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IMMUNOCHEMICAL AND FUNCTIONAL CHARACTERIZATION OF UDP-GLUCURONOSYLTRANSFERASES FROM RAT LIVER, INTESTINE AND KIDNEY*

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Abstract—Glucuronidation of various substrates in hepatic, intestinal and renal microsomes of control, phenobarbital (PB), 3-methylcholanthrene (3MC) and Aroclor-1254 (A1254) pretreated rats was investigated. UDPGT activities tested could be divided in four groups on the basis of their tissue distribution and induction by PB or 3MC in liver microsomes. GT1 activities (1-naphthol, benzo(*a*)pyrene-3,6-quinol) are induced by 3MC in liver microsomes and are present in all tissues investigated. GT2 activities (morphine, 4-hydroxybipheynl) are induced by PB in liver microsomes and appear to be restricted to the liver and the intestine. UDPGT activity towards bilirubin, although induced by PB, can be detected in hepatic, intestinal and renal microsomes. UDPGT activity towards fenoterol is restricted to the liver and intestine and is not induced by PB, 3MC or A1254.

The presence of inducible immunoreactive UDPGT isoenzymes in microsomes of liver, intestine and kidney of control and induced rats was demonstrated by immunoblot analysis using rabbit anti-rat liver-GT1 antibodies. Induction of both 54 and 56 kDa polypeptides in hepatic, intestinal and renal microsomes by 3MC or A1254 was observed. Purification of UDPGT (1-naphthol as substrate) from intestinal microsomes to apparent homogeneity yielded a polypeptide with an apparent molecular weight of 54–56 kDa.

The results indicate that 54 and 56 kDa UDPGT polypeptides are the major A1254 inducible isoenzymes in intestinal and renal microsomes. An increase in immunoreactive protein is correlated with a biochemically measurable increase in glucuronidation capacity for GT1 substrates.

UDPGT‡ activity towards various xenobiotics can be detected in a large number of organs [1, 2]. These activities are mediated by a family of isoenzymes [3, 4] and can be classified according to different criteria [5]. The model of differential induction, based on selective induction of enzyme activities in rat liver microsomes by 3MC or PB, is widely used [5–8]. Activity towards GT1 substrates is induced by 3MC; activity towards GT2 substrates by PB. The occurrence of activity towards various groups of substrates in different organs is not known in detail, but it is suggested that activity towards GT1 substrates is ubiquitous and that activity towards GT2 substrates is only present in the liver and the intestine [2].

Induction in the strict sense implicates an increase in enzyme protein. Immunological methods make it possible to measure an increase in immunoreactive protein. An increase in UDPGT-protein in rat liver microsomes by 3MC has been measured with the aid of antibodies raised against purified GT1, the 3MC-inducible isoenzyme of UDPGT [9]. Immunoblot analysis makes it possible to detect selective induction of particular isoenzymes [10, 11]. This method has recently been applied to detect 3MC inducible UDPGT isoenzymes in liver microsomes [12, 13]. We decided to use this method to study the induction of UDPGT isoenzymes in rat intestinal and renal microsomes. Immunoblot analysis was combined with the measurement of UDPGT activity towards aglycones belonging to different substrate groups (according to the model of differential induction). The results indicate that increases of 54 and 56 kDa polypeptides, recognized by rabbit anti-rat GT1 antibodies are correlated with the induction of UDPGT activity towards GT1-substrates in hepatic, intestinal as well as renal microsomes.

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‡ Abbreviations used: PB, phenobarbital; 3MC, 3-methylcholanthrene; A1254, Aroclor-1254; HPLC, high-performance liquid-chromatography; BP-3,6-quinol(MG), benzo(*a*)pyrene-3,6-quinol-(monoglucuronide); UDPGT, UDP-glucuronosyltransferase (EC 2.4.1.17); GT_1, 3-methylcholanthrene-inducible UDPGT-activities; GT_2, phenobarbital-inducible UDPGT-activities; GT_1 and GT_2 are functionally defined and do not necessarily represent UDPGT isoenzymes.

MATERIALS AND METHODS

Chemicals. 3MC was obtained from Ferak, W. Berlin (F.R.G.); Brij-58 from Atlas, Essen (F.R.G.) and A1254 from Monsanto Chemical Co., St. Louis, MO (U.S.A.). Morphine.HCl was purchased from Brocacef (Maarssen, The Netherlands), and was of Dutch pharmacopeial grade. Benzo(*a*)pyrene-3,6-quinone was obtained from the Chemical Carcinogen Reference Standard Repository, National Institutes of Health, Bethesda, MD and morphine 3-glucuronide from the National Institute on Drug Abuse, Rockville, MD (U.S.A.). Fenoterol.HBr, fenoterol-*para*-glucuronide and fenoterol-*meta*-glucuronide were kindely donated by Boehringer-Ingelheim K.G., Ingelheim/Rhein (F.R.G.). All other chemicals were of analytical grade purity and used as supplied.

Treatment of animals. Male Wistar rats (230–270 g) had access to tap water and a commercially available diet (Altromin, Lage, Lippe, F.R.G.). Pretreatment with PB, 3MC and A1254 was performed as follows. PB: An initial dose of 100 mg/kg was given once i.p. and was followed by 0.1% (w/v) in drinking water; animals were killed 4 days after treatment. 3MC: A dose of 40 mg/kg, dissolved in olive oil, was given once i.p.; animals were killed 4 days after treatment. A1254: A dose of 500 mg/kg, dissolved in olive oil, was given once i.p.; animals were killed 6 days after treatment.

Preparation of microsomes. Liver microsomes were prepared in 0.25 M sucrose/5 mM Tris pH 7.4 as described before [6]. Kidney microsomes and intestinal microsomes were prepared in the same buffer by Ultra-Turrax (Janke & Kunkel KG, Staufen im Breisgau, F.R.G.) homogenization of total kidneys and isolated intestinal epithelial cells [14], respectively. Intestinal epithelial villus cells were isolated by 10 min vibration of the everted intestine in a phosphate-buffered 5 mM EDTA-solution [15]. Microsomal preparations were diluted to 10 (intestine) or 20 (liver, kidney) mg protein/ml and stored at −20° until assay of UDPGT activities. Protein was determined according to Lowry [16] using bovine serum albumin as a protein standard.

Assays of UDP-glucuronosyltransferase. Enzyme activities towards various substrates were assayed at the following aglycone concentrations and by methods already described: 0.5 mM 1-naphthol [17]; 0.5 mM 4-hydroxybiphenyl [7]; 0.025 mM benzo(*a*)pyrene-3,6-quinol [18]; 1.0 mM morphine [19]; 0.1 mM bilirubin [20] and 1.0 mM fenoterol. Glucuronidation of fenoterol was measured as the sum of formed *meta*- and *para*-glucoronide; the glucuronides were separated by HPLC-analysis [21]. All 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 digitonin (1.5 mg/mg microsomal protein; bilirubin-assay) or Brij-58 (0.5 mg/mg microsomal protein; all other assays). 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.

Immunoblotting. SDS-polyacrylamide gel electrophoresis of microsomal proteins was carried out as described by Laemmli [22]. Proteins were transferred to nitrocellulose sheets [11] and UDPGT was made visible by reaction with rabbit antibodies, raised against purified liver-GT1 from 3MC-pretreated rats [9]. All procedures are described in detail elsewhere [23].

Purification of intestinal UDP-glucuronosyltransferase. UDP-glucuronosyltransferase was purified to apparent homogeneity from control rat intestinal microsomes using ion exchange chromatography (DEAE-Sepharose CL) and affinity-chromatography on an UDP-hexanolamine Sepharose-4B column [24]. During purification UDPGT activity towards 1-naphthol was monitored.

Experimental design and statistical analysis. UDPGT-activities were measured in duplicate each from two batches of microsomes prepared from control, PB-, 3MC- or A1254-pretreated rats. Each batch of liver, intestinal and kidney microsomes was prepared from two animals. The same batches were used for immunoblotting. Significance of induction by PB, 3MC or A1254 was tested by one-way analysis of variance, followed by the sequential Newman–Keuls test [25]. The analysis of variance was performed using logtransformed data in order to achieve homogeneity of variances; the within replicate duplicate mean square was used as error variance.

RESULTS

UDPGT activity towards all aglycones investigated could be detected in hepatic and intestinal microsomes. In kidney microsomes only glucuronidation of 1-naphthol, BP-3,6-quinol and bilirubin could be detected (Table 1). Activity towards GT1 substrates (1-naphthol, BP-3,6-quinol) is induced by 3MC in liver microsomes and can be measured in all organs investigated, while activity towards GT2 substrates (morphine, 4-hydroxybiphenyl) is induced by PB in liver microsomes and appears to be restricted to the liver and the intestine (Tables 1 and 2). A1254 induces UDPGT activities towards both groups of substrates. Induction of extrahepatic UDPGT activities is only seen after administration of A1254. Glucuronidation of fenoterol is not induced by PB, 3MC or A1254 (Table 2). The induction-factors observed (Table 2) correspond to factors observed before [2, 7, 8].

Immunoblotting of intestinal and renal microsomes suggests immunochemical similarity of some hepatic and extrahepatic UDPGTs (Fig. 1). In both extrahepatic tissues, 54 and 56 kDa polypeptides, that can be induced by 3MC or A1254, are observed similar to findings in liver microsomes [23]. It should be remarked that an increase in immunochemical recognizable 54 and 56 kDa polypeptides in intestinal and kidney microsomes by 3MC-induction is not reflected in an increase in UDPGT activities towards 1-naphthol and BP-3,6 quinol (Table 2). Furthermore, the induction by A1254 (Fig. 1) appears to be stronger than the induction of enzyme activities (Table 2).

With anti-GT_1 antibodies raised in different rabbits we observed differential affinity for the 54 and 56 kDa polypeptides. In the analysis described in Fig. 1 an antibody with preference for the 56 kDa

Table 1. Glucuronidation of various aglycones in control microsomes from rat liver, intestine and kidney

Aglycone	Liver	Intestine	Kidney
1-Naphthol	39.6 ± 6.6	21.4 ± 3.5	7.4 ± 0.9
BP-3,6-quinol	4.4 ± 0.5	0.7; 0.4	1.3 ± 0.1
BP-3,6-quinol-MG	0.20 ± 0.04	0.2; 0.3	0.05 ± 0.01
Morphine	5.8 ± 0.6	0.07 ± 0.01	n.d. (<0.01)
4-Hydroxybiphenyl	13.8 ± 2.0	2.4 ± 0.7	n.d. (<0.3)
Bilirubin	1.4 ± 0.1	0.4 ± 0.1	0.21 ± 0.04
Fenoterol	7.2 ± 0.2	6.6 ± 0.2	n.d. (<0.02)

Microsomes were prepared as described in Materials and Methods and maximal UDPGT activities were measured in the presence of 0.15% digitonin (bilirubin-assay) or 0.5 mg/mg protein Brij-58 (other assays). Units are nmole/min · mg protein ± S.E.M. of four determinations; n.d. = not detectable.

Table 2. Induction of glucuronidation in microsomes from rat liver, intestine and kidney by phenobarbital, 3-methylcholanthrene and Aroclor-1254

	Liver			Intestine			Kidney		
Aglycone	PB	3MC	A1254	PB	3MC	A1254	PB	3MC	A1254
1-Naphthol	1.5	4.3*	6.5*	0.8	0.6	2.1*	1.0	1.3	3.2*
BP-3,6-quinol	1.8*	10.4*	12.9*	1.1	1.3	2.1*	0.9	1.1	1.6*
BP-3,6-quinol-MG	2.3*	38.2*	72.0*	1.1	1.1	2.8*	1.0	1.2	2.6*
Morphine	4.3*	1.3	3.5*	1.0	0.6	1.8	—	—	—
4-Hydroxybiphenyl	3.3*	1.5	3.1*	0.8	0.4	1.3	—	—	—
Bilirubin	1.5*	0.8	0.6	0.8	0.6	1.3	1.0	1.4	2.0
Fenoterol	1.1	1.0	1.2	0.8	0.8	0.9	—	—	—

Male Wistar rats were pretreated with PB, 3MC or A1254 as described in Materials and Methods. UDPGT activities towards various aglycones were measured in fully activated microsomes. The data given in this table represent induction-factors with respect to control activities (Table 1). Comparisons between treatment groups were made with an analysis of variance, followed by the sequential Newman–Keuls test; Statistical significance ($P < 0.05$) is indicated by an asterisk.

polypeptide was used. However, using antibodies raised in other rabbits the 54 kDa polypeptide was clearly stained in intestinal microsomes from untreated controls and this polypeptide was increased in microsomes from A1254-treated rats (now shown). The reason for this differential affinity is unknown. The results demonstrate that induction of UDPGT activity towards GT1 substrates (Table 2) is correlated with an increase in the immunochemical recognizable 54 and 56 kDa polypeptides.

UDPGT (with 1-naphthol as a substrate) was purified to apparent homogeneity from intestinal microsomes. A purification factor of 70 was obtained (recovery 2.5%). The purified enzyme has an apparent molecular weight in the same range as the liver enzyme (54–56 kDa; Fig. 2).

DISCUSSION

The model of differential induction (as described in the introductory section) is supported by the present results. The distribution and induction of UDPGT activities in extrahepatic organs further suggests that UDPGT activities towards various groups of substrates are mediated by different isoenzymes.

It was observed that induction of UDPGT activity in intestinal and renal microsomes is only seen after administration of A1254. This inducer, which induces GT1 as well as GT2 activities in the liver, has a long half-life and is widely distributed in the body. It apparently attains sufficient concentration in the intestinal epithelium and the kidney to induce GT1 activities (cf. ref. 2). Absence of induction of GT1 activities in intestinal and renal microsomes by 3MC in our experiments is probably the result of the route of administration and the kinetics of 3MC because experiments with β-naphthoflavone, 2,3,7,8-tetrachlorodibenzo-*p*-dioxin or oral 3MC have shown that extrahepatic GT1 activities can be induced [26–29]. The absence of induction of renal GT2 activities is more likely due to the absence of inducible enzyme forms in the rat kidney (see below), because PB readily accumulates in the rat kidney [30]. It is not known why PB is unable to induce intestinal GT2 activities.

Differential induction combined with tissue distribution clearly supports the discrimination of four substrate groups. Activity towards GT1 substrates (1-naphthol, BP-3,6-quinol) is preferentially induced by 3MC in the liver and can be found in all tissues investigated. The wide distribution of GT1 activities in the liver [31], the intestine [32], the kidney [28, 29] and other organs [2] is elaborately documented. Activity towards GT2 substrates (morphine, 4-hydroxybiphenyl) is induced by PB in the liver and

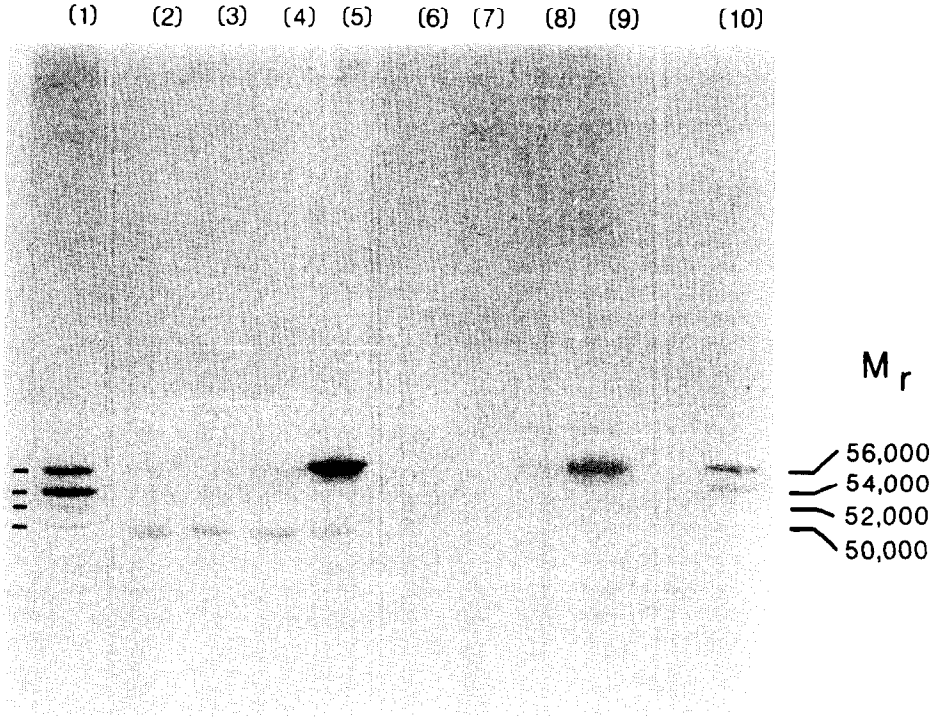

Fig. 1. Western blot analysis of UDP-glucuronosyltransferase in intestinal and renal microsomes of control, PB-treated, 3MC-treated and Aroclor-treated rats. Slab-gel electrophoresis of solubilized microsomes was performed in the presence of 0.1% (w/v) SDS according to Laemmli. After transfer to nitro-cellulose-paper the blots were developed by reaction with IgG (1:2000 dilution) raised in rabbits against GT1 purified from rat liver. Tracks 2–5, intestinal microsomes (40 μg of protein); tracks 6–9, renal microsomes (60 μg of protein); tracks 1 and 10, internal standard (hepatic microsomes of 3MC pretreated rats, 5 μg of protein in track 1, 2.5 μg in track 10. Microsomes were prepared from control (tracks 2 and 6), PB (tracks 3 and 7), 3MC (tracks 4 and 8) and A1254 (tracks 5 and 9) pretreated rats. The direction of migration was from top to bottom.

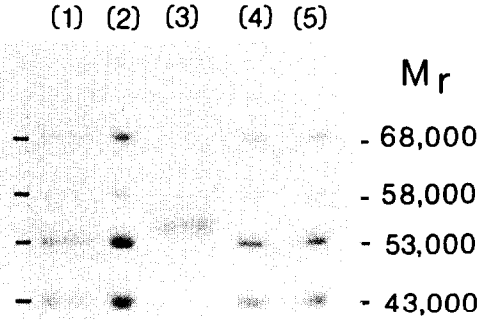

Fig. 2. SDS/PAGE of UDPGT purified from intestinal microsomes of control rats. UDPGT was purified from intestinal microsomes by DEAE-cellulose and UDP-hexanolamine chromatography. The fraction exhibiting glucuronidation activity towards 1-naphthol was subjected to SDS/PAGE (track 3; 7.5 μg of protein). Molecular markers (tracks 1, 2, 4 and 5) include bovine serum albumin (M_r 68,000), catalase (58,000), glutamate dehydrogenase (53,000) and ovalbumin (43,000). Protein was made visible by staining with Coomassie Brilliant Blue. The direction of migration was from top to bottom.

appears to be restricted to the liver and the intestine in the rat [2, 29, 32]. In the rabbit kidney, however, UDPGT activity towards morphine can be found [33]. Bilirubin has been placed in a separate group of substrates on the basis of its selective induction by clofibrate [7]. Our data warrant this separate classification because activity can be measured in hepatic [31], intestinal [34] and renal [28] microsomes and because activity can be induced by PB, but not A1254, in liver microsomes [7]. Fenoterol appears to belong to still another group of substrates. Activity towards this compound is not detected in kidney microsomes and no induction by PB, 3MC or A1254 is observed in liver and intestinal microsomes. It may be possible that fenoterol is glucuronidated by one of the constitutive steroid-UDPGTs [5, 7].

Immunochemical characterization of UDPGT isoenzymes suggests a similarity of the 54 and 56 kDa polypeptides from hepatic, intestinal and renal microsomes. The results strongly suggest that induction of UDPGT activity towards GT1 substrates and enhancement of the 54 and 56 kDa polypeptides are correlated. It was previously shown that 54 and 56 kDa polypeptides are the major 3MC inducible UDPGT forms in rat liver [12, 23]. A 3MC-inducible UDPGT isoenzyme with an apparent molecular weight of 56 kDa, which glucuronidates the GT1 substrates *p*-nitrophenol, 4-methylumbelliferone and 1-naphthol, has been purified from rat liver [35]. The apparent molecular weight of the purified

intestinal UDPGT can be estimated to be in the same range. An increase of immunochemical reactive protein and biochemically measurable enzyme activity appears not be linearly correlated, because 3MC-induction in intestinal and kidney microsomes is seen immunologically but not biochemically. A1254-induction appears to be more efficient by immunoblot analysis than by biochemical measurement. The reason for this quantitative discrepancy is unknown at the moment, but warrants further investigation.

In addition to the 3MC- and A1254-inducible 54 and 56 kDa polypeptides other non-inducible polypeptides are observed in renal and intestinal microsomes. These polypeptides are not induced by PB, 3MC or A1254 (cf. ref. 12). Whether or not these polypeptides represent distinct UDPGT isoenzymes remains to be established [23, 36].

It can be concluded that the model of differential induction is supported by the distribution and induction of UDPGT activities in the liver, the intestine and the kidney of the rat. Four different groups of UDPGT-aglycones can be delineated. A semi-quantitative estimation of immunoreactive protein by immunoblot analysis demonstrates that 54 and 56 kDa polypeptides represent the major 3MC- or A1254-inducible UDPGT isoenzymes in hepatic, intestinal as well as renal microsomes. An increase in these polypeptides is correlated with a biochemically measurable increase of glucuronidation capacity for the GT1 substrates 1-naphthol and BP-3,6-quinol. Although some inter-species comparisons of rat, rabbit and human liver UDPGTs are available [4, 12, 23] the data do not warrant a direct extrapolation of our present results.

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