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VARIABLE EFFECT OF PHENOBARBITAL TREATMENT OF MICE ON HEPATIC UDP-GLUCURONYLTRANSFERASE ACTIVITY WHEN JUDGED BY SLIGHTLY DIFFERENT ENZYME-ASSAY TECHNIQUES*

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Abstract—Somewhat conflicting results have been observed in the response of UDP-glucuronyltransferase on barbiturate pretreatment. A reinvestigation has established that differences in the enzyme-assay techniques employed could be responsible for these discrepancies.

Peroral phenobarbital pretreatment of female and male mice for 16 days significantly ($P < 0.001$) increased activity of hepatic bilirubin glucuronyltransferase, while the glucuronyltransferase activity towards *o*-aminophenol, *p*-nitrophenol, phenolphthalein and 4-methylumbelliferone was not increased as judged by enzyme assay with fresh liver homogenate without addition of detergent (non-activated enzyme). However, when assays were performed with an optimal amount of detergent added to the incubation mixtures (activated enzyme), an increase in glucuronyltransferese activity after phenobarbital treatment was found towards all five acceptor substrates in both female and male animals, the sole exception was the *o*-aminophenol glucuronyltransferase activity which was not significantly ($P > 0.025$) increased in male animals. The results thus stress the importance of doing parallel studies of both non-activated and detergent-activated enzyme.

UDP-GLUCURONYLTRANSFERASE (UDP-glucuronate glucuronyltransferase, EC 2.4.1.17) is a microsomal enzyme catalysing the transfer of glucuronic acid to several endogenous and exogenous compounds.[1] Ample evidence supports the hypothesis that several glucuronyltransferases exist,[2] although recent data on partial purification favours the concept of there being only one enzyme.[3]

Several hepatic microsomal drug-metabolizing enzymes are induced by pretreatment of animals with phenobarbital.[4–6] However, somewhat conflicting results concern the effect of barbiturates on glucuronyltransferase. Phenobarbital pretreatment has been shown to increase the activity of glucuronyltransferase measured in rat-liver homogenate and microsomal preparations with bilirubin[7] and *p*-nitrophenol[7,8] as acceptors. Similarly the activity of hepatic bilirubin and *o*-aminophenol glucuronyltransferases was significantly though not very much increased in male mice treated with sodium-barbital.[9] However, in liver-homogenates from female mice pretreated with barbital no significant increase in bilirubin glucuronyltransferase activity was seen, neither was there any increased activity of glucuronyltransferase

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(male animals) with phenolphthalein as substrate.[9] In rabbits also no increase in glucuronyltransferase activity towards *p*-nitrophenol, *o*-aminophenol or phenolphthalein was observed after phenobarbital treatment.[10]

Such discrepancies in the effect of barbiturate pretreatment on glucuronyltransferase activity measured *in vitro* might be due to species differences, lack of response of some glucuronyltransferases (if several enzymes exist) or differences in the enzyme assay techniques used. Glucuronyltransferase activity is increased severalfold *in vitro* by supplementing the enzyme-assay mixtures with detergents (Triton X-100 and digitonin).[11–13] Activation of glucuronyltransferase has also been shown to occur *in vitro* after dialysis of liver-homogenates against EDTA-mercaptoethanol at pH 9.[14] With one exception[7] earlier studies on the effect of barbiturates on glucuronyltransferase activity have been performed on non-activated enzyme, while Halac and Sicignano[7] who obtained 2-fold increased activities of bilirubin and *p*-nitrophenol glucuronyltransferases after phenobarbital treatment of rats, assayed enzyme activated *in vitro* after dialysis of the homogenates at pH 9. No comparison with results obtained by assay of non-activated glucuronyltransferase was performed.

A recent report from our laboratory[15] shows that the activation characteristics of *o*-aminophenol glucuronyltransferase *in vitro* vary according to age and sex in rats. The possibility that phenobarbital pretreatment might change the activation characteristics of glucuronyltransferase *in vitro* seemed worth exploration since this would affect the interpretation of the phenobarbital pretreatment. Mice were selected in the present study since different results were obtained with different substrates in the study of Catz and Yaffe.[9]

MATERIALS AND METHODS

The animals used were NMRI/BOM mice of either sex of comparable age. The weights of the animals when sacrificed were (mean $\pm$ S.D.): 28·3 $\pm$ 2·96 g for female controls, 28·7 $\pm$ 3·65 g for treated females, 38·7 $\pm$ 4·52 g for male controls and 36·7 $\pm$ 2·06 g for treated males. Both female and male animals were arbitrarily divided into two groups. One group served as control and was given tap water, while the other group was given a solution of sodium–phenobarbital (1 g/l.) to drink during the 16 days preceding sacrifice. All animals were fed a regular laboratory diet *ad lib.*

The animals were killed by dislocation of the vertebrae. The liver was removed at once, weighed and cooled on ice. A 20% (w/v) homogenate in ice-cold 0·154 M KCl solution was prepared in a glass homogenizer with Teflon pestle. The homogenate was spun at 2000 *g* for 15 min and the supernatant was used for the assay of enzyme activity.

Glucuronyltransferase activity was measured with the following acceptors: bilirubin, *o*-aminophenol, *p*-nitrophenol, phenolphthalein and 4-methylumbelliferone each at a final 0·5 mM concentration. The concentration of UDP-glucuronate was 2·0 mM. The buffer was 75 mM tris–maleate (pH 7·4). Incubation mixtures were kept in stoppered glass tubes and incubated aerobically for 30 min at 37° in a shaking water bath. Incubations usually were in duplicate.

Enzyme activities were assayed both in fresh homogenates without addition of detergent, and with optimal concentration of detergent added in the incubation mixture: 0·075% (w/v) Triton X-100 with bilirubin, 0·050% (w/v) Triton X-100 with

TABLE 1. THE EFFECT OF PHENOBARBITAL TREATMENT OF MICE ON HEPATIC UDP-GLUCURONYLTRANSFERASE ACTIVITY AS JUDGED BY ASSAY OF ENZYME ACTIVITY IN LIVER HOMOGENATES BOTH WITH AND WITHOUT ADDITION OF DETERGENT

		Female mice			Male mice		
Acceptor	Detergent	Controls (9)	Treated (12)		Controls (14)	Treated (12)	
Bilirubin	−	0·63 ± 0·15	1·33 ± 0·25	P<0·001	0·34 ± 0·05	0·66 ± 0·11	P<0·001
	+	2·65 ± 0·62	6·92 ± 0·92	P<0·001	1·25 ± 0·39	3·22 ± 0·76	P<0·001
o-Amino-	−	1·48 ± 0·24	1·63 ± 0·25	P>0·025	1·87 ± 0·25	1·55 ± 0·32	P<0·001
phenol	+	4·39 ± 1·03	6·93 ± 1·46	P<0·001	6·23 ± 0·89	7·10 ± 1·48	P>0·025
p-Nitro-	−	3·16 ± 0·55	3·57 ± 0·61	P>0·050	4·02 ± 0·98	3·88 ± 0·89	P>0·35
phenol	+	19·8 ± 3·65	32·3 ± 3·19	P<0·001	23·0 ± 4·17	34·2 ± 4·80	P<0·001
Phenol-	−	2·49 ± 0·42	3·50 ± 1·36	P=0·350	2·03 ± 0·51	2·92 ± 0·69	P>0·20
phthalein	+	21·7 ± 3·30	52·7 ± 8·43	P<0·001	18·2 ± 3·35	35·6 ± 7·76	P<0·001
4-Methylum-	−	7·98 ± 2·45	11·3 ± 3·50	P=0·0125	7·61 ± 3·15	10·3 ± 1·87	P>0·050
belliferone	+	117·5 ± 30·3	188·0 ± 22·2	P<0·001	109·0 ± 23·7	172·0 ± 16·3	P<0·001

The animals were treated and the enzyme activities measured as described in Materials and Methods. UDP-glucuronyltransferase activities (mean ± S.D.) are given as μmoles/g wet wt./hr. The number of animals in each group are given in parenthesis. The P value (Student's *t*-test) refers to the statistical significance of the differences between treated and control groups.

p-nitrophenol, phenolphthalein and 4-methylumbelliferone, and 0·20% (w/v) digitonin with *o*-aminophenol as substrate.

For more detailed description of methods see Ref. 13.

RESULTS

As described earlier[16,17] the liver weights and liver/body weight ratios were significantly ($P < 0{\cdot}001$) greater in the phenobarbital treated animals than in the controls. The liver weights in per cent of the body weights were (mean ± S.D.): 4·95 ± 0·38 for female controls 8·65 ± 0·48 for treated females, 5·76 ± 0·41 for male controls and 8·75 ± 0·84 for treated male animals.

With bilirubin as acceptor a highly significant increase of glucuronyltransferase activity was found in the treated group for male and female animals, and both without and with Triton X-100 added during the assay (Table 1). The increase seems, however, somewhat greater when activated enzyme was assayed. As described earlier[9] the activity of "native" bilirubin glucuronyltransferase from mouse-liver was considerably lower in males than in females. In contrast to the earlier report[9] this difference did not disappear upon phenobarbital treatment, and was even slightly more pronounced in the detergent-activated preparations.

With *o*-aminophenol, *p*-nitrophenol, phenolphthalein and 4-methylumbelliferone as substrates a slight (but not significant) increase in glucuronyltransferase activity was observed in most cases after phenobarbital treatment when the enzyme was assayed without detergent. The activity of *o*-aminophenol glucuronyltransferase from male mice was, however, significantly (though slightly) reduced after phenobarbital treatment in contrast to earlier observation.[9]

In the phenobarbital pretreated group the glucuronyltransferase activity towards the four "foreign" substrates was, however, significantly greater than that of the controls when enzyme was assayed in the presence of detergents. The sole exception was exhibited by *o*-aminophenol glucuronyltransferase from male animals where a slight, non-significant increase in enzyme activity wes revealed. The effect of phenobarbital pretreatment was greatest with bilirubin (2·6 times increase) and phenolphthalein (2·0–2·4 times increase) as acceptors. With the other acceptors the increase resulting from phenobarbital pretreatment was 1·5 to 1·6-fold in detergent-activated preparations. Expressed in another way, the activating effect of detergent on glucuronyltransferase in homogenates from phenobarbital pretreated animals was 1·13–1·74 (mean 1·37) times greater than that found with homogenates from control animals.

DISCUSSION

The present results may explain some of the discrepancies between earlier reports on the effect of barbiturate treatment on glucuronyltransferase activity, since in some cases "native" enzyme only was tested,[9,10] whereas others assayed enzyme which had been activated *in vitro*.[7] However, the reason why a greater percentage of enzyme activity remains latent in homogenates from animals pretreated with phenobarbital compared with controls is unknown.

Whether some enzyme activity remains latent in liver-slices, and whether detergents will result in increased glucuronidation if added to such preparations is unknown, but ought to be studied. The reports of increased glucuronidation of bilirubin[17] and *o*-

aminophenol[18] as measured in liver-slices from phenobarbital pretreated rats compared with controls cannot without reservation be compared with the glucuronidation obtained with homogenates supplemented with UDP-glucuronate. Hollmann and Touster[19] have shown that barbital pretreatment of rats resulted in a 2-fold increase of UDPG-dehydrogenase activity which besides the activity of UDP-glucuronyltransferase would affect the amount of glucuronide synthesized by liver slices.

A recent report[20] on the effect of phenobarbital pretreatment on *p*-nitrophenol glucuronyltransferase (assayed both with and without detergent) from male Wistar rats is in agreement with the present findings. The author[20] discussed the possibility that repeated rehomogenization of the microsomal pellet may result in release of some of the latent glucuronyltransferase activity. This again may explain why phenobarbital pretreatment of rats had a distinct stimulating effect on the *p*-nitrophenol glucuronyltransferase as assayed in microsomes.[8]

It has been shown earlier[15] that quite different conclusions on the relative glucuronyltransferase activity in different situations may be drawn when non-activated and detergent-activated enzyme are assayed. The present report again stresses the importance of doing parallel studies of both non-activated and activated enzyme until a better understanding of the activation *in vitro* is reached.

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