Stimulation of Defective Gunn-Rat Liver Uridine Diphosphate Glucuronyltransferase Activity *in vitro* by Alkyl Ketones

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Addition of alkyl ketone (10mM) to Gunn-rat liver homogenates increased UDP-glucuronyltransferase activity towards 2-aminophenol by 10–20-fold, up to enhanced values of enzyme activity observed with similarly treated Wistar-rat liver homogenates. Alkyl ketones also activate the defective enzyme purified from Gunn-rat liver. This genetic deficiency of UDP-glucuronyltransferase activity is no longer apparent when assayed in the presence of alkyl ketones.

In 1938, Gunn described a mutant strain of Wistar rat that has hereditary hyperbilirubinaemia. More recent work has shown this mutant strain to be a good animal model for the study of the human Crigler–Najjar syndrome (Cornelius & Arias, 1972). The genetic lesion in the Gunn-rat liver results in the complete absence of bilirubin UDP-glucuronyltransferase activity and very poor ability to glucuronidate 2-aminophenol [see Dutton & Burchell (1977) for references]. Stevenson *et al.* (1968) showed that the carcinogen diethylnitrosamine, added to Gunn-rat liver preparations *in vitro*, stimulated the deficient UDP-glucuronyltransferase activity towards 2-aminophenol up to the enhanced activities observed in diethylnitrosamine-treated Wistar-rat liver homogenates. The deficiency of UDP-glucuronyltransferase activity towards 2-aminophenol was no longer apparent. This effect has since been widely investigated (see Dutton & Burchell, 1977).

Diethylnitrosamine has been recently used to activate the defective UDP-glucuronyltransferase purified to apparent homogeneity from Gunn-rat liver (Weatherill & Burchell, 1978). Thus diethylnitrosamine may stimulate UDP-glucuronyltransferase activity by direct interaction with the enzyme protein.

In the present paper we have initiated a search for less hazardous compounds that can stimulate the deficient UDP-glucuronyltransferase activity towards 2-aminophenol in the same manner as diethylnitrosamine.

Materials and Methods

Pentan-2-one, pentan-3-one, butan-2-one, acetone and 2-aminophenol were all obtained from BDH Chemicals, Poole, Dorset, U.K. Diethylnitrosamine

was purchased from Eastman Kodak Co., Rochester, NY, U.S.A. UDP-glucuronic acid was from Sigma (London) Chemical Co., Kingston upon Thames, Surrey, U.K. Lubrol 12A9 was purchased from I.C.I. Organics Division, Blackley, Manchester, U.K.

Gunn and Wistar rats were from the colonies maintained in the Institute animal unit.

UDP-glucuronyltransferase with 2-aminophenol as substrate was assayed by the method of Winsnes (1969) and with bilirubin as substrate by the method of Heirwegh *et al.* (1972). Protein concentrations were determined by the methods of Lowry *et al.* (1951) and Bradford (1976).

UDP-glucuronyltransferase was purified from Gunn-rat liver by a simple modification of the procedure described by Weatherill & Burchell (1978), enabling more rapid production of purified enzyme. The procedure used merely involves changing the order of the chromatographic steps and elimination of the CM-cellulose column. Thus all of the chromatography columns are equilibrated and used with 25mM-potassium phosphate/0.05% Lubrol/5mM-2-mercaptoethanol buffer, pH7.4. A concentrated DEAE-cellulose eluate was obtained by the method previously described (Weatherill & Burchell, 1978). This material was directly applied to a UDP-hexanolamine–Sephadex-4B column and eluted with UDP-glucuronic acid (see Burchell, 1978). Finally, this concentrated eluate was applied to a DEAE-Sephadex column. UDP-glucuronyltransferase passes straight through this column and the impurities are adsorbed.

Results

All Gunn-rat liver homogenates were assayed for UDP-glucuronyltransferase activity towards bili-

Table 1. *Effect of alkyl ketones on the UDP-glucuronyltransferase activity of male Gunn- and Wistar-rat liver homogenates towards 2-aminophenol in vitro*

Enzyme assay mixtures were prepared from 20% (w/v) liver homogenates. The final concentration of activators added to enzyme assay mixtures was 10mM. One unit of enzyme activity is expressed as 1 pmol of 2-aminophenol glucuronide formed/min. Activities are presented as means $\pm$ S.E.M. for duplicate assays of samples from five rat livers. Where smaller numbers of samples were used, the number of samples is shown in parentheses and the mean enzyme activity values are reported.

Activator	UDP-glucuronyltransferase activity (units/mg of protein)		Ratio of activities of enzymes from Gunn rat/ Wistar rat
	Gunn	Wistar	
Strain of rat ...			
Propan-2-one (2)	24	39	0.61
Butan-2-one (2)	42	43	0.98
Pentan-2-one	55 $\pm$ 4	59 $\pm$ 4	0.93
Pentan-3-one	53 $\pm$ 3	57 $\pm$ 4	0.93
Diethyl- nitrosamine (3)	75	66	1.14
None	2.5 $\pm$ 0.3	30 $\pm$ 4	0.08

rubin. This enzyme activity was completely absent from all of the Gunn-rat livers used in the present studies. Table 1 shows that UDP-glucuronyltransferase activity of Gunn-rat liver towards 2-aminophenol, as expected, was only 8.3% of the activity in the equivalent Wistar-rat liver preparation. Addition of 10mM-pentan-2-one, -pentan-3-one or -butan-2-one to Gunn-rat liver homogenate assay mixtures stimulated UDP-glucuronyltransferase activity 10–22-fold when using 2-aminophenol as substrate (Table 1), whereas addition of these compounds to assays of UDP-glucuronyltransferase activity in the equivalent Wistar-rat liver preparations increased enzyme activity only 1.4–2.0-fold. Thus, like diethylnitrosamine, these compounds were able to overcome the inability of the enzyme to glucuronidate 2-aminophenol, though diethylnitrosamine was slightly more effective than the ketones.

Gunn-rat liver UDP-glucuronyltransferase was not significantly activated by the detergent Lubrol 12A9 at concentrations that activate the Wistar-rat liver enzyme (Weatherill & Burchell, 1978). UDP-glucuronyltransferase was solubilized and purified from Gunn- and Wistar-rat livers by using Lubrol 12A9 (Table 2). UDP-glucuronyltransferase activity towards 2-aminophenol was stimulated by pentan-3-one at all stages of the enzyme-purification procedure. Although UDP-glucuronyltransferase activity was not detected in the final purified Gunn-rat liver preparation, the enzyme activity can be generated by the presence of diethyl ketone in the assay (Table 2).

Table 2. *Activation of purified UDP-glucuronyltransferase by pentan-3-one*

Pentan-3-one (10mM final concentration) was added to the enzyme assay mixtures immediately before incubation. Results shown were obtained from six female Gunn-rat livers. One unit of enzyme activity is expressed as 1 nmol of 2-aminophenol glucuronide formed/min. Abbreviation: N.D., not detectable.

Purification fraction	UDP-glucuronyltransferase activity (units/mg of protein)	
	–Pentan-3-one	+Pentan-3-one
25–60%-satn.- (NH ₄) ₂ SO ₄ precipitate	0.03	
DEAE-cellulose eluate	0.02	0.23
UDP-glucuronic acid eluate from UDP- hexanolamine- Sephadex-4B	0.42	13.6
DEAE-Sephadex eluate	N.D.	77.6

Discussion

Pentan-2-one, pentan-3-one and butan-2-one increase Gunn-rat liver UDP-glucuronyltransferase activity towards 2-aminophenol up to a value similar to that existing in Wistar rats, thereby eliciting the same response as diethylnitrosamine (Stevenson *et al.*, 1968). However, compounds such as pentan-2-one are obviously less hazardous than the hepatocarcinogen diethylnitrosamine. Derivatives of these compounds may prove to be of therapeutic value.

Alkyl ketones (Table 2) and diethylnitrosamine (Weatherill & Burchell, 1978) generate UDP-glucuronyltransferase activity of the enzyme purified from Gunn-rat liver, and these compounds also stimulate the activity of the purified Wistar-rat liver enzyme 2–3-fold (results not shown; Burchell, 1978). Thus these compounds are apparently interacting directly with the enzyme protein and may prove to be useful for structural examination of the active site of UDP-glucuronyltransferase.

The previously reported stimulatory effect of diethylnitrosamine on Gunn-rat liver UDP-glucuronyltransferase has been shown to be limited to only a few substrates, 2-aminophenol, paracetamol (Stevenson *et al.*, 1968), 2-aminobenzoate (Zakim *et al.*, 1973), and 4-nitrophenol (Weatherill & Burchell, 1978), that have been tested. Certainly this effect has not been observed with other drug-metabolizing enzymes, where diethylnitrosamine has proved to be a good inhibitor of hexobarbital oxidation (Stevenson & Greenwood, 1968). As yet we have not tested the effect of 10mM-pentan-2-one on other drug-metabolizing enzymes.

Clearly a therapeutically useful compound must be capable of activating deficient UDP-glucuronyltransferase activity towards bilirubin and must not be an inhibitor of other drug-metabolizing enzymes (see Dutton & Burchell, 1977). Preliminary experiments revealed that pentan-2-one did not stimulate bilirubin UDP-glucuronyltransferase activity in either Gunn- or Wistar-rat liver homogenates. Bilirubin UDP-glucuronyltransferase may be truly absent from Gunn-rat liver or perhaps the correct activator has not yet been found. However, the results reported in the present paper reveal a large number of new compounds that require further study.

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