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Demonstration of Functional Heterogeneity of Hepatic Uridine Diphosphate Glucuronosyltransferase Activities after Administration of 3-Methylcholanthrene and Phenobarbital to Rats

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After the administration of 3-methylcholanthrene to adult male rats, activities of hepatic UDP-glucuronosyltransferase towards six from a group of 12 substrates were stimulated by 250–350%. Activities towards the remaining six substrates were unaffected. Conversely, after phenobarbital administration, activities formerly stimulated by 3-methylcholanthrene remained unchanged, and the other six activities were stimulated by 160–280%. The relationship of these two groups of transferase activities to other evidence suggesting the same heterogeneity of the enzyme is discussed.

We have previously shown that during rat perinatal development, hepatic activities of the microsomal conjugating enzyme UDP-glucuronosyltransferase (EC 2.4.1.17) for at least 12 substrates may be divided into two groups: those achieving adult values by the 20th foetal day (the 'late-foetal' group) and those, remaining negligible at this time, which rise to adult, or near-adult, values during the first 2 postnatal days (the 'neonatal' group) (Wishart, 1978).

Furthermore activities in the late-foetal group may be induced by glucocorticoids in foetal liver *in utero* and in culture, under which conditions neonatal-group activities remain negligible (Wishart, 1978). Bock *et al.* (1973) found that 3-methylcholanthrene when administered to rats showed a tendency to stimulate preferentially activities towards 1-naphthol and 4-nitrophenol rather than towards bilirubin and chloramphenicol, whereas phenobarbital had a tendency to stimulate preferentially activity towards the latter two substrates.

We have previously noted that the former two substrates are in our late-foetal group of transferase activities, whereas the latter two are in our neonatal group (Wishart *et al.*, 1977). We have further pursued the possible correlation between groups of transferase activities found during development and after xenobiotic treatment in rats by examining the effect of phenobarbital and 3-methylcholanthrene on rat liver transferase activities towards the 12 substrates used in our developmental study (Wishart, 1978). We find an exact correlation between the two groups.

Methods

Male Wistar rats of 350–400g weight were injected intraperitoneally with either 3-methylcholanthrene (15mg in 0.4ml of arachis oil on day 1) or sodium phenobarbitone (30mg in 0.3ml of 0.9% NaCl on days 1, 2 and 3). Control animals were injected with carrier only. UDP-glucuronosyltransferase assays were performed on day 5. Assay of UDP-glucuronosyltransferase and protein, and sources of materials were as before (Wishart, 1978), except that for assay of chloramphenicol glucuronidation, 0.5mM-substrate was used, with ethanol to a final concentration of 5% (v/v) in the incubation medium.

Results

On injection under the conditions chosen, 3-methylcholanthrene stimulated by 250–350% transferase activities towards all six substrates in the late-foetal group of Wishart (1978), but had no significant effect on the neonatal activities, which remained at 90–120% of control values (see Table 1). Conversely, injection of phenobarbital stimulated activities in the neonatal group by 160–280%, and late-foetal activities remained at 90–110% of the controls.

Control values for both regimens of injections were similar. The correlation of the late-foetal group with the 3-methylcholanthrene-stimulated group of activities and of the neonatal group with the phenobarbital-stimulated group of activities is thus complete.

Discussion

This work confirms and extends the differential stimulation of transferase activities observed by Bock *et al.* (1973) to a greater number of substrates, and correlates the two groups of transferase activities thus distinguished with two groups separated by their

Table 1. *Effect of phenobarbital and 3-methylcholanthrene on adult male rat liver UDP-glucuronosyltransferase activities towards 12 substrates*

Phenobarbital, 3-methylcholanthrene and control injections, transferase and protein assays were performed as described in the Methods section. Activities are presented as means ± S.E.M. of samples from the numbers of rats shown in parentheses.

	3-Methylcholanthrene-treated rats			Phenobarbital-treated rats		
	UDP-glucuronosyltransferase activity (nmol of glucuronide formed/h per mg of protein)			UDP-glucuronosyltransferase activity (nmol of glucuronide formed/h per mg of protein)		
Substrate	Control	Test	Percentage stimulation	Control	Test	Percentage stimulation
Late-foetal group						
2-Aminophenol	29.9 ± 0.9 (3)	91.3 ± 13.6 (3)	310	32.8 ± 5.2 (5)	29.3 ± 3.1 (6)	90
2-Aminobenzoate	19.4 ± 0.8 (3)	65.0 ± 1.1 (3)	330	19.1 ± 4.6 (5)	18.6 ± 1.7 (6)	100
4-Nitrophenol	318 ± 32 (5)	795 ± 68 (5)	250	311 ± 24 (5)	288 ± 11 (6)	90
1-Naphthol	131 ± 28 (3)	420 ± 35 (3)	320	136 ± 26 (5)	160 ± 35 (6)	120
4-Methylumbelliferone	400 ± 45 (3)	882 ± 75 (3)	220	359 ± 49 (3)	368 ± 25 (3)	100
5-Hydroxytryptamine	209 ± 22 (3)	597 ± 115 (3)	290	180 ± 15 (4)	184 ± 28 (3)	100
Neonatal group						
Bilirubin	7.9 ± 0.9 (3)	8.5 ± 0.2 (3)	110	12.0 ± 0.5 (5)	19.0 ± 1.7 (6)	160
Testosterone	37.0 ± 1.4 (5)	34.0 ± 1.2 (5)	90	33.4 ± 1.6 (6)	54.8 ± 2.2 (6)	160
Oestradiol	6.5 ± 1.1 (3)	6.2 ± 1.1 (3)	100	7.3 ± 1.0 (5)	12.7 ± 1.8 (6)	170
Morphine	241 ± 20 (3)	251 ± 25 (3)	100	212 ± 8 (5)	603 ± 32 (6)	280
Phenolphthalein	140 ± 25 (3)	141 ± 40 (3)	100	130 ± 9 (5)	212 ± 15 (6)	160
Chloramphenicol	48.1 ± 6.3 (3)	42.6 ± 5.0 (3)	90	39.4 ± 5.8 (3)	88.1 ± 5.0 (3)	220

differential development and glucocorticoid inducibility in rats (Wishart, 1978).

Stimulation by phenobarbital and 3-methylcholanthrene is not an absolute characteristic of the described groups of transferase activities. With higher doses, both groups of activities may be stimulated by both xenobiotics (Bock *et al.*, 1973) and we also have found that with increased dosage or period of treatment, phenobarbital will eventually stimulate the late-foetal activities. However, conditions were chosen in this work to allow the group of transferase activities less sensitive to either xenobiotic to remain unaltered while the more sensitive group was markedly stimulated.

Functional heterogeneity of two groups within rat hepatic transferase activities towards 12 substrates has now been demonstrated by evidence from several different systems: time of onset of development and glucocorticoid inducibility (Wishart, 1978), and, in the present work, sensitivity to stimulation by phenobarbital and sensitivity to stimulation by 3-methylcholanthrene. Further, Lucier & McDaniel (1977) have found, in rats, a 'non-steroidal' group of three transferase activities which are high at birth and stimulated, in the adult, by 2,3,7,8-tetrachlorodibenzo-*p*-dioxin, in contrast with a 'steroidal' group of five activities which are low at birth and not stimulated.

Whether these now well-documented groups reflect two or more enzyme proteins, or indeed one enzyme protein subject to differential regulation is not yet clear. They do, however, imply that there are two functionally discrete hepatic systems for glucuronidation of lipophilic compounds, and it may well be relevant that at least three substrates from the late-foetal group of transferase activities are excreted largely (>90%) in the urine, whereas all six substrates of the neonatal group are excreted predominantly in the bile (see Smith, 1973).

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