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EFFECTS OF PHENOBARBITAL AND 3-METHYLCHOLANTHRENE ON SUBSTRATE SPECIFICITY OF RAT LIVER MICROSOMAL UDP-GLUCURONYLTRANSFERASE

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SUMMARY

1. Substrate specificity of liver microsomal UDPglucuronyltransferase (UDP-glucuronate glucuronyltransferase, EC 2.4.1.17) towards *p*-nitrophenol, 1-naphthol, bilirubin and chloramphenicol changes after treatment of rats with phenobarbital and 3-methylcholanthrene. Phenobarbital mainly increases the glucuronidation of chloramphenicol, whereas 3-methylcholanthrene only stimulates the glucuronidation of the two phenolic substrates. These changes have been observed using 'native', Triton X-100-treated, or deoxycholate-solubilized microsomes as well as partially purified enzyme preparations.

2. Treatment with phenobarbital or 3-methylcholanthrene did not alter the apparent K_m values for UDPglucuronic acid (0.16 mM) and *p*-nitrophenol (0.34 mM).

3. The results suggest that several forms of UDPglucuronyltransferase exist in rat liver microsomes which are selectively induced by either phenobarbital or 3-methylcholanthrene.

INTRODUCTION

The synthesis of glucuronides by liver microsomal UDPglucuronyltransferase (UDPglucuronate glucuronyltransferase, EC 2.4.1.17) is a major pathway by which the body inactivates both endogenous and foreign compounds. The enzyme catalyses the transfer of activated glucuronic acid to a variety of lipophilic hydroxyl, carboxyl and amine acceptors, thus forming *O*- and *N*-glucuronides. It has been possible to separate *O*- and *N*-glucuronyltransferases[1]. In addition, many other observations favour the idea of a heterogeneous group of closely related enzymes with a preferential specificity for certain classes of substrates[2]; *e.g.* homozygous Gunn rats are unable to form bilirubin glucuronides whereas their ability to glucuronidate *p*-nitrophenol is not impaired. However, the multiplicity of UDPglucuronyltransferase is still debated; *e.g.* competitive inhibition between bilirubin and *p*-nitrophenol glucuronidation has been found suggesting that both substrates are conjugated at the same

active site[3]. So far all attempts have been unsuccessful to completely purify the enzyme or to separate *O*-glucuronyltransferases. Purification of this enzyme is hampered because its properties are intimately related to interactions with the membrane environment especially with phospholipids[4].

In the present report the substrate specificity of liver UDPglucuronyltransferase is studied after treatment of rats with either phenobarbital or 3-methylcholanthrene, two different types of inducers of cytochrome P-450-dependent monooxygenase[5] as well as of UDPglucuronyltransferase[6–8]. *p*-Nitrophenol and 1-naphthol have been used as phenolic, chloramphenicol as alcoholic and bilirubin as carboxylic acceptor substrates. The results, which have been presented in a preliminary form[9] indicate that UDPglucuronyltransferase is affected differently after treatment of rats with phenobarbital or 3-methylcholanthrene. This suggests that several UDPglucuronyltransferases exist in microsomes. However, it cannot be ruled out that the membrane environment influences substrate specificity.

METHODS

Male Sprague–Dawley rats (150–180 g) were fed *ad libitum* a standard diet containing 20% protein (Altromin-R, Lage-Lippe, Germany). Phenobarbital was given by 3 daily intraperitoneal injections of 100 mg/kg and liver microsomes were prepared on the 5th day. 3-Methylcholanthrene was dissolved in olive oil and injected intraperitoneally as a single dose of 80 mg/kg. Liver microsomes were prepared on the 4th day.

Preparation of 'native' and activated microsomal fractions

Livers of rats under ether anesthesia were perfused through the portal vein with 0.9% (w/v) NaCl, excised, then minced with scissors and homogenized in 4 vol. of 0.25 M sucrose using a motor-driven homogenizer with a Teflon pestle. The homogenate was centrifuged at 10 000 × *g* for 15 min to sediment nuclei and mitochondria. The ensuing supernatant was spun at 100 000 × *g* for 45 min and the pellet suspended in 0.25 M sucrose. This fraction is called the 'native' microsomal fraction. In order to activate microsomal UDPglucuronyltransferase, 'native' microsomes were treated *in vitro* in a number of different ways: (a) Sonication: microsomes were sonicated at 0 °C with an MSE sonifier at 1.5 A (8 times for 30 s each time). This resulted in the maximal increase of UDPglucuronyltransferase activity. (b) Triton X-100-treated microsomes: unless otherwise indicated 0.05% (w/v, final concentration) Triton X-100 was added to the incubation mixture used for the assay of UDPglucuronyltransferase. (c) Deoxycholate solubilization: the microsomal pellet was suspended in 0.05 M Tris–HCl (pH 8.0) containing 1 mM EDTA. After addition of 0.23% (w/v, final concentration) sodium deoxycholate the mixture was centrifuged at 100 000 × *g* for 90 min. The supernatant was passed through a Sephadex G-25 column (20 cm × 2.5 cm) equilibrated with 0.05 M Tris–HCl (pH 8.0) containing 1 mM EDTA. Microsomal proteins were collected as the coloured fraction eluted in the void volume. (d) Trypsin treatment: this was carried out as described by Omura *et al.*[10]. The 100 000 × *g* sediment was suspended in 0.25 M sucrose and used for the enzyme assay.

Assays of UDPglucuronyltransferase

Assays were performed at 37 °C in a total volume of 1 ml containing 100 mM Tris–HCl (pH 7.4), 5 mM $MgCl_2$ as well as different acceptor substrates and microsomal protein as indicated. The enzyme reaction was started by the addition of 3 mM UDPglucuronic acid. In controls, UDPglucuronic acid was omitted. (a) *p*-Nitrophenol glucuronidation: 1 μmole *p*-nitrophenol and 1 mg microsomal protein were added to the assay mixture. The reaction was linear between 1 and 6 min. *p*-Nitrophenol was determined in 0.1 M NaOH essentially as described by Hollmann and Touster[11] using a mmolar extinction coefficient of 18 (ref. 1). In order to determine initial reaction velocities at low substrate concentrations, the decrease of *p*-nitrophenol was monitored continuously at pH 7.4 in cuvettes having an optical path length of 0.1 cm, using a mmolar extinction coefficient of 15.5 for calculation. (b) 1-Naphthol glucuronidation was assayed with the radioactive method of Lucier *et al.*[12] using 0.5 mM 1-naphthol (dissolved in 0.3% (v/v, final concentration) dimethylsulphoxide) and 0.5 mg microsomal protein. The reaction was linear between 1 and 6 min. (c) Bilirubin glucuronidation was determined with the method of Van Roy and Heirwegh[13]. 0.3 mM bilirubin dissolved in 0.1 M Na_2CO_3 was added together with 2 mg microsomal protein. The reaction was linear up to 10 min. (d) Chloramphenicol glucuronidation: 1.5 mM chloramphenicol (dissolved in 0.6% (v/v, final concentration) dimethylsulphoxide) was added together with 5 mg microsomal protein. Glucuronidation was linear up to 20 min. The reaction was terminated with 2.4 ml 0.2 M trichloroacetic acid. After centrifugation the supernatant was adjusted to pH 6.0 and free chloramphenicol was extracted twice with 25 ml chloroform–ethyl acetate (2:1, v/v). From the remaining aqueous phase 3 ml were carefully removed and mixed with 0.4 ml 1 M NaOH and 25 mg sodium dithionite for reduction of the nitro group. After 15 min at room temperature chloramphenicol glucuronide was determined as described by Levine and Fischbach[14]. The glucuronide could be measured with the same method as the unconjugated chloramphenicol since the decrease of chloramphenicol in the chloroform–ethyl acetate extract corresponded well with the increase of chloramphenicol glucuronide in the aqueous phase. Practically all the chloramphenicol detected in the aqueous phase could be extracted into the organic phase, after treatment of the aqueous phase with β-glucuronidase for 24 h.

Protein was determined with the method of Lowry *et al.*[15] using bovine serum albumin as standard. For determination of cytochrome P-450, the method of Omura and Sato[16] was used. Phospholipids were extracted from microsomes with the procedure of Folch *et al.*[17]. After evaporation and ashing of the extract inorganic phosphate was measured by the method of Ames and Dubin[18] and μmoles of inorganic phosphate were multiplied by 775 to obtain μg of phospholipid. Radioactivity was determined by liquid scintillation counting either in Brays solution[19] or in a toluene-based scintillation fluid containing 0.5% (w/v), 2,5-diphenyloxazol and 0.05% (w/v) *p*-bis-2-(5-phenyloxazolyl)benzene. Quenching was monitored by the addition of an internal standard.

RESULTS

Activation of microsomal UDPglucuronyltransferase in vitro

A stimulation of microsomal UDPglucuronyltransferase activity has been ob-

served by a variety of treatments affecting membrane integrity, *e.g.* ageing[20], sonication[4,21], addition of detergents[8,20,22,23] or treatment of microsomes with trypsin[23]. This activation has to be taken into account in studies on a possible induction of this enzyme. The determination of enzyme activity in a maximally activated state possibly eliminates this problem and probably best reflects the amount of enzyme present in the membrane.

The extent of activation varies with different substrates (Table I). After the addition of Triton X-100 the conjugation of *p*-nitrophenol or 1-naphthol was activated about 10-fold whereas the glucuronidation of chloramphenicol was stimulated only

TABLE I

ACTIVATION OF RAT LIVER MICROSOMAL UDPGLUCURONYLTRANSFERASE *in vitro*

Microsomes were prepared from phenobarbital-treated rats. See Methods for the treatment of microsomes *in vitro* and for the enzyme assays. Data are listed as the mean ±S.D. The number of experiments is given in parenthesis.

Treatment in vitro	*UDPglucuronyltransferase (nmoles/min per mg protein)*			
	p-Nitrophenol	*1-Naphthol*	*Chloramphenicol*	*Bilirubin*
None	5 ± 2 (6)	11 ± 3 (6)	0.8 ± 0.2 (4)	0.7 ± 0.08 (7)
Triton X-100	61 ± 9 (18)	86 ± 18 (18)	2.6 ± 0.4 (4)	0.6 (3)
Sonication	56 ± 3 (4)	—	—	1.6 (3)
Deoxycholate (100 000 × *g* supernatant)	79 ± 14 (4)	118 ± 19 (4)	4.0 ± 0.5 (4)	2.5 ± 0.3 (4)
Trypsin (100 000 × *g* sediment)	75 ± 5 (4)	—	—	—

3-fold. In our hands bilirubin glucuronidation could not be activated by Triton X-100 in contrast to several earlier reports[3,24]. After sonication and in deoxycholate-solubilized microsomal preparations, however, bilirubin glucuronidation was markedly activated.

Several methods have been compared to find a fully activated form of the enzyme (Table I). A similar maximal activity of *p*-nitrophenol conjugation was obtained with Triton X-100-treated or sonicated microsomes. Deoxycholate-solubilized or trypsin-treated microsomal preparations are not necessarily comparable with Triton X-100-treated or sonicated microsomes. Trypsin treatment removes about 30% of microsomal proteins (but not UDPglucuronyltransferase) without affecting the bilayer structure of the microsomal vesicles[10]. When a 30% loss of proteins is taken into account the corrected specific activity corresponds well with that of Triton X-100-treated and sonicated microsomes. The higher specific activity in deoxycholate-solubilized enzyme preparations is also partly due to the removal of non-enzyme proteins during centrifugation. However, some additional activation due to treatment with EDTA[22] may also account for the higher specific activity of this preparation compared with the others.

Detergents exert two different effects on membrane-bound glucuronyltransferase. At low concentrations an activating effect is dominant probably due to an alteration of membrane structure; at high concentrations the enzyme is inhibited[22–25]. Maximal stimulation was achieved with 0.05% Triton X-100 in agreement with

Winsnes[24]. With the deoxycholate-solubilized microsomal preparation only the inhibiting effect of Triton X-100 was observed (Fig. 1). The deoxycholate-solubilized preparation was considered most reliable for the studies on enzyme induction since with all substrates the highest specific activity was obtained. However, it was always compared with 'native' and Triton X-100-treated microsomes. Solubilization denotes here that the enzyme preparation does not sediment during centrifugation for 90 min at 100 000 × *g* although the enzyme is still part of a macromolecular structure[26].

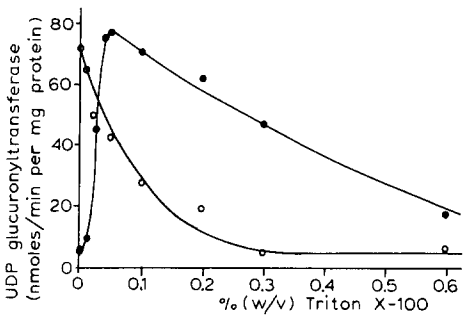

Fig. 1. Influence of Triton X-100 on membrane-bound and deoxycholate-solubilized rat liver UDPglucuronyltransferase. ●—●, "native" microsomes; ○—○, deoxycholate-solubilized microsomes. Microsomes were prepared from phenobarbital-treated rats and the enzyme was assayed with *p*-nitrophenol as acceptor substrate as described in Methods.

Induction of UDPglucuronyltransferase after treatment of rats with phenobarbital or 3-methylcholanthrene

After treatment of rats with the inducers, UDPglucuronyltransferase activity is affected differently depending on the substrate. 3-Methylcholanthrene markedly increases the glucuronidation of *p*-nitrophenol whereas the conjugation of chloramphenicol decreases. In contrast, phenobarbital mainly stimulates chloramphenicol glucuronidation (Fig. 2). Methylcholanthrene is slowly absorbed from the peritoneal

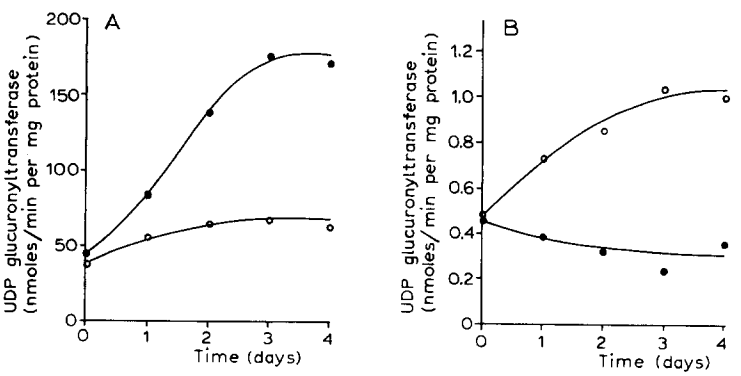

Fig. 2. Time course of UDPglucuronyltransferase induction by 3-methylcholanthrene (●—●) and phenobarbital (○—○) with *p*-nitrophenol (A) and chloramphenicol (B) as acceptor substrates. Male Sprague–Dawley rats (150 g) were injected once at 0 time with phenobarbital (100 mg/kg) or 3-methylcholanthrene (80 mg/kg). Enzyme activity with *p*-nitrophenol and chloramphenicol was determined in Triton X-100-treated liver microsomes as given in Methods. The mean of 3 experiments is listed.

TABLE II

UDPGLUCURONYLTRANSFERASE AFTER TREATMENT OF RATS WITH PHENOBARBITAL OR 3-METHYLCHOLANTHRENE IN DIFFERENT MICROSOMAL PREPARATIONS

Treatment of rats with phenobarbital or 3-methylcholanthrene, the preparation of liver microsomal fractions and enzyme assays were performed as described in Methods. Data are listed as the mean of at least 4 preparations ±S.D.

Microsomal preparation	*Treatment in vivo*	*UDPglucuronyltransferase (nmoles/min per mg protein)*	
		p-Nitrophenol	*Chloramphenicol*
"Native" microsomes	—	3 ± 2	0.2 ± 0.1
	Phenobarbital	5 ± 2	0.8 ± 0.2
	Methylcholanthrene	12 ± 5	—
Triton X-100-	—	38 ± 5	0.5 ± 0.1
-treated	Phenobarbital	61 ± 9	2.6 ± 0.4
microsomes	Methylcholanthrene	167 ± 20	0.5 ± 0.1
Deoxycholate-	—	57 ± 10	1.1 ± 0.1
-solubilized	Phenobarbital	79 ± 14	4.0 ± 0.5
microsomes	Methylcholanthrene	174 ± 20	0.7 ± 0.1

cavity and is present in the liver for a long time period after a single injection[27]. Phenobarbital has to be given continuously in order to obtain maximal stimulation which is reached after 3–4 days. Similar changes in the catalytic properties of UDP-glucuronyltransferase are observed in 'native' and Triton X-100-treated microsomes as well as in deoxycholate-solubilized preparations (Table II). The relative changes in substrate specificity after treatment of rats with the different types of inducers are clearly seen in deoxycholate-solubilized enzyme preparations (Table III). The glucuronidation of 1-naphthol was affected similarly to that of *p*-nitrophenol while bilirubin more closely resembled chloramphenicol, *e.g.* its conjugation was not stimulated by 3-methylcholanthrene. Despite this similarity between the glucuronidation of chloramphenicol and bilirubin, studies with homozygous Gunn rats revealed clear differences. The genetically defective Gunn rats are incapable of forming bilirubin glucuronides[28–30]. However, they retain their ability to glucuronidate chloramphenicol which can be stimulated by phenobarbital. Specific activities of

TABLE III

RELATIVE ACTIVITIES OF LIVER MICROSOMAL UDPGLUCURONYLTRANSFERASE AFTER TREATMENT OF RATS WITH PHENOBARBITAL OR 3-METHYLCHOLANTHRENE

The relative activity was calculated from deoxycholate-solubilized enzyme preparations by setting the specific activity in untreated controls as 100%. Data for *p*-nitrophenol and chloramphenicol were taken from Table II. Specific activities for 1-naphthol and bilirubin in controls were 76 ± 15 and 1.2 ± 0.1 nmoles/min per mg protein, respectively.

Substrate	*Relative activity (%)*	
	Phenobarbital	*3-Methylcholanthrene*
p-Nitrophenol	139	305
1-Naphthol	155	173
Bilirubin	208	100
Chloramphenicol	364	64

0.5 and 1.0 nmole chloramphenicol conjugated per min per mg protein were found in Triton X-100-treated microsomes from untreated and phenobarbital treated Gunn rats, respectively, under the conditions listed in Table II.

When protein synthesis was inhibited with cycloheximide, the increase of *p*-nitrophenol glucuronidation after treatment with 3-methylcholanthrene was significantly reduced (Table IV). At the dosage of cycloheximide (0.5 mg/kg) protein

TABLE IV

INFLUENCE OF CYCLOHEXIMIDE ON THE INDUCTION OF UDPGLUCURONYLTRANSFERASE (*p*-NITROPHENOL AS SUBSTRATE) BY 3-METHYLCHOLANTHRENE

Sprague–Dawley rats were treated intraperitoneally with 3-methylcholanthrene (as listed in Methods) or with cycloheximide (0.5 mg/kg, every 12 h) or with both drugs. Controls received olive oil only. After 24 h liver microsomes were prepared from 2 animals. The mean of 4 experiments ±S.D. is listed.

Treatment in vivo	*UDPglucuronyltransferase p-nitrophenol as substrate (nmoles/min per mg protein)*
—	38 ± 4
Cycloheximide	39 ± 6
3-Methylcholanthrene	73 ± 8
3-Methylcholanthrene + cycloheximide	48 ± 7

synthesis was reduced by about 40% during a period of 12 h (as judged from the incorporation of [^{14}C]leucine into liver proteins[31]). Secondary changes of organ functions (azotemia, acid–base changes and histological abnormalities) which have been clearly detectable with 2.5 mg/kg cycloheximide were not observed with the lower dosage[31].

Kinetic analysis of p-nitrophenol glucuronidation

The increased enzyme activity after treatment with phenobarbital or 3-methylcholanthrene could be either caused by an altered K_m value for the substrates or by an increased amount of enzyme. In order to distinguish between these possibilities kinetic studies were performed. K_m values for *p*-nitrophenol and UDPglucuronic acid have been determined graphically with the method described by Cleland[32], similar to the studies of Vessey and Zakim[33] with beef liver and guinea pig liver microsomes who found a random sequential reaction mechanism. Initial rates of enzyme activity were measured as a function of the concentration of *p*-nitrophenol at several fixed concentrations of UDPglucuronic acid. The data were plotted according to Lineweaver and Burk (Fig. 3, A) and the intercepts on the ordinate ($1/V$) were used to determine the K_m value for UDPglucuronic acid (Fig. 3, B). Using this method apparent K_m values determined in microsomal preparations from untreated rats were compared with those from animals treated with the inducers. As shown in Table V the K_m values did not significantly change after treatment with either phenobarbital or 3-methylcholanthrene.

Partial purification of UDPglucuronyltransferase

Partial purification of UDPglucuronyltransferase has been reported with rabbit

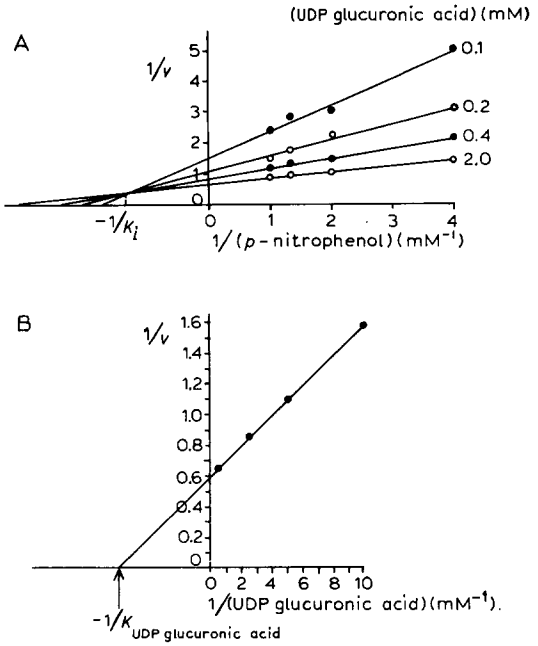

Fig. 3. Bisubstrate kinetic analysis of UDPglucuronyltransferase, *p*-nitrophenol as substrate. A. Double reciprocal plots of initial rates of UDPglucuronyltransferase activity as a function of varying concentrations of *p*-nitrophenol at different fixed concentrations of UDPglucuronic acid. B. Secondary plots of the intercepts on the $1/V$ axis *versus* 1/(UDPglucuronic acid). The data were obtained with a deoxycholate-solubilized liver microsomal preparation form phenobarbital-treated rats.

and guinea pig liver microsomes[1,26]. The extent of enzyme purification may have been overestimated because processes of activation were not taken into account. After solubilization with phospholipase, unstable enzyme preparations have been obtained[1].

Solubilization of the enzyme with cholate and subsequent $(NH_4)_2SO_4$ fractionation according to Lu *et al.*[34] however, yielded a partially purified enzyme

TABLE V

KINETIC CONSTANTS OF RAT LIVER MICROSOMAL UDPGLUCURONYLTRANSFERASE, *p*-NITROPHENOL AS SUBSTRATE

Microsomal preparations are the same as those listed in Table II. Apparent K_m values for UDP-glucuronic acid (UDPGA) and *p*-nitrophenol were determined graphically as described in Fig. 3. The mean of two experiments is listed.

Microsomal preparation	*Treatment in vivo*	K_{UDPGA} *(mM)*	$K_{p\text{-nitrophenol}}$ *(mM)*
Triton X-100-	—	0.13	0.35
-treated	Phenobarbital	0.24	0.40
microsomes	3-Methylcholanthrene	0.18	0.37
Deoxycholate-	—	0.10	0.33
-solubilized	Phenobarbital	0.16	0.28
microsomes	3-Methylcholanthrene	0.16	0.31

preparation stable for months, partly separated from cytochrome P-450 (Table VI). 3-Methylcholanthrene induces a special form of cytochrome P-450, called P_1-450 (ref. 35) or P-448 (ref. 34). In sodium dodecyl sulphate–polyacrylamide gels, according to Schimke and Dehlinger[36], a prominent protein band with a molecular weight of 50 000 was substantially reduced. The best separation between glucuronyltransferase and cytochrome P-450 was obtained with microsomes from methylcholanthrene-

TABLE VI

PARTIAL PURIFICATION OF RAT LIVER MICROSOMAL UDPGLUCURONYLTRANSFERASE

Microsomes were prepared from 3 rats treated with 3-methylcholanthrene. They were solubilized with cholate and fractionated with $(NH_4)_2SO_4$ as described by Lu *et al.*[34]. Determinations were performed as described in Methods.

Preparation	*UDPglucuronyltransferase, p-nitrophenol as substrate (nmoles/min per mg protein)*	*Cytochrome P_1-450 (nmoles per mg protein)*	*mg phospholipid per mg protein*	*Total protein (mg)*
Cholate-solubilized microsomes	165	1,5	0.4	300
$(NH_4)_2SO_4$ fraction				
0–40%	25	1.3	0.1	98
40–50%	73	2.8	0.2	47
>50%	324	0.9	1.0	71

treated animals. No separation between the glucuronidation of different substrates could be achieved by the $(NH_4)_2SO_4$ fractionation. However, the altered substrate specificity seen in microsomes after induction was retained in the partially purified enzyme preparation, showing that the substrate specificity was not influenced by the removed proteins. The activity of UDPglucuronyltransferase could not be influenced by the addition of lecithin micelles prepared from soybean or egg yolk lecithin by sonication in contrast to enzyme preparations obtained after treatment with phospholipase A and C (refs 37–39).

DISCUSSION

Studies on a possible induction of microsomal UDPglucuronyltransferase are complicated by the marked activation of this enzyme following various treatments which affect membrane integrity. The term induction is used in an operational sense as an increase in the amount of a protein regardless of the mechanism causing this increase. Two models have been proposed to explain the activation of UDPglucuronyltransferase: (a) The enzyme might exist in different conformational forms[4]. (b) The active site of the enzyme might be buried within the lipid phase of the membrane or may be located on the inside of microsomal vesicles[8,23] thus constituting a permeability barrier for the substrates. To avoid problems caused by these types of activation, UDPglucuronyltransferase was assayed in a maximally activated form which probably best reflects the amount of enzyme.

Depending on the acceptor substrate, UDPglucuronyltransferase was affected differently after treatment of rats with phenobarbital or 3-methylcholanthrene. After methylcholanthrene the glucuronidation of *p*-nitrophenol and 1-naphthol was

markedly stimulated (305 and 173%, respectively), while the conjugation of bilirubin and chloramphenicol was unchanged or slightly decreased. In contrast, after phenobarbital, the glucuronidation of chloramphenicol and bilirubin were mainly stimulated (364 and 208%, respectively), whereas the conjugation of the phenolic substrates was only increased about 150% of controls. Thus, by our induction studies two groups of substrates for UDPglucuronyltransferase can be distinguished: the conjugation of one group (*e.g.* *p*-nitrophenol, 1-naphthol) is preferentially stimulated by 3-methylcholanthrene whereas the glucuronidation of the second group (*e.g.* chloramphenicol, bilirubin) is mainly increased by phenobarbital. However, despite this similarity between chloramphenicol and bilirubin, studies with homozygous Gunn rats revealed clear differences. These genetically defective rats are incapable of forming bilirubin glucuronides[28–30] but are still able to conjugate chloramphenicol.

The increase of *p*-nitrophenol glucuronidation after treatment with 3-methylcholanthrene could be either attributed to an alteration of the enzyme as reflected by the apparent K_m values or to an increased amount of enzyme. However, the K_m values for UDPglucuronic acid (0.16 mM) and *p*-nitrophenol (0.34 mM) were not significantly altered after treatment with the inducer. Furthermore, when protein synthesis was diminished during treatment with cycloheximide the stimulation of *p*-nitrophenol glucuronidation by methylcholanthrene was significantly reduced suggesting that enzyme synthesis was responsible for the increase in enzyme activity.

The induction of UDPglucuronyltransferase and the regulation of its substrate specificity seems to be species dependent. In guinea pig liver the specific activity of microsomal bilirubin glucuronyltransferase could be stimulated by 3-methylcholanthrene but not with phenobarbital[40] in contrast to our findings with rats.

The observed changes in the catalytic properties of UDPglucuronyltransferase after treatment of rats with phenobarbital or 3-methylcholanthrene can be most easily explained by assuming different UDPglucuronyltransferases. These observations thus add to a variety of other evidences in favour of a multiplicity of these enzymes[2,22]. However, unless more purified enzyme preparations are available, it cannot be ruled out that the change of substrate specificity is caused by alterations of the membrane environment influencing one single enzyme. A major argument against heterogeneity of UDPglucuronyltransferase is the competitive inhibition between the glucuronidation of *p*-nitrophenol and bilirubin[3]. It is conceivable, however, that the unspecific binding site for the different lipophilic aglycons may well be occupied by compounds which are not substrates of the enzyme. In fact an inhibition of UDPglucuronyltransferase could be detected with aminopyrin, SKF 525 A and metapyrone (unpublished).

Similar changes of substrate specificity after phenobarbital and 3-methylcholanthrene have also been observed with the cytochrome P-450-dependent monooxygenase[5,41], a microsomal enzyme system functionally linked to UDPglucuronyltransferase. Likewise, in the case of microsomal monooxygenase there is accumulating evidence for a multiplicity of related enzyme systems[35,41–43].

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