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ON NON-SPECIFIC INHIBITION OF RAT LIVER MICROSOMAL UDP GLUCURONYLTRANSFERASE BY SOME DRUGS

GERARD J. MULDER

Department of Pharmacology, State University of Groningen, Groningen, The Netherlands

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Abstract—The effect of imipramine, desipramine, harmol, harmalol and some monoamine oxidase inhibitors of the hydrazine type on rat liver UDP glucuronyltransferase (EC 2.4.1.17) activity has been investigated. Substrates of the enzyme were *p*-nitrophenol and bilirubin; with *p*-nitrophenol only Triton X-100 activated microsomes were used as enzyme source, with bilirubin both not-activated and Triton X-100 activated microsomes were used. The degree of inhibition obtained with the various inhibitors was dependant on the substrate used and on the pretreatment of the microsomes. It is concluded that most of the effects were caused by the action of the compounds on microsomal membrane structure, which affects UDP glucuronyltransferase activity, and that the physiological relevance of inhibition of the enzyme *in vitro* is questionable.

A NUMBER of drugs are known to cause jaundice of the unconjugated type. The mechanism of this effect is reported to be inhibition of UDP glucuronyltransferase activity towards bilirubin. This has extensively been investigated and discussed by Hargreaves.[1,2]

However, the results of experiments concerning this problem are rather confusing. Thus, Hargreaves studied inhibition of *o*-aminophenol and bilirubin glucuronidation by a number of monoamine oxidase inhibitors of the hydrazine type.[3] Some of these compounds activated glucuronidation of both substrates, some of only one of the substrates (being inhibitory towards the other) and one of them (iproniazide) activated UDP glucuronyltransferase activity towards *o*-aminophenol at low concentrations (1 mM), whereas at 10 mM there was inhibition of this activity.

As suggested previously[4,5] results of experiments with UDP glucuronyltransferase may depend on the pretreatment of the microsomal enzyme preparation. Thus, activation of the enzyme activity *in vitro* can be obtained by a number of pretreatments which seem to affect the membrane structure. Detergents,[4,6,7] sonication[8] and phospholipase treatment[9–11] can increase activity considerably. These increases have been attributed to effects on the microsomal membrane conformation, which affect UDP glucuronyltransferase activity. Therefore, any compound which has an effect on microsomal membrane conformation may affect UDP glucuronyltransferase activity and it may depend on the status of the enzyme, that is, already optimally activated by e.g. detergents or nonactivated, whether such a compound then causes an increase or decrease of enzyme activity.

In this work the effects of some compounds (Fig. 1) have been investigated with *p*-nitrophenol and bilirubin as substrates of UDP glucuronyltransferase, the latter both with and without activation of the microsomal enzyme preparation by Triton X-100, a non-ionic detergent. The most likely interpretation of the present results is that many compounds inhibit UDP glucuronyltransferase *in vitro* due to effects on microsomal membrane conformation and not through an effect directly on the enzyme-protein.

MATERIALS AND METHODS

Drugs. Harmol and harmalol were obtained from Fluka, Buchs (Switzerland). Imipramine and desipramine were a gift from Propharma N.V., Haarlem (The Netherlands). Benzoylhydrazine was obtained from Schuchardt, Munich (Germany) and the other hydrazines were from Aldrich Chem. Co. Milwaukee (U.S.A.). The structural formulas of the compounds are shown in Fig. 1.

The compounds could be dissolved in water; in the case of benzylhydrazine, phenelzine and iproniazide, 100 mM solutions of the hydrazines were adjusted to pH 6·5 with a NaOH solution so that the pH of the incubation medium was not affected by the addition of these hydrazines. The addition of the other compounds did not affect this pH.

Enzyme preparation. The enzyme preparation used was a microsomal fraction, prepared in 0·15 M KCl as described before,[5] from the liver of male rats (Wistar, TNO,

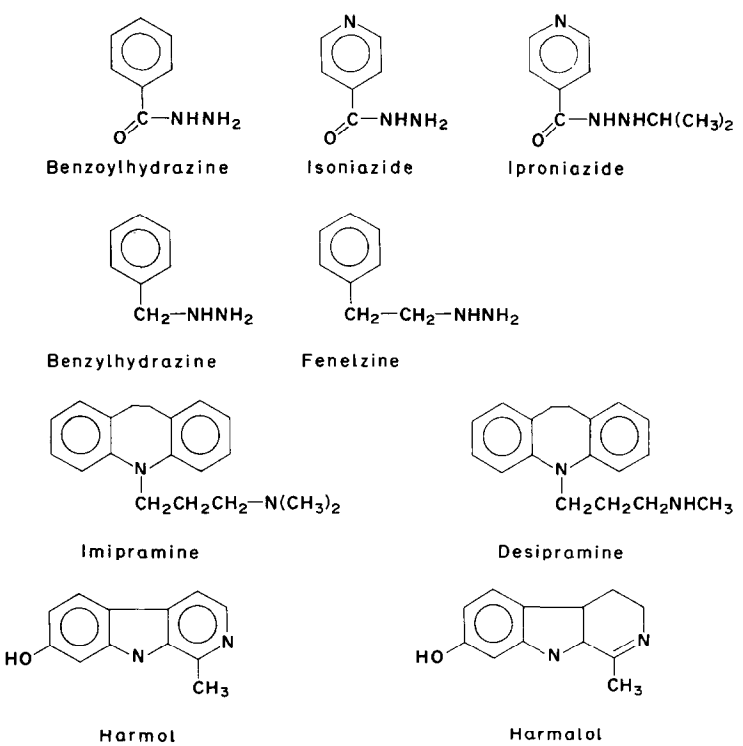

FIG. 1.

Zeist, the Netherlands) weighing 200–300 g. The microsomes were resuspended in 0·15 M KCl to a final concentration of about 12 mg of microsomal protein per ml and kept at −40°. In all experiments unless stated otherwise, this preparation was activated by addition of the detergent Triton X-100 (final concentration in the microsomal preparation 0·25% v/v). In the experiments the microsomal protein concentration in the incubation medium was 200–600 μg/ml.

UDP glucuronyltransferase estimation. *p*-Nitrophenol and bilirubin glucuronidation were measured as described before[5,12] in 75 mM Tris–HCl buffer pH 7·3 containing 5 mM $MgCl_2$. UDP glucuronate concentration was 1·5 mM and substrate concentration was 0·6 mM for *p*-nitrophenol and 120 μM for bilirubin. Bilirubin was used in the albumin solubilized form;[5] albumin concentration was about 40 μM. All concentrations mentioned are final concentrations in the incubation medium. Incubation time was in most experiments 15 min at 37°.

Protein determination. Protein was determined by the method of Lowry *et al.*[13] as described by Layne[14] with bovine serum albumin (Poviet, Amsterdam, The Netherlands) as standard.

RESULTS

Monoamine oxidase inhibitors. The glucuronidation of *p*-nitrophenol was only marginally affected by the hydrazines at a concentration of 10 mM in the incubation medium (Table 1). It was important to use the hydrazines immediately after dissolving them, because at pH 7·3 the solution gradually developed a bad smell indicating that they were unstable. As can be seen in Fig. 2 the presumed hydrolysis products of phenelzine, which appear during preincubation of phenelzine in the Tris–$MgCl_2$ buffer, pH 7·3, were more inhibitory than phenelzine itself, if this compound is at all inhibitory: during the enzymatic incubation phenelzine will also be hydrolyzed and the 8 per cent inhibition found with phenelzine may be due to this hydrolysis. Therefore, during all experiments the hydrazines were dissolved immediately before use and were not allowed to stand for more than 5 min, before the incubation started.

With bilirubin as substrate of UDP glucuronyltransferase the situation was more complex. From Table 1 it seems that isoniazide and benzoylhydrazine were unable to inhibit bilirubin glucuronidation, both in Triton X-100 activated and non-activated microsomes. Phenelzine, iproniazide and benzylhydrazine were inhibitory when non-activated microsomes were used. The differences between the incubation with activated and non-activated microsomes were, (1) with non-activated microsomes twice the microsomal protein concentration was used, (2) the incubation lasted for 30 min in this case in order to get a reliable conversion of bilirubin, and (3) the presence of Triton X-100.

Phenelzine and benzylhydrazine slightly inhibited the colour reaction for the assay of bilirubin-glucuronide (by 5–12 per cent), whereas the three other hydrazines gave a slight increase (about 7 per cent). The inhibition percentages shown in Table 1 have been corrected for these effects.

Imipramine and desipramine. The inhibition of UDP glucuronyltransferase with *p*-nitrophenol as substrate by imipramine and desipramine is shown in Table 2. The compounds were inhibitory from 0·5 mM. They were equally inhibitory and for desipramine the inhibition was independent of UDP glucuronate and *p*-nitrophenol concentrations (Table 3), indicating non-competitive inhibition of the enzyme activity.

TABLE 1. INHIBITION OF GLUCURONIDATION BY MONOAMINE OXIDASE INHIBITORS*

Inhibitor	mM	Activity as % of control: *p*-Nitrophenol as substrate			Bilirubin as substrate: Triton X-100 activated			Bilirubin as substrate: Non-activated		
Phenelzine	10	92	(91–94)†	(3)‡	93	(92–95)†	(3)‡	61	(59, 63)†	(2)‡
Isoniazide	10	99	(97–100)	(4)	109	(103–112)	(3)	105	(98, 112)	(2)
Iproniazide	10	89	(87–91)	(3)	80	(75–84)	(3)	66	(57, 75)	(2)
Benzylhydrazine	10	95	(90–100)	(3)	80	(75–85)	(3)	56	(55, 57)	(2)
Benzoylhydrazine	10	90	(87–92)	(3)	94	(86–100)	(3)	106	(101, 110)	(2)

* For incubation conditions: see Methods section.
† The range of the values.
‡ The number of separate determinations

TABLE 2. EFFECT OF IMIPRAMINE AND DESIPRAMINE ON GLUCURONIDATION*

Inhibitor	mM	Activity as % of control: *p*-Nitrophenol as substrate			Bilirubin as substrate: Triton X-100 activated			Bilirubin as substrate: Non-activated		
Imipramine	2·0	46	(35–52)	(3)	82	(81–83)	(3)	173	(169, 176)	(2)
Desipramine	2·0	44	(38–49)	(4)	74	(72–76)	(3)	177	(169, 185)	(2)

* See legend to Table 1.

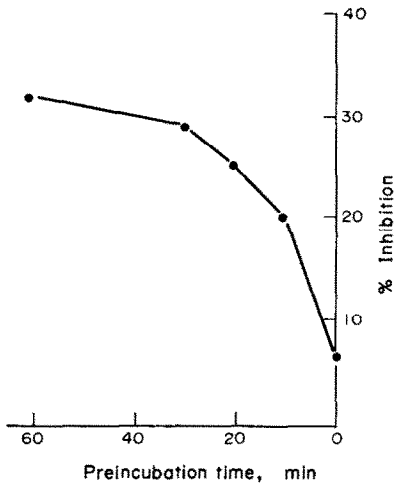

FIG. 2. Effect of preincubation of a phenelzine solution at pH 7·3 on the inhibition of UDP glucuronyltransferase by this phenelzine solution. A phenelzine solution (about 17 mM) in 125 mM Tris–HCl pH 7·3, containing 8·3 mM $MgCl_2$ and 1·0 mM *p*-nitrophenol was incubated for 0–60 min at 37°. UDP glucuronate (or water, control) and microsomes were then added to give normal concentrations of tris, $MgCl_2$, *p*-nitrophenol and phenelzine (10 mM). The percentage inhibition of *p*-nitrophenol glucuronidation is given as a function of preincubation time.

With bilirubin as substrate the situation was again more complex (Fig. 3). When non-activated microsomes were used there was a strong activation of bilirubin glucuronidation by desipramine. When, however, Triton X-100 activated microsomes were used only slight activation occurred at low concentrations, followed by an inhibition at higher concentrations. Imipramine and desipramine behaved similarly in this experiment (Table 2). Neither compound had any influence on colour development in the bilirubin–glucuronide assay. *p*-Nitrophenol glucuronidation was not stimulated by desipramine in non-activated enzyme preparations. In these experiments it was found that desipramine caused aggregation of microsomes, from 0·5 mM very clearly visible.

Harmol and harmalol. These compounds are glucuronidated *in vivo* in the rat[16] and the glucuronidation *in vitro* has been investigated by Wong.[17,18] Both compounds inhibit the glucuronidation of *p*-nitrophenol (Table 4). The results shown in Table 3 suggest a non-competitive inhibition for harmol.

The compounds were much more inhibitory towards bilirubin; *p*-nitrophenol glucuronidation was inhibited about 16 per cent by 2·5 mM harmalol, bilirubin glucuronidation was inhibited 40 per cent by 0·1 mM harmalol. The inhibition by harmol was 32 per cent (at 2·5 mM) and 19 per cent (at 0·1 mM) respectively. Both compounds at 1 mM completely inhibited bilirubin glucuronidation by Triton X-100 activated microsomes. This may have been influenced by the fact that the compounds displaced bilirubin from its albumin binding sites in the presence of Triton X-100. When no Triton X-100 was present in the incubation, harmol and harmalol could not displace bilirubin from this binding. Thus, Triton X-100 seems to labilize bilirubin binding by albumin. With non-activated microsomes harmol and harmalol were still more inhibitory. Neither compound had any influence on colour development in the bilirubin–glucuronide assay.

TABLE 3. INHIBITION OF *p*-NITROPHENOL GLUCURONIDATION BY HARMOL AND DESIPRAMINE*

Inhibitor	mM	UDPGA (mM)	*p*-Nitrophenol (mM)	v (without inhibitor present)†	% Inhibition
Desipramine	1·0	0·5	0·6	44	42 (42–44)‡
		1·5	0·6	63	37 (35–39)
		1·5	0·3	45	33 (29–37)
		1·5	0·8	76	42 (35–47)
Harmol	1·0	0·5	0·6	44	30 (25–35)
		1·5	0·6	63	24 (19–27)
		1·5	0·3	45	25 (22–26)
		1·5	0·8	76	30 (25–32)

* All results are the means of three separate experiments. For incubation conditions: see Methods section.

† The velocity (v) is given as nmoles converted/min/mg microsomal protein, without inhibitor present.

‡ The range of the values.

DISCUSSION

The results show that it is very difficult to obtain consistent results with UDP glucuronyltransferase. The fact that different effects are found when different substrates are used is well known and has often been ascribed to the existence of different UDP glucuronyltransferases with different substrate specificity. We found that pretreatment of the enzyme preparation influenced the effect obtained (Triton X-100 activated and non-activated enzyme preparations). This has been shown also by other authors.[4,19–21] It complicates the reliability of the interpretations of the results and

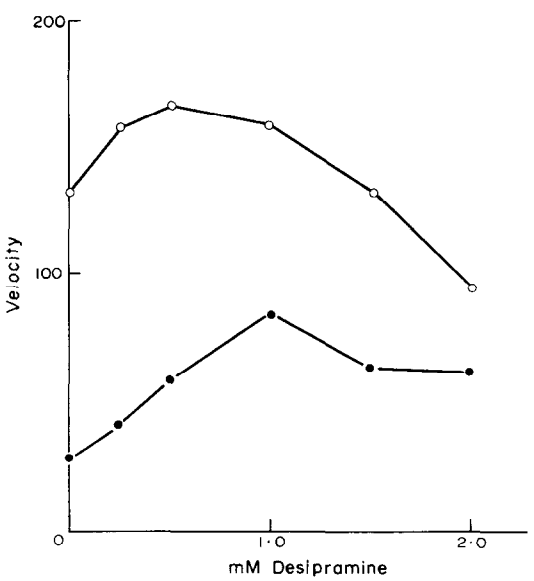

FIG. 3. Effect of desipramine on bilirubin glucuronidation. The effect of various concentrations of desipramine on bilirubin glucuronidation by Triton X-100 activated (○) or non-activated (●) rat liver microsomes is shown. The velocity of bilirubin glucuronidation is expressed as the absorbance at 530 nm developed during the incubation for 15 min, with about 500 μg microsomal protein per ml of incubation medium.

TABLE 4. INHIBITION OF GLUCURONIDATION BY HARMOL AND HARMALOL*

Inhibitor	mM	Activity as % of control								
					Bilirubin as substrate					
		p-Nitrophenol as substrate			Triton X-100 activated			Non-activated		
Harmol	2·5	68	(63–75)	(5)						
Harmalol	2·5	84	(83–85)	(3)						
Harmol	0·1				81	(80–83)	(4)	47	(47, 47)	(2)
Harmalol	0·1				61	(56–65)	(4)	33	(33, 33)	(2)

* See legend to Table 1.

conclusions must be drawn with caution. This is illustrated by the present results of inhibition of UDP glucuronyltransferase by some drugs.

As far as the hydrazine type monoamine oxidase inhibitors are concerned it is important to ensure that their effects are really due to the unchanged hydrazines and not to their hydrolysis products. Thus, it is not clear whether the inhibition by phenelzine of morphine glucuronidation as shown by Yeh and Mitchell[22] and which they claim to be competitive, is in fact an inhibition due to phenelzine or to its hydrolysis products appearing during incubation for 30 min at pH 8·0. The results presented above suggest that phenelzine will be partly hydrolyzed during the incubation.

It should be noted that the three most hydrophobic hydrazines inhibit the glucuronidation of bilirubin (Fig. 1). Mixed function oxidase activity (*N*-demethylation of meperidine) is more sensitive to phenelzine and iproniazide than UDP glucuronyltransferase; with this enzyme system they are very inhibitory at 0·06–0·5 mM.[23] The activation of bilirubin glucuronidation by iproniazide as found by Hargreaves[3] could not be reproduced in the present experiments. Hargreaves, however, used guinea pig liver microsomes, as the enzyme source. It has been shown that these have properties rather different from those of rat liver microsomes used in the present study.[25]

The effect of desipramine and imipramine (Table 2 and Fig. 3) may be due to a "Triton X-100-like" effect on microsomal membrane conformation[4] which at first is favourable to enzyme activity but becomes inhibitory when this conformational change goes beyond a certain limit. Miller and Dingell,[24] however, found different effects of imipramine and desipramine on tetrahydrocortisone glucuronidation at a concentration of 0·5 mM. Desipramine inhibited this glucuronidation (about 30 per cent) and imipramine stimulated it (about 40 per cent), in non-activated rat liver microsomes. Hargreaves[15] found no effect of desipramine, even at 10 Mm, on bilirubin glucuronidation in non-activated guinea-pig liver homogenates, and a 30 per cent inhibition by 10 mM imipramine.

Finally, inhibition by harmol and harmalol of *p*-nitrophenol glucuronidation unexpectedly showed non-competitive characteristics (Table 2). Bilirubin glucuronidation was considerably more sensitive to harmol and harmalol than *p*-nitrophenol conjugation. An explanation might be that with *p*-nitrophenol the inhibition (at high inhibitor concentrations) is due to effects on microsomal membrane structure, whereas with bilirubin possibly competitive inhibition is seen at much lower concentrations of both inhibitors.

As suggested above, any compound which has an effect on microsomal membrane conformation may thereby have an effect on UDP glucuronyltransferase activity. This may be activation in non-activated microsomes, followed by inhibition at higher concentrations, or only inhibition in optimally activated microsomes. An effect on microsomal membranes is to be expected from compounds which readily dissolve in or bind to membranes, due to their lipid-solubility. Therefore, it will not be surprising if a hydrophobic compound affects UDP glucuronyltransferase activity. It remains, however, rather questionable whether these effects are of any significance to the *in vivo* situation or whether they are only a consequence of the artefacts introduced by taking the enzyme from its natural environment by isolation of microsomes.

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