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SIMILARITY OF RAT INTESTINAL AND HEPATIC MICROSOMAL 7-HYDROXYCOUMARIN-UDP-GLUCURONYLTRANSFERASE: IN VITRO ACTIVATION BY TRITON-X100, UDP-N-ACETYLGLUCOSAMINE AND $MgCl_2$

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UDP-glucuronyltransferase is one of the major enzymes responsible for phase II metabolism of xenobiotics [1]. Although the hepatic UDPGT seems to be of paramount importance for *in vivo* glucuronidation of xenobiotics, extrahepatic sites can also contribute to the total conjugative capacity [2]. The study of conjugation-reactions in the intestinal wall is particularly interesting because bioavailability of orally administered drugs can be affected to a large extent [3-5] Predictions of the *in vivo* glucuronidation-capacity can be made by investigating microsomal preparations [1, 6, 7]. Since the microsomal UDPGT can be activated during isolation or *in vitro*-incubation, it is important to know if and to what extent the enzyme is activated in order to make valid extrapolations [1]. In contrast with the hepatic UDPGT, the intestinal UDPGT is considered to be inactivatable *in vitro* or, conversely, to be fully activated during the isolation procedure [1, 6-8]. We found, however, that the microsomal UDPGT, prepared from rat intestinal mucosal cells, could be activated *in vitro* by both the nonionic detergent Triton-X100 and UDPNAG. Some further experiments made clear that there is a qualitative similarity of the intestinal and hepatic microsomal UDPGT from the rat, of which evidence will be given below.

METHODS

Male Wistar rats (Cpb: WU, 200-250 g) were obtained from T.N.O. (Zeist, The Netherlands) and allowed free access to water and a commercially available diet (RMH-B, Hope Farms, Woerden, The Netherlands). After killing the animals by cervical dislocation, the small intestine was immediately flushed with saline (154 mM NaCl). Mucosal cells were isolated by a high-frequency low-amplitude vibration-method (Chemap-E1 Vibromixer, 100 Hz, amplitude 2 mm) [9]. After 2 minutes vibration in saline in order to remove excessive mucus, mucosal cells were isolated by 40 minutes vibration in chelating medium (9 mM KH_2PO_4, 34 mM $Na_2HPO_4 \cdot 2H_2O$, 5 mM EDTA-Na_2, 90 mM NaCl, pH 7.4). After washing twice with 154 mM NaCl the cells were suspended (cells of 0.5 g intestine/ml) in phosphate buffer (9 mM KH_2PO_4, 34 mM $Na_2HPO_4 \cdot 2H_2O$, 0.1 mM EDTA-Na_2, pH 7.4) and homogenized using an Ultra-Turrax (Janke-Kunkel, 1100 rpm, 4 bursts of 10 seconds). Intestinal microsomes were prepared by differential centrifugation (20 min at 9000 g, 60 min at 100.000 g), resuspended in phosphate buffer and stored at -80°C until use. Livers were immersed

abbreviations used: 7-HC = 7-hydroxycoumarin, UDPGA = UDP-glucuronic acid, UDPGT = UDP-glucuronyltransferase (E.C.2.4.1.17), UDPNAG = UDP-N-acetylglucosamine.

in phosphate-buffer immediately after removal from the animal and homogenized using a glass-Teflon Potter-Elvehjem-homogenizer (1800 rpm, 4 up-and-down strokes). Hepatic microsomes were centrifuged and stored as described for intestinal microsomes. All preparations were carried out at 0-5°C. Incubations were done at 37°C in a shaking waterbath in phosphate buffer (final volume 1 ml) with 0.10-0.15 mg protein. Cofactors, activators and microsomes were preincubated for 10 minutes, after which the reaction was started by adding 7-HC (Fluka; final concentration 20-200 µM) in Polysorbate-80 (final concentration 0.002%). The reaction was stopped after 10 minutes with 125 µl 20% trichloric acetic acid and the remaining 7-HC was extracted and quantitated fluorimetrically [10]. Reactions were linear for at least 15 minutes and incubation blanks (no UDPGA added) showed no reaction. In preliminary experiments it was established that all disappeared 7-HC could be recovered by treatment with β-glucuronidase. Protein was determined [11] using crystalline bovine serum albumine (Poviet) as a standard. UDPGA and UDPNAG (sodium salt) were obtained from Sigma. All other chemicals were of analytical grade purity and used as supplied.

RESULTS AND DISCUSSION

The activation-characteristics of the rat intestinal and hepatic microsomal UDPGT were investigated with three activators which are considered to exert their effects by different mechanisms. Divalent cations are usually considered to function as a cofactor of the UDPGT [12] but more recent evidence indicates that these ions may act by removing of end product-inhibition [13] or by directly affecting the microsomal membrane [14]. Triton-X100 has a general membrane perturbing effect [14, 15]. UDPNAG is generally considered to be an allosteric

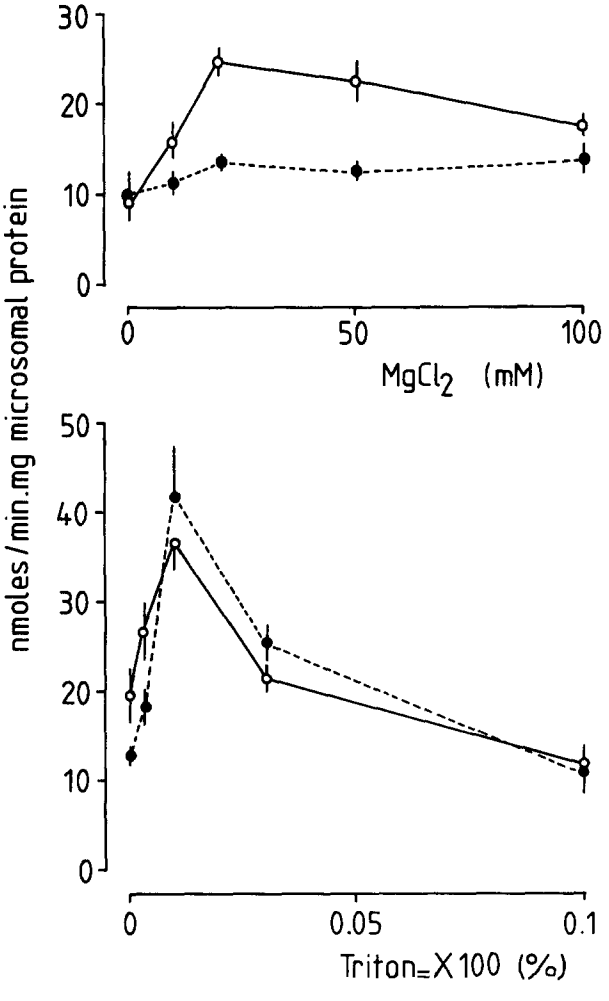


Figure 1.
Influence of $MgCl_2$ and Triton-X100 on the glucuronidation-rate of 7-HC in rat intestinal (o) and hepatic (•) microsomes. Incubations were carried out in the presence of 20 mM $MgCl_2$ (in the Triton-X100 experiment), 3 mM UDPGA and 200 µM 7-HC. Data points represent the means (± S.E.M.) of duplicate determinations with three batches of microsomes, each prepared from four (intestine) or two (liver) rats.

Table 1. Influence of UDPNAG on the glucuronidation-rate of 7-HC. Incubations were carried out in the presence of 20 mM $MgCl_2$ and 3 mM UDPGA. Further conditions as described in the caption to Fig. 1.

7-HC-concentration	addition	intestinal microsomes	hepatic microsomes
40 µM	none	6.4 ± 0.5	2.3 ± 0.7
	3 mM UDPNAG	9.0 ± 0.5†	8.4 ± 0.8†
200 µM	none	21.6 ± 2.7	17.3 ± 2.1
	3 mM UDPNAG	22.4 ± 3.9	20.7 ± 3.4

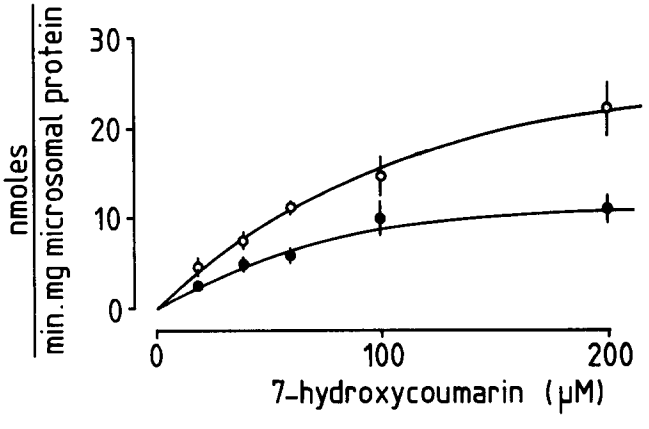
units are nmoles/min·mg microsomal protein

†significantly different from control ($p < 0.001$, Student's paired-sample test).

modifier of the UDPGT [16], but other mechanisms have been put forward recently [17]. The results of our experiments with $MgCl_2$, Triton-X100 and UDPNAG are given in Fig. 1 and Table 1. Experiments with Triton-X100 and UDPNAG were carried out in the presence of $MgCl_2$ because activation by these agents is dependent on the presence of divalent cations [12, 15, 17]. From the results given it can be concluded that both the intestinal and the hepatic enzyme can be activated by Triton-X100 and UDPNAG. These findings are in contrast with the results given by Josting et al. [7]. However, these authors used a saturating concentration of 1-naphthol, which may explain why they failed to see stimulation by UDPNAG (cf. Table 1). Furthermore, they did not use a range of Triton-X100 concentration, which is essential to recognize the rather narrow activation-optimum (Fig. 1 and [14]). Finally, Josting et al. isolated intestinal cells by scraping off the mucosa, which can cause contamination of microsomal preparations or may liberate more activating substances from the intestinal wall than does our procedure [8, 9]. Activation of UDPGT by $MgCl_2$ appears to be only significant in intestinal microsomes (Fig. 1). In view of the relatively high optimum $MgCl_2$ concentration, a non-specific membrane-effect seems to be the more likely explanation for this finding. On the basis of our results no discrimination can be made between different activation-mechanisms of UDPNAG.

Pseudonormal estimates of the kinetic parameters for UDPGA and 7-HC were determined with the aid of a non-linear least-squares fitting programme [18] (using the S.D.-supplied and bisquare- weighting options), followed by jackknifing the results [19]. Apparent K_m values (± S.E.M.) for UDPGA were 726 ± 86 µM and 624 ± 96 µM for the intestinal and hepatic enzyme

Figure 2.
Glucuronidation-rate in rat intestinal (o) and hepatic (•) microsomes as a function of 7-HC-concentration, determined in the presence of 20 mM $MgCl_2$ and 3 mM UDPGA. Further conditions as described in the caption to Fig. 1.



respectively. Apparent K_m and V_{max} values, obtained for 7-HC (K_m = 143 ± 23 μM and V_{max} = 38.3 ± 3.6 nmoles/min·mg microsomal protein for intestinal UDPGT; K_m = 67 ± 13 μM and V_{max} = 14.2 ± 1.4 nmoles/min·mg microsomal protein for hepatic UDPGT; Fig. 2) are comparable with values obtained for the glucuronidation of 1-naphthol [20].

Summarizing, it can be concluded that the rat intestinal microsomal UDPGT, prepared by the presently described procedure, can be activated *in vitro* by Triton-X100, UDPNAG and $MgCl_2$. This result suggests that the intestinal microsomal UDPGT is qualitatively similar to the hepatic enzyme. Further experiments are necessary to describe quantitatively the relation between the glucuronidation-capacity of the intestinal wall *in vivo* and the glucuronidation-capacity of microsomes, prepared from intestinal mucosal cells.

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