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COMPARISON BETWEEN *o*-AMINOPHENOL GLUCURONIDATION IN LIVER SLICES AND HOMOGENATES FROM CONTROL AND PHENOBARBITAL-TREATED WISTAR AND GUNN RATS

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Abstract—Glucuronidation rates were studied in sliced and homogenised liver from control and phenobarbital-treated Gunn and Wistar rats of both sexes, aglycone concentration not being significantly rate-limiting. Kinetic parameters of *o*-aminophenol UDP-glucuronyltransferase (EC 2.4.1.17) were determined in fresh ("native") homogenates and those activated by UDP-*N*-acetylglucosamine, digitonin and diethylnitrosamine added singly or together. Apparent $K_{\text{UDP-glucuronate}}$-values were increased by both UDP-*N*-acetylglucosamine and digitonin, but not by the specific activator diethylnitrosamine; values were similar for both strains and not significantly affected by phenobarbital pretreatment; sex differences were encountered. Apparent V_{max}-values were increased by 30- to 65-fold for Wistar and 400- to 1600-fold for Gunn rats in maximally-activated homogenates. Phenobarbital pretreatment approximately doubled V_{max} of maximally-activated enzyme in all animals, but had little effect on V_{max} of "native" or partially-activated preparations. Slices of Gunn rat liver glucuronidated *o*-aminophenol at rates 30–44 per cent those of Wistar rats; rates in both strains exhibited a consistent sex difference and increased after phenobarbital treatment. Comparison of results from slices and homogenates containing physiological concentrations of UDP-glucuronate suggested that in both sexes and strains UDP-glucuronyltransferase activity *in vivo* is higher than that observed in "native" unactivated homogenates, presumably because of endogenous activators; however, as glucuronidation in similar, but maximally-activated, homogenates was well above that in slices, the enzyme may still remain partially "latent" *in vivo*.

A WIDE range of endogenous and exogenous compounds, including many drugs and their metabolites, are glucuronidated by the microsomal enzyme UDP-glucuronyltransferase (UDP-glucuronate glucuronyltransferase [acceptor unspecific] EC 2.4.1.17). Because of its pharmacological importance and its marked variation with age and species, this enzyme has been much studied.[1] Many recent reports indicate that considerable UDP-glucuronyltransferase activity can remain latent in liver homogenates or microsomal preparations,[2–7] especially in the rat, where only some 10 per cent of the optimal activity towards *o*-aminophenol is observed in "native" unactivated homogenate, even with relatively high concentrations of UDP-glucuronate present.[8] In the Gunn rat strain, genetically defective in this enzyme, latency can be even more marked.[9,10] Activation varies with sex, age and phenobarbital-pretreatment.[5,8,11] The more specific activator diethylnitrosamine[9] activates further the enzyme already activated by detergents or UDP-*N*-acetylglucosamine.[4]

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These phenomena suggest regulation of UDP-glucuronyltransferase activity *in vivo*. They cause difficulty in the pharmacological interpretation of UDP-glucuronyltransferase assays, especially as the degree of latency present in the intact cell is unknown. Moreover, comparisons between glucuronidation rates in broken- and intact-cell preparations have been few and conflicting.[12–14] Partly these discrepancies are due to comparison of slice-activities with "native"[12,13] or activated[14] (sonicated) enzyme alone.

The present investigation, which compares for the first time glucuronidation rates in intact cells (slices) and in homogenates fortified with the different activators, therefore seemed desirable. Wistar and Gunn rats of both sexes, normal and phenobarbital-treated, were employed, for the latency of UDP-glucuronyltransferase varies greatly between these animals.

MATERIALS AND METHODS

Chemicals were obtained as described earlier.[4] Wistar and Gunn rats were 200–300 g (females) and 300–400 g (males) body wt; one group received sodium phenobarbital solution (1 g/l) to drink for 16 days before sacrifice.

UDP-glucuronyltransferase activity was measured in liver homogenates buffered with Tris–maleate at pH 7·4 as described by Winsnes,[4] except that 10 mM-EDTA was present during incubation to inhibit nucleotide pyrophosphatase (EC 3.6.1.9)[15,16]. UDP-glucuronate was present at 0·5, 1·0 and 2·0 mM, together with 0·5 mM-*o*-aminophenol, which gave approximate saturation of enzyme with aglycone (see Discussion); amount of tissue present corresponded to 15, 30 or 45 mg wet wt liver depending on whether fully-activated, partially-activated or non-activated enzyme was assayed.

Glucuronidation of *o*-aminophenol in liver slices was performed in sulphate-free Ringer substrate containing *o*-aminophenol as described by Storey and Dutton,[17] except that 0·5 mM substrate was employed to ensure maximal glucuronidation and readier comparison with the homogenate studies. Glucuronide formed in both homogenate and slice media was assayed by the same diazotisation technique.[4,17]

RESULTS

Liver weights. Confirming earlier studies,[18,19] liver weights as percentage of body weight increased in all phenobarbital-treated animals, the figures being 24 per cent for female and 48 per cent for male Wistar rats; 22 and 38 per cent were the respective figures for Gunn rats.

$K_{\text{UDP-glucuronate}}$ *determinations.* Results are in Table 1. For the enzyme from Wistar rats, both UDP-*N*-acetyl glucosamine and digitonin increased the apparent $K_{\text{UDP-glucuronate}}$-values considerably, agreeing with earlier work.[20] However, diethylnitrosamine, which activates the enzyme differently from UDP-*N*-acetylglucosamine or digitonin,[4] did not increase the apparent $K_{\text{UDP-glucuronate}}$ of "native" enzyme; according to Stevenson *et al.*[9] the apparent $K_{\text{o-aminophenol}}$ also remains unchanged on activation by diethylnitrosamine. $K_{\text{UDP-glucuronate}}$-values were similar in males and females only if the enzyme had been activated by both digitonin and diethylnitrosamine; after most other enzyme treatments they were higher in females.

TABLE 1. APPARENT KINETIC PARAMETERS OF *o*-AMINOPHENOL UDP-GLUCORONYLTRANSFERASE

Animal source	$K_{UDP\text{-}glucuronate}$ (mM)					V_{max} (μmoles /g wet wt/hr)				
	"Native"	UDPNAG	DEN	DEN + UDPNAG	DEN + DIG	"Native"	UDPNAG	DEN	DEN + UDPNAG	DEN + DIG
Control, female Wistar rat	0·60	2·1	0·61		2·4 ± 0·1	0·17	0·74	0·65		6·9 ± 0·3
Phenobarbital-treated female Wistar rat	0·41	0·7	0·47		2·6 ± 0·2	0·21	0·61	0·79		13·5 ± 0·7
Control, male Wistar rat	0·17	1·3	0·14		2·6 ± 0·1	0·31	1·05	0·69		9·3 ± 0·6
Phenobarbital-treated male Wistar rat	0·21	0·7	0·19		2·5 ± 0·2	0·31	1·09	0·93		17·4 ± 0·6
Control, female Gunn rat	N.C.		0·63	1·0 ± 0·05	2·8 ± 0·3	(0·02)		0·72	3·8 ± 0·11	9·0 ± 0·7
Phenobarbital-treated female Gunn rat	N.C.		0·24	0·9 ± 0·03	2·6 ± 0·3	(0·01)		0·72	5·4 ± 0·10	16·4 ± 0·9
Control, male Gunn rat	N.C.		0·13	0·7 ± 0·04	2·2 ± 0·3	(0·02)		0·72	4·1 ± 0·05	10·3 ± 0·7
Phenobarbital-treated male Gunn rat	N.C.		0·14	0·5 ± 0·01	2·1 ± 0·1	(0·04)		0·83	5·8 ± 0·15	16·4 ± 0·7

The animals were treated and the enzyme activities (at 0·5, 1·0 and 2·0 mM UDP-glucuronate) measured as mentioned in Materials and Methods. Apparent $K_{UDP\text{-}glucuronate}$- and V_{max}-values were calculated from Lineweaver–Burk plots. The values are mean ±S.E.M. of results from seven and five animals of Wistar and Gunn rat strain, respectively. Several of the values are given without S.E.M. because of too low accuracy in single experiments.[20] The "native" enzyme activity of Gunn rats given in parentheses are mean values at 0·5, 1·0 and 2·0 mM UDP-glucuronate. No $K_{UDP\text{-}glucuronate}$- or V_{max}-determinations were possible at this low level of activity. Abbreviations: (UDPNAG), UDP-*N*-acetylglucosamine; (DEN), diethylnitrosamine; (DIG), digitonin; (N.C.), not calculable; and ( ), not performed.

Results with Gunn rats were similar except that low activities did not permit measurement of $K_{\text{UDP-glucuronate}}$ for "native" enzyme.

Pretreatment with phenobarbital had little effect on apparent $K_{\text{UDP-glucuronate}}$-values. However, in female rats they could be lowered by such pretreatment (Table 1); this may be related to the increase in microsomal lecithin after phenobarbital treatment,[21] for lecithin can lower apparent $K_{\text{UDP-glucuronate}}$-values.[20]

V_{max} *determinations.* Results are in Table 1. When diethylnitrosamine was present during enzyme incubation there were no great differences between V_{max}-values from Wistar and Gunn rats. Simultaneous addition of diethylnitrosamine and digitonin to "native" enzyme from Wistar rats increased V_{max} 30- to 65-fold, and from Gunn rats some 400- to 1600-fold; the greatest increases were in the phenobarbital-treated groups.

In agreement with earlier studies[11] no significant increase in V_{max} of the "native" enzyme occurred after phenobarbital treatment of Wistar rats; neither was any experimentally significant increase observed in the almost unmeasureably low activities of the "native" Gunn rat enzyme. In addition, neither the UDP-*N*-acetylglucosamine nor the diethylnitrosamine-activated enzymes were much increased by the drug. Only when diethylnitrosamine was added together with digitonin (or UDP-*N*-acetylglucosamine) did V_{max} increase notably (1·6- to 2·0-fold) for the enzyme from phenobarbital-treated animals.

Glucuronidation in liver-slices. Liver-slice glucuronidation was twice as high in males as in females of both strains. Phenobarbital pretreatment increased these activities some 2-fold further. Throughout, Gunn rat liver-slices conjugated *o*-aminophenol at rates as high as 30–45 per cent of those found in Wistar rats (Table 2).

Comparison of slices and homogenates. For both strains, activity of UDP-glucuronyltransferase in liver-slices, judged by rate of glucuronidation, was much lower than the maximal activity of the activated forms of the eznyme (Table 1), but were higher than activities of "native" enzyme; this suggests that the degree of enzyme latency in the intact cell preparations could not be as high as in homogenates.

However, the intracellular levels of UDP-glucuronate are 0·20–0·30 mM.[22–24] Enzyme activities at this low UDP-glucuronate level were therefore compared (Table 2) with the results from liver-slices, EDTA being present in the homogenates to inhibit nucleotide pyrophosphatase.

As activation by UDP-*N*-acetylglucosamine or digitonin greatly increased apparent $K_{\text{UDP-glucuronate}}$-values, the marked differences seen above in V_{max}-values of the different enzyme forms became much less pronounced at the low UDP-glucuronate level. However, the maximally-activated enzyme always exhibited activities well above those suggested by the slices, this difference being especially prominent in the Gunn rats. In the Wistar rats, the enzyme, if activated only by UDP-*N*-acetylglucosamine or diethylnitrosamine, could not (at 0·25 mM UDP-glucuronate) adequately account for the liver-slice activities.

The sex differences in activity, not always notable at V_{max} (Table 1) became more obvious at the low UDP-glucuronate levels because of higher apparent $K_{\text{UDP-glucuronate}}$-values in female than male rats; this adequately accounts for the sex difference displayed in liver-slices.

TABLE 2. COMPARISON BETWEEN GLUCURONIDATION RATE OF *o*-AMINOPHENOL IN LIVER-SLICES AND HOMOGENATES

Animal source	Liver-slice activities (μmoles/g wet wt/hr)	Homogenate enzyme activities at 0·25 mM UDP-glucuronate (μmoles/g wet wt/hr)				
		"Native"	UDPNAG	DEN	DEN + UDPNAG	DEN + DIG
Control, female Wistar rat	0·29 ± 0·02	0·05	0·08	0·19		0·65
Phenobarbital treated female Wistar rat	0·58 ± 0·06	0·08	0·16	0·27		1·18
Control, male Wistar rat	0·55 ± 0·04	0·19	0·17	0·44		0·81
Phenobarbital-treated male Wistar rat	1·28 ± 0·11	0·17	0·29	0·53		1·59
Control, female Gunn rat	0·12 ± 0·01	(0·02)		0·20	0·76	0·74
Phenobarbital-treated female Gunn rat	0·21 ± 0·01	(0·01)		0·37	1·20	1·44
Control, male Gunn rat	0·24 ± 0·02	(0·02)		0·47	1·08	1·05
Phenobarbital-treated male Gunn rat	0·39 ± 0·01	(0·04)		0·54	1·84	1·74

Animal treatment, assay of enzyme and assay of glucuronidation were as mentioned in Materials and Methods. UDP-glucuronyltransferase activities in homogenates (0·25 mM UDP-glucuronate present) were calculated by extrapolation from V_{max}- and apparent $K_{UDP\text{-}glucuronate}$-values, except for those in parentheses (see legend for Table 1).

DISCUSSION

Since phenolic substrates at high concentration can activate UDP-glucuronyltransferase,[4,22] it is not possible in practice to saturate completely the enzyme with *o*-aminophenol. However, the concentration of *o*-aminophenol employed (0·5 mM) only slightly limited glucuronidation rate in homogenates, and to increase its concentration further would obscure comparison with glucuronidation rate in intact cells, which is maximal with 0·2–0·5 mM in the medium[23] (Winsnes and Rugstad, unpublished work).

The intracellular level of UDP-glucuronate[24–26] in the range 0·20–0·30 mM, is similar to the apparent $K_{\text{UDP-glucuronate}}$-values found above for "native" and diethylnitrosamine-activated enzyme, but much less than the apparent $K_{\text{UDP-glucuronate}}$ levels of maximally-activated UDP-glucuronyltransferase. For this reason the great differences in homogenate activities at V_{max} between the differently-treated enzyme preparations become comparatively much smaller at more physiological UDP-glucuronate levels. The "native" enzyme activities (even at V_{max}) cannot adequately account for the activity present in slices; thus the enzyme in the intact cell may be subject to some degree of activation, mediated for instance by UDP-*N*-acetylglucosamine and/or endogenous detergents. However, as enzyme activities (at 0·25 mM UDP-glucuronate) when maximally-activated are mostly far above the activity in slices, then a certain degree of latency may also exist in the intact cell.

These possibilities are strengthened by the comparison of Gunn and Wistar rats. The "native" enzyme is much lower in Gunn rat than the relative activities in slices from the two strains would suggest. Again, the liver-slices from Gunn rats are still only 30–44 per cent as active as those from Wistar rats although the V_{max}-values of the maximally-activated enzyme in homogenates are similar in the two strains.

It is also interesting that the V_{max}-values of maximally-activated enzyme do not exhibit the sex differences in glucuronidation rate found in slices, whereas the homogenate activities at physiological levels of UDP-glucuronate do show this difference. The somewhat higher apparent $K_{\text{UDP-glucuronate}}$-values of females, seen especially with "native" and diethylnitrosamine-activated enzyme, may thus reflect a true difference between the enzyme of males and females as it exists in the cell; the higher UDPG-dehydrogenase (EC 1.1.1.22) activity reported[27] in male rats is therefore not necessarily responsible for observed sexual differences in glucuronidation.

Higher UDPG dehydrogenase activity in liver follows barbiturate treatment according to some workers,[28,29] but not to others.[30] Increase in this activity could lead to increased tissue levels of UDP-glucuronate. Our results suggest that any such increase in UDP-glucuronate levels need not be wholly responsible for the higher rates of glucuronidation observed in liver slices from phenobarbital-treated rats. In liver homogenates from these rats, although the V_{max} of native or partially-activated UDP-glucuronyltransferase was increased by pretreatment only slightly or not at all, the corresponding apparent $K_{\text{UDP-glucuronate}}$-values were lowered (Table 1). Therefore the increased glucuronidation in liver slices from treated rats could be partly accounted for by the UDP-glucuronyltransferase activity displayed in corresponding homogenates fortified with only the normal tissue level of UDP-glucuronate (Table 2).

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