Biochemical Pharmacology, Vol. 27, pp. 369–371. Pergamon Press, 1978. Printed in Great Britain.

UDP-glucuronyltransferase in perfused rat liver and in microsomes: V. Studies with Gunn rats

(*Received* 11 *May* 1977; *accepted* 11 *July* 1977)

Conjugation with glucuronic acid by microsomal UDP-glucuronyltransferase (EC 2.4.1.17) is a prominent pathway by which the body inactivates and eliminates a wide variety of xenobiotics and endogenous compounds [1]. Many problems of this important enzyme reaction remain unresolved, (a) the multiplicity of this enzyme, (b) the enzyme induction* by xenobiotics and endogenous inducers, and (c) mechanisms and significance of the marked constraint or latency* of the enzyme activity in the microsomal membrane [2]. Recently we were able to separate and partially purify UDP-glucuronyltransferases conjugating 1-naphthol and morphine which are chiefly induced by 3-methylcholanthrene and phenobarbital, respectively [3, 4]. These two inducing agents have been characterized as two different types of inducing agents of microsomal monooxygenases [5–8]. We particularly focussed on the glucuronidation of the lipid-soluble 1-naphthol since sensitive and convenient assays have been developed to study this conjugation reaction *in vitro* [9, 10], in perfused liver and intestine [9, 11] and hepatocytes [10]. A series of comparative studies in perfused liver and in liver homogenates or microsomes indicated that the enzyme was operating largely in a constrained form within the cell [9, 12–14]. In this form the enzyme can be allosterically regulated by UDP-*N*-acetylglucosamine (UDPG1cNAc) [15, 16].

In the present report we extend our studies to the Gunn strain of rat. This mutant strain of Wistar rats is characterized by a severe genetically determined defect of bilirubin glucuronidation [17, 18]. In liver microsomes from homozygous Gunn rats bilirubin glucuronyltransferase cannot be detected although traces of bilirubin glucuronides were recently identified in the bile of these rats [19]. The defect in the Gunn rat is very similar to that found in Crigler–Najjar patients [18]. On the other hand the glucuronidation of a variety of other substrates of UDP-glucuronyltransferase appears to be unaffected in the mutant strain adding to the growing evidences in favour of a multiplicity of UDP-glucuronyltransferases [18]. The glucuronidation of a third group of substrates (e.g. *o*-aminophenol and *o*-aminobenzoic acid) is partially defective in the mutant strain [18]. The low *in vitro* glucuronidation of these substrates could be activated to the normal level by the addition of both detergents and diethylnitrosamine (DENA) [20–23] suggesting abnormal enzyme-phospholipid interactions, i.e., an abnormal constraint of the firmly membrane-bound enzyme. A lowered affinity of the Gunn rat enzyme for UDP-glucuronic acid was recently suggested from kinetic studies [24]. Preliminary experiments indicated an abnormal constraint of UDP-glucuronyltransferase (1-naphthol as substrate) in Gunn rat microsomes. Therefore we studied 1-naphthol glucuronidation in perfused livers and microsomes from Gunn rats in order to explore the consequences of the altered enzyme constraint for glucuronide synthesis by the intact tissue.

MATERIALS AND METHODS

Homozygous Gunn rats were kindly provided by Dr. K. P. M. Heirwegh, Laboratory of Hepatology, Department of Medical Research, University of Leuven, Belgium. Details about this colony of Gunn rats are given in Ref. 19. Brij 58 was obtained from Atlas Chemie, Essen, Germany.

Male Gunn and Wistar rats (200–250 g) were fed *ad lib.* a standard diet containing 20 per cent protein (Altromin, Lage-Lippe, Germany). The liver:body weight ratio (about 4 per cent) was not significantly different in both rat strains. In phenobarbital-treatment, rats received 100 mg/kg/day i.p. for 3 days. 3-Methylcholanthrene was given once i.p. at a dosage of 40 mg/kg, dissolved in olive oil. Treated animals were sacrificed at the 4th day. Liver microsomes were prepared as described [9]. Microsomal protein was determined by the method of Lowry *et al.* [25] using bovine serum albumin as standard. Previously described methods were used for the assay of UDP-glucuronyltransferase with 1-naphthol [10], 4-nitrophenol, chloramphenicol, [13] and morphine [26] as substrates. Instead of Triton X-100, Brij 58 (0.05%, w/v) was used to activate UDP-glucuronyltransferase. Brij 58 activates the enzyme similar to other detergents but it is less inhibitory at higher concentrations. Hence the fully activated form of the enzyme can be better approximated.

Livers of Gunn and Wistar rats were perfused with 70 ml Eagle's minimal essential medium containing 25% (v/v) washed bovine erythrocytes, 2% (w/v) bovine serum albumin and 0.1% (w/v) heparin. [^{14}C]-1-naphthol (35 μmoles) was added to the perfusion medium, 1-naphthol glucuronide and sulfate in the perfusion medium and in liver tissue and the level of UDP-glucuronic acid in liver tissue were determined by previously described methods [9].

RESULTS AND DISCUSSION

In perfused livers from Gunn and Wistar rats the formation of 1-naphthol glucuronide and sulfate was found to be similar (Fig. 1). In addition, similar levels of 1-naphthol glucuronide (0.2 μmole/g tissue) and of UDP-glucuronic acid (0.3–0.4 μmole/g tissue) were found in liver tissue of both strains at the end of the perfusion experiments. However UDP-glucuronyltransferase (1-naphthol as substrate) was markedly decreased in Gunn rat microsomes with and without activation (Table 1). In contrast to other substrates such as *o*-aminophenol [20] the glucuronidation of 1-naphthol was only slightly activated by DENA in Gunn rats and not at all in Wistar rats. Similar results to those described in Table 1 were obtained when a more physiological concentration of UDP-glucuronic acid (0.3 mM) was used in the enzyme assays, when Triton X-100 was added instead of Brij 58, or when 4-nitrophenol glucuroni-

* Induction is used operationally to describe the long term enhancement of both the constrained and activated enzyme which can be prevented by inhibitors of protein synthesis. Release of enzyme constraint or latency, i.e. activation, denotes the short term increase of catalytic activity of UDP-glucuronyltransferase caused by diverse methods altering the microsomal membrane structure.

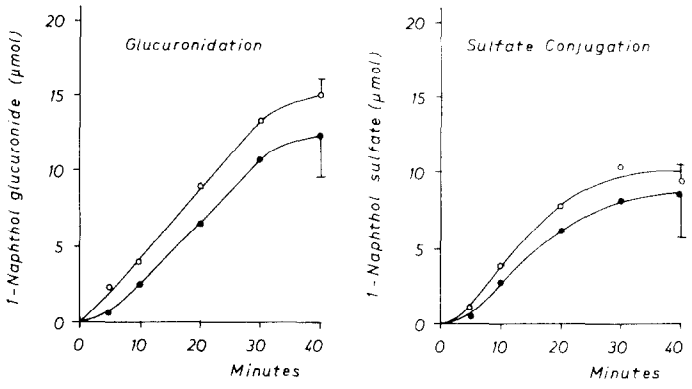

Fig. 1. Appearance of 1-naphthol conjugates in perfused livers of Gunn (○) and Wistar (●) rats. The mean of 4 liver perfusions ± S.D. is shown.

dation was determined (data not shown). Furthermore, after solubilization of the Gunn rat enzyme by cholate and partial purification with ammonium sulfate [3] the enzyme (1-naphthol as substrate) could still be activated by DENA but the specific activity was only about 20 per cent compared with Wistar rats. The solubilized Gunn rat enzyme appeared to be less stable upon storage than the corresponding enzyme preparations from Wistar rats.

The *in vitro* studies can be explained by assuming either a defective UDP-glucuronyltransferase (1-naphthol as substrate) and/or abnormal phospholipid-enzyme interactions. It is conceivable that the basal enzyme content is decreased in our colony of Gunn rats. In Gunn rat homogenates Brij 58 plus DENA-activated UDP-glucuronyltransferase (1-naphthol as substrate) was decreased to about one third, as in microsomes. However in native and UDPGlcNAc-activated homogenates no significant difference between the two strains was observed (Table 1). The lower enzyme level and/or the abnormal phospholipid-enzyme interactions did not lead to impaired glucuronide synthesis in the perfused Gunn rat liver possibly due to a compensatory activation of the latent enzyme. Intracellular activation of UDP-glucuronyltransferase in Gunn rat liver tissue was also suggested with *o*-aminophenol as substrate [21]. One possible activator could be bilirubin which is present at high levels in the blood (about 10 mg% [19]) and tissues of the Gunn rat. Bilirubin is known to activate liver microsomal UDP-glucuronyltransferase [27]. This was confirmed by us with 1-naphthol as substrate both with Wistar and Gunn rat liver microsomes. However in perfused livers from Wistar rats, the formation of 1-naphthol glucuronide was unaltered by the addition of 7 μmoles bilirubin to the perfusion medium and decreased to about 70 per cent by the addition of 43 μmoles bilirubin. Thus in perfused livers of normal Wistar rats no acute activating effect of bilirubin on 1-naphthol glucuronidation could be detected.

In order to further characterize the defects of UDP-glucuronyltransferase (1-naphthol as substrate) in Gunn rats we studied the induction of this enzyme by phenobarbital and 3-methylcholanthrene. Induction was studied in the activated form of the microsomal enzyme, i.e. in the presence of Brij 58 plus DENA, because it is in this form that an increased enzyme content can be distinguished from enzyme activation. In contrast to the marked enzyme induction by 3-methylcholanthrene in Wistar rats (the specific enzyme activity in microsomes was increased about 5-fold) Gunn rats were nonresponsive to this inducer, in agreement with the results of Vainio and Hietanen [28]. However the enzyme was inducible by phenobarbital in the mutant strain to the same extent as in Wistar rats (about 2-fold).

Microsomal UDP-glucuronyltransferase (morphine and chloramphenicol as substrates) was not significantly different in Gunn and Wistar rats; both in the basal enzyme level (7 ± 2 and 1.0 ± 0.2 nmoles/min/mg protein, respectively) and in the inducibility by phenobarbital (about 4-fold and 2-fold, respectively). The glucuronidation of

Table 1. Effects of membrane perturbants and UDPGlcNAc on liver microsomal UDP-glucuronyltransferase (1-naphthol as substrate) of Gunn and Wistar rats

Enzyme source	Additions to assay *in vitro*	UDP-glucuronyltransferase Gunn rats	Wistar rats
		(nmol/min/mg protein)	
Microsomes	native	0.7 ± 0.1	1.5 ± 0.4
	+ UDPGlcNAc	2.4 ± 0.8	5.0 ± 0.7
	+ DENA	1.1 ± 0.2	1.8 ± 0.4
	+ Brij 58	10.0 ± 1.2	50.8 ± 11.7
	+ Brij 58 + DENA	14.6 ± 2.5	50.1 ± 9.7
		(nmol/min/g tissue)	
Homogenates	native	39 ± 11	42 ± 19
	+ UDPGlcNAc	78 ± 16	80 ± 18
	+ Brij 58 + DENA	277 ± 57	999 ± 194

In the experiments with microsomes UDP-glucuronic aicd and UDPGlcNAc were present close to enzyme saturation (3mM) whereas in homogenates a more physiological concentration of (0.3mM) was used. Membrane perturbants were added at concentrations leading to optimal enzyme activation in both rat strains:
Brij 58 (0.05%, w/v), DENA (10mM).
The mean ± S.D. of 4 experiments is shown.

morphine and chloramphenicol was studied in the presence of Brij 58 but without DENA since these enzyme reactions were inhibited by DENA in both Gunn and Wistar rat microsomes. These comparative studies of various substrates are in line with recent evidences indicating that morphine and chloramphenicol are glucuronidated by an enzyme differing from UDP-glucuronyltransferase (1-naphthol as substrate) [3, 4, 26].

The results of the comparative studies in perfused liver and microsomes indicate that the markedly decreased UDP-glucuronyltransferase (1-naphthol as substrate) in the Gunn strain of rats does not lead to impaired glucuronide formation in perfused livers probably due to compensatory activation of the latent enzyme.

Acknowledgements—The authors are grateful to Dr. K. P. M. Heirwegh for generously providing us with Gunn rats, to Mrs. B. Frank for expert technical assistance and to the Deutsche Forschungsgemeinschaft for financial support.

Section on Biochemical Pharmacology, Institute of Pharmacology and Toxicology, University of Göttingen, D-3400 Göttingen
KARL WALTER BOCK*
ULRICH C. V. CLAUSBRUCH
and
Institute of Toxicology, University of Tübingen, D-7400 Tübingen, Germany
HUBERT OTTENWÄLDER

* To whom correspondence and reprint requests should be addressed.

REFERENCES

1. G. J. Dutton, in *Glucuronic Acid, Free and Combined* (Ed. G. J. Dutton), p. 185. Academic Press, New York (1966).
2. G. J. Dutton, *Biochem. Pharmac.* **24,** 1835 (1975).
3. K. W. Bock, W. Fröhling, H. Remmer and B. Rexer, *Biochim. biophys. Acta* **347,** 46 (1973).
4. K. W. Bock, U. C. von Clausbruch, D. Josting and H. Ottenwälder, *Biochem. Pharmac.* **26,** 1097 (1977).
5. A. H. Conney, *Pharmac. Rev.* **19,** 317 (1967).
6. A. Y. H. Lu and W. Levin, *Biochem. biophys. Res. Commun.* **46,** 1334 (1972).
7. A. P. Alvares and P. Siekevitz, *Biochem. biophys. Res. Commun.* **54,** 923 (1973).
8. D. A. Haugen, M. J. Coon and D. W. Nebert, *J. biol. Chem.* **251,** 1817 (1976).
9. K. W. Bock and I. N. H. White, *Eur. J. Biochem.* **46,** 451 (1974).
10. K. W. Bock, G. van Ackeren, F. Lorch and F. W. Birke, *Biochem. Pharmac.* **25,** 2351 (1976).
11. K. W. Bock and D. Winne, *Biochem. Pharmac.* **24,** 859 (1975).
12. M. M. Abou-El-Makarem and K. W. Bock, *Eur. J. Biochem.* **62,** 411 (1976).
13. G. Otani, M. M. Abou-El-Makarem and K. W. Bock, *Biochem. Pharmac.* **25,** 1293 (1976).
14. K. W. Bock, E. Huber and W. Schlote, *Naunyn-Schmiedeberg's Arch. Pharmak.* **296,** 199 (1977).
15. A. Winsnes, *Biochim. biophys. Acta* **289,** 88 (1972).
16. D. Zakim and D. A. Vessey, in *The Enzymes of Biological Membranes* (Ed. A. Martonosi), Vol. 2, p. 443. Plenum Publ. Corp., New York (1976).
17. C. K. Gunn, *J. Hered.* **29,** 137 (1938).
18. R. Schmid, in *The Metabolic Basis of Inherited Diseases* (Eds J. B. Stanbury, J. B. Wyngaarden and D. S. Fredrickson), p. 1141. McGraw-Hill, New York (1972).
19. N. Blanckaert, J. Fevery, K. P. M. Heirwegh and F. Compernolle, *Biochem. J.* **164,** 229 (1977).
20. I. Stevenson, D. Greenwood and J. McEwen, *Biochem. biophys. Res. Commun.* **32,** 866 (1968).
21. A. Winsnes and G. J. Dutton, *Biochem. Pharmac.* **22,** 1765 (1973).
22. D. Zakim, J. Goldenberg and D. A. Vessey, *Biochim. biophys. Acta* **297,** 497 (1973).
23. D. Nakata, D. Zakim and D. A. Vessey, *Biochem. Pharmac.* **24,** 1823 (1975).
24. D. Nakata, D. Zakim and D. A. Vessey, *Proc. Natn. Acad. Sci. U.S.A.* **73,** 289 (1976).
25. O. H. Lowry, N. J. Rosebrough, A. L. Farr and R. J. Randall, *J. biol. Chem.* **193,** 265 (1951).
26. E. Del Villar, E. Sanchez, A. P. Autor and T. R. Tephly, *Molec. Pharmac.* **11,** 236 (1975).
27. E. Sanchez and T. R. Tephly, *Life Sci.* **13,** 1483 (1973).
28. H. Vainio and E. Hietanen, *Biochim. biophys. Acta* **362,** 92 (1974).