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FACTORS AFFECTING THE RESPONSE OF MICROSOMAL UDP-GLUCURONYLTRANSFERASE TO MEMBRANE PERTURBANTS

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SUMMARY

Evidence is presented indicating a species difference in the levels of UDP-glucuronyltransferase (EC 2.4.1.17) activity of rat and guinea pig liver microsomal fractions. The specific activities of microsomal fractions prepared in 0.154 M KCl are higher than those of fractions prepared in 0.25 M sucrose. The specific activity of UDPglucuronyltransferase in microsomal fractions and the concentration of UDP-glucuronate during assay influence the enzyme's response to membrane perturbation by phospholipase A (EC 3.1.1.4) and detergents.

INTRODUCTION

Mammalian liver UDPglucuronyltransferase (EC 2.4.1.17) is firmly bound to microsomal membranes and its activity is markedly dependent upon membranous structure and phospholipid composition. This information has been gained mainly from studies of the effects of membrane perturbing agents like phospholipases and detergents[1–9]. However, although these agents unquestionably affect the enzyme's activity there is considerable disagreement in the literature concerning the magnitude of their effect and even, in some cases, as to whether the enzyme is activated or inactivated. For example, whereas several workers have found that Triton X-100 activates UDPglucuronyltransferase[4,8–10], others report that the detergent has no significant effect[2,7]. Recently we have shown[3] that a major factor contributing to these apparent discrepancies is a difference in the responses to detergents of the enzymes from rat and guinea pig, the most frequently used sources of UDPglucuronyltransferase. Nevertheless, even when attention is restricted to a single animal species a number of discrepancies persist. For example, whereas we found that phospholipase A (EC 3.1.1.4) and phospholipase C (EC 3.1.4.3) inactivate guinea pig liver microsomal *p*-nitrophenol UDPglucuronyltransferase[1,2], Vessey and Zakim[6] reported that the enzyme from the same source and with the same acceptor is activated by similar treatments with these phospholipases. The most likely causes of these discrepancies are differences in the methods by which microsomal fractions were prepared and in the concentrations of one of the enzyme's substrates (UDPglucuronate) during assay. In this communica-

tion we examine the effects of these parameters on the response of microsomal UDP-glucuronyltransferase from rat and guinea pig to phospholipase A and detergents.

MATERIALS AND METHODS

Male rats (Wistar, 150–200 g) and guinea pigs (Hartley, 250–400 g), purchased from Fisons Pharmaceuticals, Loughborough, Leicestershire, Great Britain, were starved overnight and liver microsomal fractions prepared by one of two methods. (a) The livers were excised, washed with ice-cold 0.154 M KCl, blotted dry and homogenised in 4 vol. of the same solution for two 1-min periods, with cooling on ice. The homogenates were fractionated and the microsomal fractions washed twice with 0.154 M KCl, essentially according to Pogell and Krisman[11]. Microsomal fractions prepared by this method were used in our previous work[1–3] and are referred to as KCl-treated microsomes. (b) Washed microsomal fractions were also prepared by the method of Vessey and Zakim[6] which employs homogenisation and washing in 0.25 M sucrose. These are referred to as sucrose-treated microsomes.

The washed microsomal pellets were resuspended in sufficient 0.154 M KCl or 0.25 M sucrose to yield a protein concentration of 20 mg/ml as measured with a biuret reagent[12], standardised with bovine serum albumin. Unless otherwise stated, the microsomal suspensions were stored at −18 to −23 °C[1,6] and used within 72 h of preparation.

Microsomal suspensions (5 mg of protein/ml, final concentration) were digested with a purified phospholipase A preparation, from *Crotalus adamanteus* venom[1], with shaking at 20 °C in a medium containing 12.5 mM Tris–HCl buffer, pH 8.0, and 2.5 mM $CaCl_2$. The phospholipase concentration was either 10 or 50 μg/mg of microsomal protein and the reaction was terminated by adding EDTA (final concentration, 10 mM).

Other microsomal fractions (5 mg of protein/ml) were treated with Triton X-100 (0.1%) or sodium deoxycholate (0.06%) for 10 min at room temperature in the presence of 12.5 mM Tris–HCl buffer, pH 8.0.

Samples (0.2–0.5 mg of protein) of the treated microsomal suspensions and of control suspensions which had been subjected to similar treatment except that phospholipase and detergent had been omitted, were assayed for UDPglucuronyl-transferase activity at pH 7.1 with 4 mM UDPglucuronate and *p*-nitrophenol (0.6 mM) as acceptor using Vessey and Zakim's[5] method with the following slight modification. After deproteinisation and centrifugation 1-ml portions of the supernatant solutions were added to 0.02 ml of 10 M KOH and the absorbances determined at 400 nm. Enzyme activity (nmoles of *p*-nitrophenol glucuronidated/min per mg of microsomal protein) was calculated from initial velocity.

RESULTS AND DISCUSSION

In agreement with previous workers[13,6] the UDPglucuronyltransferase activities of guinea pig microsomal fractions prepared by either method are considerably higher than those of corresponding rat liver preparations (Table I). This finding is also in accord with our earlier results[3] which showed that the activities of KCl-treated

TABLE I

THE UDP-GLUCURONYLTRANSFERASE ACTIVITIES OF MICROSOMAL FRACTIONS PREPARED IN 0.154 M KCl OR IN 0.25 M SUCROSE

Values in parentheses give the number of experimental observations

Animal	*Enzyme activity (nmoles/min per mg of protein)*	
	KCl	*Sucrose*
Rat	6.0–15.2 (10)	2.0–3.5 (6)
Guinea pig	15.8–28.7 (10)	4.9–7.9 (6)

microsomes from the two species, determined at a much lower concentration of UDPglucuronate (0.167 mM), were similarly related.

Table I also shows that the UDPglucuronyltransferase activities of KCl-treated microsomes from either species are 3–4 times higher than those of sucrose-treated microsomes. However, when sucrose-treated microsomes are repeatedly washed with 0.154 M KCl their activities increase about 2-fold, *i.e.* to levels approaching those of KCl-treated microsomes. Since only a small percentage of the microsomal protein is removed by this washing, we suggest that preparation or washing of microsomal fractions in 0.154 M KCl renders the enzyme more active either by increasing the permeability of the microsomal membrane to substrates[8,9], or by releasing the enzyme from a conformational restraint[4] present in sucrose-treated microsomes.

The activity of KCl-treated microsomes from rat liver is enhanced 4-fold by treatment for up to 10 min with phospholipase A (Fig. 1). Longer treatment results in return of activity towards the control value. The activity of guinea pig KCl-treated microsomes treated in the same way is only increased by 60%. Digestion with a higher concentration of phospholipase does not magnify this activation and prolonged treatment results in progressive inactivation.

These results with guinea pig liver KCl-treated microsomes are not exactly the same as those we obtained earlier using a lower concentration of UDPglucuronate (0.167 mM) in enzyme assays. In those experiments, phospholipase A caused only a progressive loss of UDPglucuronyltransferase activity[1,2]. Repetition of those earlier experiments confirmed this result and, in addition, showed that the enzyme activity of rat liver KCl-treated microsomes at the low UDPglucuronate concentration was enhanced 3-fold by the phospholipase. Thus, the difference between the enzymes of rat and guinea pig KCl-treated microsomes in response to phospholipase A is more pronounced at low UDPglucuronate concentrations than at the much higher concentration of the substrate used in the present experiments. Our finding that the response of UDPglucuronyltransferase to phospholipase A is very dependent upon UDP-glucuronate concentration is consistent with recent kinetic data on the effects of membrane perturbants[14].

The difference between the responses to phospholipase A of rat and guinea pig UDPglucuronyltransferase activity observed with KCl-treated microsomes is not apparent in sucrose-treated microsomes (Fig. 2). Enzyme activities are increased to

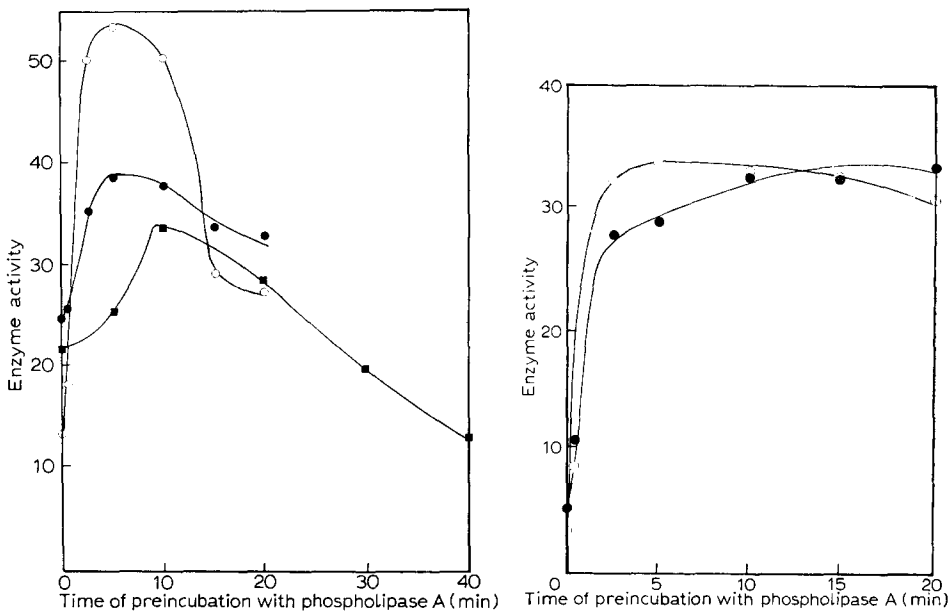

Fig. 1. The effect of phospholipase A on the UDPglucuronyltransferase activities of microsomal fractions from rat and guinea pig prepared in 0.154 M KCl. ○, rat microsomal fraction, 10 μg of phospholipase A per mg of protein; ●, guinea pig microsomal fraction, 10 μg of phospholipase A per mg of protein; ■, guinea pig microsomal fraction, 50 μg of phospholipase A per mg of microsomal protein. Enzyme activity is quoted as nmoles/min per mg of protein.

Fig. 2. The effect of phospholipase A on the UDPglucuronyltransferase activities of microsomal fractions from rat and guinea pig prepared in 0.25 M sucrose. Microsomal fractions were digested with 10 μg of phospholipase A per mg of microsomal protein. ○, rat microsomal fraction; ●, guinea pig microsomal fraction. Enzyme activity is quoted as nmoles/min per mg of protein.

about the same value and there is no significant inactivation even after prolonged digestion. Moreover, the UDPglucuronyltransferase activities of both sucrose- and KCl-treated microsomes from both species observed after phospholipase treatment for about 15 min are approximately the same (28–34 nmoles/min per mg of protein). Thus differences in enzyme activity between sucrose- and KCl-treated microsomes and between rat and guinea pig preparations (Table I) are greatly diminished by treatment with phospholipase A.

The enzyme activities of the various microsomal preparations also respond differently to detergents. Earlier work[3] showed that low concentrations of Triton X-100 or deoxycholate activated the enzyme in KCl-treated microsomes from rats but had no significant effect on that in identical preparations from guinea pigs, when UDPglucuronyltransferase activity was measured at a low UDPglucuronate concentration (0.167 mM). With rats, activation depended on surfactant concentration and was maximal at 0.1% Triton X-100 or 0.06% deoxycholate. When KCl-treated microsomes are preincubated with these concentrations of detergents and UDP-glucuronyltransferase activity determined at 4 mM UDPglucuronate essentially the same effects are observed (Table II). In contrast, when sucrose-treated microsomes are used both rat and guinea pig enzymes are strongly activated by the detergents. In

TABLE II

EFFECTS OF DETERGENTS ON THE UDP-GLUCURONYLTRANSFERASE ACTIVITIES OF RAT AND GUINEA PIG MICROSOMAL FRACTIONS

Animal	*Microsomal fraction prepared in*	*Period of storage at −18 to −23 °C (h)*	*Enzyme activity (nmoles/min per mg of protein)*		
			Untreated	*With 0.1% Triton X-100*	*With 0.06% deoxycholate*
Rat	0.154 M KCl	0	8.1	42.1	38.2
		48	6.0	38.2	38.2
Guinea pig	0.154 M KCl	0	25.7	27.2	34.1
		48	15.8	15.8	25.0
Rat	0.25 M sucrose	24	2.9	24.3	17.6
Guinea pig	0.25 M sucrose	24	6.5	14.9	25.2

addition, it is observed (Table II) that low concentrations of the detergents, like phospholipase A-treatment, greatly diminish the differences in UDPglucuronyltransferase activity between sucrose- and KCl-treated microsomes and between rat and guinea pig preparations.

Thus, there appears to be a marked difference in the UDPglucuronyltransferase activities of intact rat and guinea pig microsomal fractions prepared by either method, and differences in the responses of the enzymes from these various preparations to phospholipase A and detergents. We conclude that these phenomena, together with the dependence on UDPglucuronate concentration of the responses of enzyme activity to perturbants, account for a number of discrepancies in the literature, including that between our previous work[1,2] and the results of Vessey and Zakim[6] on the effects of phospholipase A.

Of the preparations studied here, guinea pig KCl-treated microsomes have the highest activity. Phospholipase A and detergents have comparatively little effect on the UDPglucuronyltransferase activity of these preparations when assayed at a high UDPglucuronate concentration. Rat sucrose-treated microsomes have the lowest activity and, like the other preparations which have intermediate activities, are activated by phospholipase A and detergents up to the level of guinea pig KCl-treated microsomes which already show most of their potential activity.

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