration.

Products in the aqueous phase were identified by thin-layer chromatography on cellulose plates with ethanol–1 M ammonium acetate (9:1, v/v) as the solvent system and by detection of 1-naphthol or naphthalene dihydrodiol after hydrolysis with β-glucuronidase.

RESULTS AND DISCUSSION

Glucuronidation of 1-naphthol by isolated hepatocytes. In order to test the glucuronide-forming capacity of isolated hepatocytes the rate of 1-naphthol glucuronide formation was determined. 1-Naphthol was chosen since there are only two major pathways of its metabolism, leading to the glucuronide and sulfate conjugates [21]. These metabolites were determined by a rapid radioassay as previously described for the isolated perfused liver system [13]. Substrate concentration and the ratio packed cells/incubation volume were selected to allow a comparison with the liver perfusion system as specified in Methods.

Release of 1-naphthol glucuronide from hepatocytes was linear between 5 and 20 min. From this linear initial increase a rate of 0.13 ± 0.01 μmole min^{-1} ml packed $cells^{-1}$ (n = 4) was calculated. This rate is in good agreement with the value previously obtained in the perfused liver system (0.10 ± 0.04 μmole min^{-1} g $liver^{-1}$ [13]). Vitality criteria such as stimulation of respiration by succinate and the respiratory control ratio were unaltered by 1-naphthol under our incubation conditions.

The comparative studies on naphthol glucuronidation suggest that the glucuronide-forming capacity of isolated hepatocytes is similar to the intact organ.

Conversion of naphthalene into naphthalene dihydrodiol glucuronide in isolated hepatocytes and in the microsomal system. When isolated hepatocytes were incubated with 100 nmole naphthalene for 40 min, 45 nmole were metabolized. 15.1 and 26.4 per cent of the metabolites could be identified as free naphthalene dihydrodiol and its glucuronide, respectively (Table 1). Pilot experiments demonstrated that glucuronides increased linearily up to 40 min. A minor

Table 1. Conversion of naphthalene into naphthalene dihydrodiol, 1-naphthol and their glucuronides in isolated hepatocytes and in liver microsomes*

System	Addition of UDPGlcNAc *in vitro*	Naphthalene dihydrodiol		1-Naphthol	
		Free	Glucuronide	Free	Glucuronide
		(% of naphthalene metabolites)			
Hepatocyte	–	15.1 ± 5.6	26.4 ± 5.1	0.7 ± 0.2	1.1 ± 0.2
Liver microsomes (5 mg protein/ml)	–	40.8	4.4	1.8	14.0
	+	20.0	18.0	1.0	13.8
Liver microsomes (13 mg protein/ml)	–	32.6	15.7	2.0	5.7
	+	18.0	35.4	1.7	7.7

* Isolated hepatocytes or liver microsomes were incubated for 40 min with 100 nmole naphthalene, and metabolites were determined as described in Methods. Naphthalene was metabolized to the extent of 45, 50 and 30 per cent in the experiments with hepatocytes, microsomes (5 mg protein/ml) and microsomes (13 mg protein/ml), respectively.

amount of naphthalene dihydrodiol (1.6%) was present as the sulfate ester conjugate (not shown). Hence naphthalene dihydrodiol and its conjugates comprised 43 per cent of total naphthalene metabolites. A small fraction of the dihydrodiol (about 7 per cent) was converted into 1,2-dihydroxynaphthalene and its conjugates, and into 1,2-naphthoquinone. The accumulation of free naphthalene dihydrodiol indicates that at least in the rat further metabolism of the dihydrodiol by dihydrodiol dehydrogenase is slow. Non-enzymatic conversion of naphthalene 1,2-oxide into 1-naphthol and its conjugates was also slow in intact hepatocytes (2 per cent). A considerable amount of naphthalene metabolites (about 15 per cent) was identified as glutathione conjugates and their hydrolysis products. The percentage of glutathione conjugates represents a minimal figure since the yield of our isolation procedure is not known. The radioactive material remaining in the aqueous phase after the extraction procedures possibly includes glutathione conjugates not absorbed to charcoal as well as naphthalene 1,2-oxide or 1,2-naphthoquinone covalently bound to tissue macromolecules. Two per cent of the radioactivity appeared to be covalently bound to protein [22]. Prolonged extraction procedures were required to remove all the non-covalently bound radioactive material. In conclusion the balance sheet of naphthalene metabolites in isolated hepatocytes indicates that naphthalene dihydrodiol and its glucuronide are major products of naphthalene metabolism.

Conversion of naphthalene into naphthalene dihydrodiol glucuronide was also studied in liver microsomes under various conditions. Microsomal suspensions, containing 5 or 13 mg microsomal protein/ml, were incubated with an NADPH regenerating system and 3 mM UDP-glucuronic acid in the presence and absence of 3 mM UDPGlcNAc which has been characterized as a positive allosteric effector of GT [23–26]. Concentrations of the nucleotides in the microsomal system were higher than those found in the liver tissue (0.3 μmole UDP-glucuronic acid/g liver [13, 27]; 0.4 μmole UDPGlcNAc [28]). These nucleotide concentrations were used since, in contrast to the intact cell, the nucleotides are not regenerated but rapidly hydrolyzed by nucleotide pyrophosphatase. The level of UDP-glucuronic acid in isolated hepatocytes is not known. From the close correspondence of naphthol glucuronidation in hepatocytes and in the perfused liver, shown above, it can be inferred, that the nucleotide level may be similar to the intact liver.

The formation of naphthalene dihydrodiol glucuronide was very slow in the microsomal system (Table 1). Addition of UDPGlcNAc markedly increased the proportion of dihydrodiol glucuronide. Increased glucuronidation after the addition of UDPGlcNAc to the rat liver microsomal system is not solely due to allosteric activation of GT but also the result of competitive inhibition of nucleotide pyrophosphatase [29]. Under our conditions the latter enzyme decreases the concentration of UDP-glucuronic acid from 3 mM to 1.7 mM during 40 min incubation of microsomal suspensions (5 mg protein/ml). However partial inhibition of the hydrolysis of UDP-glucuronic acid in the presence of UDPGlcNAc cannot solely account for the 4-fold increase of naphthalene dihydrodiol glucuronide. Thus allosteric activation is most probably the major cause for the increased glucuronide formation.

When the concentration of microsomal suspensions was increased non-enzymatic conversion of naphthalene 1,2-oxide into 1-naphthol and its glucuronide was reduced (Table 1). Hence the concentration of microsomal membranes may alter the proportion of

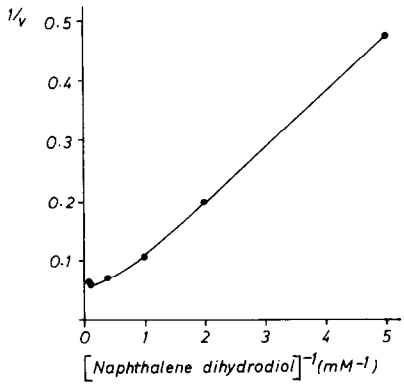

Fig. 3. Double reciprocal plots of initial rates of naphthalene dihydrodiol glucuronidation. Initial rates were determined with liver microsomes activated by the addition of Triton X-100 (0.05%, v/v) as described in Methods. Velocity v is given in nmole min^{-1} mg $protein^{-1}$. Values represent the mean of 3 experiments.

Table 2. Influence of phenobarbital and 3-methylcholanthrene on the glucuronidation of naphthalene dihydrodiol and 1-naphthol*

Treatment *in vivo*	Addition of Triton X-100 *in vitro*	UDP-glucuronyltransferase (nmole min^{-1} mg $protein^{-1}$) Naphthalene dihydrodiol	1-Naphthol
—	−	2 ± 1	3 ± 1
—	+	22 ± 3	63 ± 15
Phenobarbital	−	2 ± 1	5 ± 2
Phenobarbital	+	26 ± 5	91 ± 20
3-Methylcholanthrene	−	2 ± 1	8 ± 2
3-Methylcholanthrene	+	26 ± 3	208 ± 49

* Rats were treated with phenobarbital or 3-methylcholanthrene, and UDP-glucuronyltransferase was assayed in liver microsomes as described in Methods. The mean of 4 experiments ± S.D. is listed.

drug metabolites: 0.15 ml packed hepatocytes contain about 5 mg microsomal protein, based on 31 mg microsomal protein/g liver [30]. It should be noted however that in the microsomal system the membranes are equally distributed in 1 ml incubation medium, whereas in incubations with hepatocytes they are confined to 0.15 ml, i.e. the concentration of microsomal membranes is much higher in the intact cell system.

Our comparative studies on the conversion of naphthalene dihydrodiol glucuronide indicate that glucuronide formation is very efficient in the intact hepatocyte in contrast to the microsomal system. When UDPGlcNAc is added to the microsomal system the proportion of free naphthalene dihydrodiol to its glucuronide is similar to that found in hepatocytes. Hence it is conceivable that UDPGlcNAc is an important intracellular effector of GT. In the absence of UDPGlcNAc the potentially toxic naphthalene dihydrodiol would accumulate. Other known activators of GT such as membrane perturbants lead to a form of GT which is inhibited by UDP and other UDP-sugars [31]. This form does not favour glucuronide formation under conditions presumed to exist *in vivo*.

Effects of phenobarbital and 3-methylcholanthrene on the glucuronidation of naphthalene dihydrodiol. When GT was studied in microsomes with naphthalene dihydrodiol as substrate initial reaction rates increased linearly up to a concentration of 1 mM (Fig. 3). The glucuronidation rate in native microsomes was markedly activated by membrane perturbants such as Triton X-100 (Table 2). Treatment of rats with phenobarbital or 3-methylcholanthrene did not significantly stimulate the glucuronidation of naphthalene dihydrodiol both in native and activated microsomes. Similar results were obtained with microsomal preparations solubilized by deoxycholate [32]. In contrast, the glucuronidation of 1-naphthol was markedly enhanced by treatment with 3-methylcholanthrene, in agreement with previous studies [32]. Cytochrome P-450 dependent monooxygenase, epoxide hydratase and GT are known to be inducible by phenobarbital or 3-methylcholanthrene. There is accumulating evidence for the existence of multiple substrate specific forms of cytochrome P-450 [33–38] and GT [32, 39, 40] which are selectively induced by the above inducing agents. It is surprising that GT (naphthalene dihydrodiol as substrate) and the functionally linked monooxygenase–epoxide hydratase system are not simultaneously induced. A lack of correlation between the induction of the monooxygenase system and GT was also observed in cultured chick embryo liver [41]. Even in the case of the coupled monooxygenase and epoxide hydratase, evidence has been obtained that these two enzymes are under independent genetic control [42].

The three microsomal enzymes catalyzing the conversion of naphthalene into naphthalene dihydrodiol glucuronide (monooxygenase, epoxide hydratase and GT) are confined to the same membranes. A more detailed study of this reaction sequence may lead to a better understanding of the role of the membrane in the linkage of sequential enzyme reactions. The membrane may facilitate the communication of the enzymes. In addition it may prevent the leakage of lipid-soluble intermediary products out of the membrane and thus concentrate substrates for subsequent enzyme reactions. The described model system may be useful to study microsomal enzymes involved in the metabolism of polycyclic hydrocarbons in extrahepatic tissues such as kidney, lung, intestine and skin.

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