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Stimulation of Steroid Glucuronidation by Uridine Diphosphate *N*-Acetylglucosamine and by Microsomal Disruption

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Glucuronidation of oestrone and of oestradiol in microsomal fractions was markedly and significantly stimulated by UDP-*N*-acetylglucosamine and by ultrasonication: Triton X-100 also stimulated. This is consistent with compartmentation of UDP-glucuronyltransferase. Stimulation by UDP-*N*-acetylglucosamine may be physiologically significant.

The activator UDP-*N*-acetylglucosamine is a feasible physiological regulator for UDP-glucuronyltransferase (Winsnes, 1972; Zakim & Vessey, 1974, 1976a; Otani *et al.*, 1976). However, a recent report questions the generality of UDP-*N*-acetylglucosamine action, since it did not stimulate glucuronidation of oestrone, oestradiol or testosterone in guinea-pig liver microsomal fractions (Zakim & Vessey, 1976b). In the same study, detergent treatment or brief exposure to phospholipase A₂ also failed to stimulate steroid glucuronidation, and this was cited as evidence against compartmentation or structural latency for the transferase.

Elsewhere we have briefly described a model wherein UDP-*N*-acetylglucosamine assists in regulating glucuronyltransferase, which is latent because it is depicted as being embedded in the cisternal face of the endoplasmic reticulum (Berry & Hallinan, 1976). Here we report a study of steroid glucuronidation in microsomal preparations from male guinea-pig liver, in which both UDP-*N*-acetylglucosamine and ultrasonication markedly and significantly stimulated oestrone glucuronidation; detergent treatment also stimulated. In liver microsomal preparations from female and male guinea pigs, UDP-*N*-acetylglucosamine and ultrasonication also stimulated glucuronidation of oestrone and of oestradiol respectively. Stimulations were all large, and those caused by UDP-*N*-acetylglucosamine may be physiologically relevant.

Materials and Methods

Microsomal preparation

Male guinea pigs (Duncan–Hartley strain; 300–500 g), starved overnight, were used. Livers were homogenized in 3 vol. of 0.25 M-sucrose, the homogenate was centrifuged for 10 min at 10000g and microsomal components were sedimented in 60 min at 104000g from the supernatant. Pellets were stored

for up to 10 days at –10°C and were suspended in 0.25 M-sucrose immediately before use. *p*-Nitrophenol glucuronidation and protein were determined as described by Berry *et al.* (1975). Steroid glucuronidation was assayed exactly as described by Rao *et al.* (1977), but at pH 7.4 and in a volume of 0.4–0.8 ml. After stopping the reaction by boiling, 0.1 M-Tris/HCl buffer, pH 7.4, containing 83–250 μM-EDTA was added to make the volume to 1 ml, before extraction with dichloromethane.

Results and Discussion

Rates of glucuronidation of oestrone and oestradiol by untreated control microsomal fractions from male and female guinea pigs averaged 1.5–3.9 nmol/min per mg of microsomal protein (Table 1). Addition of 1 mM-UDP-*N*-acetylglucosamine stimulated 2.8–4.9 times. This increase in activity was statistically significant for oestrone ($P < 0.001$), with microsomal preparations from seven separate guinea pigs, and for oestradiol ($P < 0.01$). There was no detectable conjugation of either steroid, as *N*-acetylglucosaminide, in blanks that contained 1 mM-UDP-*N*-acetylglucosamine plus all other components of the assay mixture, but without added UDP-glucuronic acid. Ultrasonication also markedly stimulated steroid glucuronidation (4.5–7 times), with all animals tested (Table 1). For oestrone, this increase was highly statistically significant ($P < 0.001$). Triton X-100 (0.005–0.02%, w/v) likewise stimulated oestrone glucuronidation in fractions from male guinea pigs, confirming recent (Rao *et al.*, 1977) and earlier reports (Jacobson *et al.*, 1975) that many detergents stimulated glucuronidation of oestrone and other steroids in rat microsomal fractions. With liver microsomal preparations from male rats, UDP-*N*-acetylglucosamine and ultrasonication also markedly stimulated glucuronidation of oestradiol in the present study.

Table 1. *Stimulation of steroid glucuronidation by UDP-N-acetylglucosamine or by membrane disruption in guinea-pig liver microsomal preparations*

Assays were done at pH 7.4 in 0.1 M-Tris/HCl buffer and contained 30 μ M-steroid, 1 mM-UDP-glucuronic acid and 10 mM-MgCl₂. Triton X-100 was added to microsomal preparations at four times the assay concentrations indicated. Ultrasonication was for 6 min, intermittently, with cooling (Berry *et al.*, 1975). Numbers of experiments are shown in parentheses. Statistical significance was assessed by Student's *t* test.

Sex	Substrate	Treatment	Activity (nmol of steroid glucuronidated/ min per mg of protein) ($\pm$ S.D.)	P
Male	Oestrone	Control	2.2 $\pm$ 0.44 (7)	
		UDP-N-acetylglucosamine	7.9 $\pm$ 1.5 (7)	<0.001
Male	Oestrone	Control	1.8 $\pm$ 0.64 (7)	
		Ultrasonicated	9.3 $\pm$ 1.2 (7)	<0.001
Male	Oestrone	Control	3.85	
		Triton X-100 (% w/v)		
		0.005	7.7	
		0.01	9.5	
		0.02	8.0	
Male	Oestradiol	Control	2.0 $\pm$ 0.26 (6)	
		UDP-N-acetylglucosamine	5.8 $\pm$ 1.9 (5)	<0.01
		Ultrasonicated	14.5	
Female	Oestrone	Control	1.45 $\pm$ 0.39 (3)	
		UDP-N-acetylglucosamine	5.0, 7.1 (2)	
		Ultrasonicated	6.5	

These results clearly indicate that steroid glucuronyltransferase activity is significantly latent and is normally responsive to UDP-N-acetylglucosamine. This differs from the findings of Zakim & Vessey (1976b), who apparently prepared microsomal fractions in 0.25 M-sucrose/5 mM-EDTA and subsequently washed these twice in 0.25 M-sucrose (Zakim *et al.*, 1973), and incorporated a 15 min preincubation at 37°C with steroids into their glucuronidation assay. To try to determine the effect of these conditions, preliminary experiments were done on glucuronidation of *p*-nitrophenol and oestrone by both types of microsomal preparation. These suggest that washed 'EDTA-microsomal preparations' may be especially prone to 'spontaneous' activation (Lueders & Kuff, 1967), so that substantial activation and loss of latency can occur during the 15 min preincubation at 37°C, in the assay of Zakim & Vessey (1976b).

Half of a guinea-pig liver was used to prepare washed 'EDTA-microsomal fractions' (Zakim *et al.*, 1973), and the other half was used to prepare fractions (termed 'standard preparations') as described in the Materials and Methods section. *p*-Nitrophenol (0.4 mM), with 4 mM-UDP-glucuronic acid and 4 mM-Mg²⁺, was glucuronidated 41–45% faster by washed 'EDTA-preparations' than by 'standard preparations', but this difference may partly reflect an increase in specific transferase activity when other proteins are washed from the microsomal preparations. Adding 0.5, 1, 2 or 4 mM-UDP-N-acetylglucosamine stimulated similarly (3.1–5.4-fold) in both cases. However, the response of the two pre-

parations to a 15 min preincubation at 37°C, before assaying, was markedly different. Preincubation had no detectable effect on the 'standard preparation', but it stimulated *p*-nitrophenol glucuronidation by a further 80% in the washed 'EDTA-preparation', which then responded less well to stimulation by 0.5, 1, 2 or 4 mM-UDP-N-acetylglucosamine (1.8–2.8-fold stimulations), whereas preincubated 'standard preparations' were still stimulated 3.2–6.5-fold. Similarly, after loading microsomal vesicles with oestrone, by preincubation for 15 min at 37°C (Zakim & Vessey, 1976b), addition of 1 or 2 mM-UDP-N-acetylglucosamine stimulated oestrone glucuronidation less well in washed 'EDTA-preparations' (1.8-, 3.1- and 2-fold) than in paired 'standard preparations' (2.3-, 5.6- and 3.7-fold). Oestrone glucuronidation was also 1.5–2.8-fold faster, without added UDP-N-acetylglucosamine, in the washed 'EDTA-preparations'. Therefore there seems no reason to exclude UDP-N-acetylglucosamine as a likely physiological regulator of the glucuronidation of steroids, as well as of other aglycones.

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