Further Studies on the Effect of Imipramine and Desipramine on Uridine Diphosphate Glucuronyl Transferase

Imipramine is an iminodibenzyl derivative used extensively as an antidepressant drug[1]. Desipramine is the N-demethyl derivative of imipramine and is thought to be its pharmacologically active form[2]. A conjugated hyperbilirubinaemia, which may be transient, can follow imipramine administration and has been recorded in 0.5% to 1.0% of patients. This normally disappears on cessation of therapy[1,3]. One fatal case of jaundice following mixed imipramine-desipramine therapy has been described[4]. Our previous work suggested that imipramine probably inhibited the transferase enzyme (UDP glucuronate glucuronyl transferase, acceptor unspecific, EC 2.1.4.17.) although results with desipramine were inconsistent[5]. The most striking feature reported was the very large increase in unconjugated bilirubin in liver slices after 2 h incubation in a bilirubin-containing medium, when compared to control values. It was suggested that the amphipathic nature of the drugs might play a part in producing this unusual phenomenon[5,6].

Experimental and results. We decided to investigate further the effect of a fuller range of drug concentrations on bilirubin conjugation by UDP glucuronyl transferase. The methods used were those of VAN ROY and HEIRWEGH[7]. Reagent concentrations for system 1 were bilirubin in serum/EDTA medium, 0.4 m*M* and uridine diphosphate glucuronate (UDPGA), 8.0 m*M*. For system 2, bilirubin in ethanolamine/ethane diol was 0.068 m*M* and UDPGA, 2.0 m*M*. The principle advantage of the VAN ROY and HEIRWEGH technique is that the colour reagent employed reacts only with conjugated bilirubin.

At drug concentrations below 1.0 m*M* no significant difference in reaction rates from control values was detected. Between 1.0 m*M* and 5.0 m*M* an activating effect was evident. This was particularly enhanced using system 1 (Tables I and II). Above 5.0 m*M* reaction rates fell sharply but this proved to be due to precipitation of bilirubin from the medium by the drug. This was independent of microsomal suspension and therefore not enzyme inhibition as first thought[5].

Both imipramine and desipramine have amphipathic structures and it was thought that the drugs might have surfactant properties[5]. The osmolality of various concentrations of imipramine hydrochloride in distilled water was

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Table I. Effect of imipramine and desipramine on bilirubin conjugation in rat liver microsomes

Drug concentration (m*M*)	Imipramine		Desipramine	
	Reaction rate (μmol/g liver/h)	Activation (%)	Reaction rate (μmol/g liver/h)	Activation (%)
0	0.55 ± 0.03		0.57 ± 0.01	
1	0.83 ± 0.11	51[b]	1.00 ± 0.02	75[b]
2	1.52 ± 0.05	176[b]	1.90 ± 0.02	227[b]
3	2.01 ± 0.20	265[b]	2.08 ± 0.11	258[b]
4	1.99 ± 0.20	262[b]	1.74 ± 0.35	200[a]
5	1.44 ± 0.11	162[b]	1.28 ± 0.02	120[b]
10	0.35 ± 0.10	[a]	0.25 ± 0.07	[a]

Method of VAN ROY and HEIRWEGH[7]. System 1. [a] $P < 0.01$. [b] $P < 0.001$.

Table II. Effect of imipramine and desipramine on bilirubin conjugation in rat liver microsomes

Drug concentration (m*M*)	Imipramine		Desipramine	
	Reaction rate (μmol/g liver/h)	Activation (%)	Reaction rate (μmol/g liver/h)	Activation (%)
0	0.60 ± 0.04		0.37 ± 0.05	
1	0.68 ± 0.05	13[a]	0.61 ± 0.07	65[a]
2	0.76 ± 0.09	27[a]	0.82 ± 0.02	121[b]
3	0.65 ± 0.15		0.62 ± 0.03	67[b]
4	0.60 ± 0.08		0.42 ± 0.02	
5	0.43 ± 0.09		0.28 ± 0.04	

Method of VAN ROY and HEIRWEGH[7]. System II. [a] $P < 0.01$. [b] $P < 0.001$.

determined using a Knauer osmometer (Table III). The results show that imipramine has surfactant properties. Equipment for precise determinations of critical micelle concentration was not available but it would appear to lie between 50 and 80 m*M*.

The turbidity of various solutions containing bilirubin and desipramine was measured at 650 nm (a minimum in the bilirubin absorption spectrum). Between 10 and 50 m*M* desipramine the solution was grossly turbid but above 80 m*M* the solution was clear, probably because bilirubin was taken up into desipramine micelles (Table IV).

Discussion. The results show that both imipramine and desipramine exhibit a strong activating effect on UDP glucuronyl transferase. The effect occurs over a narrow range of drug concentrations and was not due to a direct chemical effect of the drugs on bilirubin since it was not observed in control experiments carried out in the absence of microsomal suspension.

Imipramine and desipramine are surfactants and the effects may be explained on the basis of surfactant-induced absorption. In vitro, imipramine molecules will concentrate at interfaces between the microsomes and the surrounding liquid medium. Polar groups will be orientated towards the aqueous medium and the non-polar parts of the molecules directed towards the lipid protein matrix of the microsomes. This may accelerate the absorption of bilirubin by the microsomes. When the surfaces become saturated with imipramine molecules the molecules must enter the main bulk of the solution and at this stage bilirubin is precipitated.

At drug concentrations exceeding the critical micelle concentration bilirubin is taken up into the imipramine micelles. Unfortunately, it was not possible to investigate this latter condition in the full reaction medium so the effect on the production of bilirubin conjugates is not known.

Marked activation of glucuronyl transferase and other microsomal enzymes by surfactants has been reported[8–12]. Since the completion of this work MULDER[13] has also reported activation of UDP glucuronyl transferase by desipramine. Our work has shown that imipramine and desipramine act as cationic surfactants in aqueous media. It is possible that the drugs produce changes in the microsomal environment of the enzyme and hence make it more accessible for the substrate.

The activation effect was more pronounced when bilirubin was solubilized in a serum-EDTA medium. This may indicate that other factors are concerned in the reaction. It is possible that the drugs are bound to serum proteins, notably albumin, in preference to bilirubin and this may cause more rapid transfer of the bilirubin to microsomal protein.

The effects measures in this in vitro system may not be of significances in vivo, particularly since high drug concentrations were used. However, imipramine and desipramine can produce a conjugated hyperbilirubinaemia often accompanied by cell damage, and jaundice may disappear without cessation of therapy. It is possible that imipramine and desipramine may cause a transient over production of conjugated bilirubin by the factors described above.

Table III. Osmolality of imipramine solutions

Imipramine concentration (m*M*)	Osmolality (m.osmol./kg)
1	2
2	4
5	10
10	20
25	50
50	78
100	85
150	95
200	100
250	100
300	100

Table IV. Effect of desipramine on the turbidity of a bilirubin solution

Desipramine concentration (m*M*)	E_{560}
0	0.065
1	0.540
10	0.650
25	0.650
50	0.650
75	0.650
100	0.174
150	0.125
200	0.140

Résumé. Nous avons recherché l'action de l'imipramine et de la désipramine sur l'UDP glucuronyle transférase dans les microsomes hépatiques. Les concentrations de ces drogues entre 1 et 5 m*M* ont activé l'enzyme. Ce sont des surfactants cationiques.

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Glucose Uptake by *Aspergillus nidulans*, Purification and Properties of Glucose Binding Protein

Inspite of significant advance in elucidation of uptake systems in bacteria[1–3] and yeast[4,5], relatively little is known concerning sugar transport in fungi. An impressive body of evidence has accumulated which indicates that sugar transport in bacteria[6] and yeast[7,8] is coupled with phosphorylation; however, this is not true in case of filamentous fungi *A. nidulans*[9] and *Neurospora crassa*[10].

Studies conducted in our laboratory indicated the relationship between biotin status of *A. nidulans* and the cellular permeability to ammonium ions[11,12]. In an earlier