20. K. W. Bock and I. N. H. White, *Eur. J. Biochem.* **46**, 451 (1974).
21. G. J. Mulder, E. Scholtens and D. K. F. Meijer, in *Methods in Enzymology*, (Ed. W. B. Jakoby), Vol. 77, p. 21, Academic Press, New York (1981).
22. G. J. Mulder and A. Hagedoorn, *Biochem. Pharmac.* **23**, 2101 (1974).
23. J. Jorritsma, J. H. N. Meerman, R. J. Vonk and G. J. Mulder, *Xenobiotica* **9**, 247 (1979).
24. H. Koster, I. Halsema, E. Scholtens, M. Knippers and G. J. Mulder, *Biochem. Pharmac.* **30**, 2569 (1981).
25. K. R. Krijgsheld, H. J. Koster, E. Scholtens and G. J. Mulder, submitted for publication.
26. G. J. Mulder, (Ed.) *Sulfation of Drugs and Related Compounds*. CRC Press, Boca Raton, FL (1981).

Biochemical Pharmacology, Vol. 31, No. 10, pp. 1944–1946, 1982.
Printed in Great Britain.

0006-2952/82/101944-03 $03.00/0

The effect of activators of glucuronyltransferase in the streptozotocin-induced diabetic rat

(*Received* 1 *October* 1981; *accepted* 11 *January* 1982)

The activity of UDP-glucuronyltransferase has been shown to be decreased in the streptozotocin-induced diabetic male rat, although no change was observed in female rats [1]. Using hepatic "native" microsomes from streptozotocin-induced diabetic male rats glucuronidation of *p*-nitrophenol proceeded at only half the rate observed in "native" microsomes from control rats. Furthermore, treatment with insulin abolished the effect of streptozotocin, but had no significant effect on hepatic glucuronyltransferase activity in control rats. This communication discusses the mechanisms by which these changes may occur, using the results from "native" and activated microsomal preparations.

Materials and methods

Male Sprague–Dawley rats (180–220 g) were injected i.v. with 60 mg/kg streptozotocin in acetate buffer, pH 4.5. The rats were used five days after the injection of streptozotocin (blood glucose 380 ± 26 mg/100 ml) or of acetate buffer (blood glucose 99 ± 3 mg/100 ml). Hepatic microsomes were prepared by a modification of the conventional ultracentrifugation method [2] involving the addition of 15% glycerol to the buffer in which the liver is homogenised [3]. Glucuronidation of *p*-nitrophenol was measured by the disappearance of substrate using the method of Chhabra and Fouts [4]. This was validated by direct measurement of *p*-nitrophenolglucuronide by high pressure liquid chromatography [5].

The microsomal incubations, which contained 500 nmole *p*-nitrophenol, 125 nmole saccharo-1,4-lactone, 5 μmole UDP-glucuronic acid and 0.2 ml microsomal suspension (equivalent to 3–5 mg protein), were made up to a final volume of 2.5 ml with 0.1 M Tris buffer, pH 7.4. For activation the microsomes were either preincubated with Triton X-100 for 10 min at room temperature or UDP *N*-acetylglucosamine was added directly to the enzyme incubation in the presence of 1 mM $MgCl_2$. The effect of 1 mM $MgCl_2$ alone on glucuronyltransferase activity was also measured. The concentrations of Triton X-100 and UDP *N*-acetylglucosamine used are as indicated in the figures. All reactions, which were found to be linear with respect to time up to 30 min, were carried out for 15 min at 37° unless otherwise stated. Michaelis–Menten constants for the enzyme kinetics were calculated by non-linear regression analysis using BMDP/PAR [6].

Results and discussion

Maximal activation of glucuronyltransferase by Triton X-100 occurred at a concentration of 0.25% (v/v) Triton X-100 in the incubation mixture whereas for UDP *N*-acetylglucosamine activation was maximal at a 4 mM concentration, [Figs. 1(a) and 1(b)]. The activity of "native"

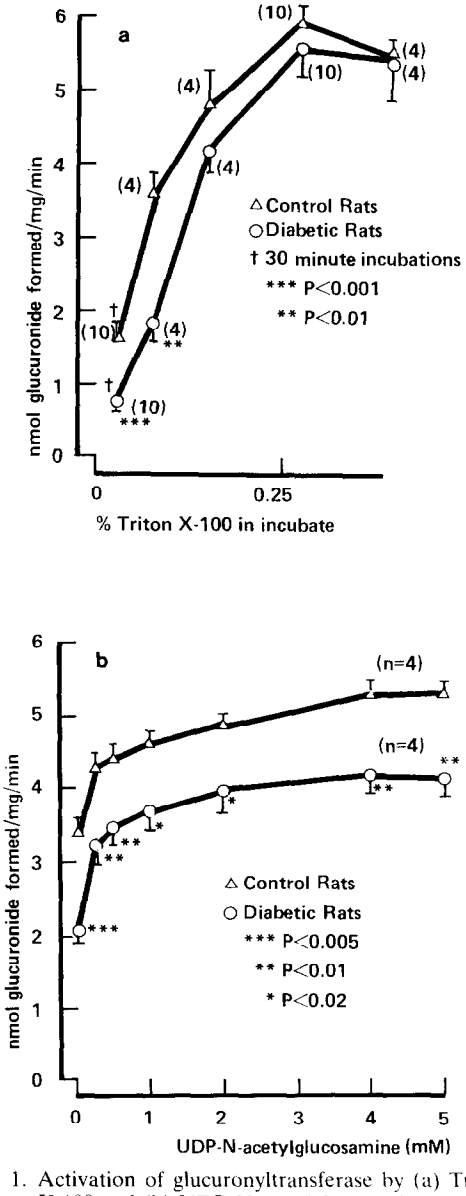

Fig. 1. Activation of glucuronyltransferase by (a) Triton X-100 and (b) UDP *N*-acetylglucosamine.

Table 1. Maximal effect of activators on UDP-glucuronyltransferase activity

	Control (nmole glucuronide formed/mg/min)	Diabetic (nmole glucuronide formed/mg/min)
"Native" enzyme activity	1.45 ± 0.12‡ (10)	0.82 ± 0.11†‡ (10)
"Native" enzyme activity + Mg^{2+}	3.32 ± 0.20 (4)	2.03 ± 0.14† (4)
Triton X-100 activation (0.25%, v/v)	5.98 ± 0.22 (10)	5.57 ± 0.34 (10)
UDP *N*-acetylglucosamine activation (4 mM)	5.27 ± 0.14 (4)	4.14 ± 0.25* (4)

All values are given as mean ± S.E. with the number of determinations in parentheses.
* $P < 0.01$, † $P < 0.005$. Significance values refer to the difference between control and diabetic animals using a non-paired *t*-test.
‡ 30 min incubation.

glucuronyltransferase was greater in control rats than in diabetic rats ($P < 0.005$). After maximal activation with Triton X-100 there was no significant difference in enzyme activity between control and diabetic rats, whereas with maximal activation by UDP *N*-acetylglucosamine enzyme activity was still significantly higher in control rats ($P < 0.01$). Table 1 shows the maximum enzyme activity attained with these activators in control and diabetic rats. The inclusion of 1 mM Mg^{2+} in the incubations in the absence of UDP *N*-acetylglucosamine increased enzyme activity 2–3 fold in both control and diabetic rats, but did not abolish the difference between the two groups of rats.

One millimolar $MgCl_2$ was included in some incubations since it has been shown that activation of *p*-nitrophenol glucuronidation by UDP *N*-acetylglucosamine is dependent on the presence of divalent metal ions in the incubation. From the work of Zakim *et al.* [7] it has been shown that metal ions modify glucuronyl transferase activity as a result of direct binding to the enzyme. Streptozotocin treatment does not appear to alter the effect of Mg^{2+} on glucuronyltransferase.

Activation by either Triton X-100 (0.25%, v/v) or UDP *N*-acetylglucosamine (4 mM) decreased the K_m for UDP-glucuronic acid (UDPGA) and when both activators were used there was an additive effect as shown in Table 2. A wide range of kinetic parameters has been reported in the literature for the affinity of UDPGA using *p*-nitrophenol as substrate [8]. Vessey and Zakim [9, 10] found that the affinity was increased after activation by Triton X-100 or UDP *N*-acetylglucosamine, whereas Winsnes [8] found decreased affinity. In the latter case the parameters were determined from biphasic Lineweaver–Burk plots, showing atypical Michaelis–Menten kinetics.

The K_m values for UDPGA, with *p*-nitrophenol as substrate, are similar for both activators in control and diabetic rats, but V_{max} is increased 7–8 fold by Triton X-100 activation compared with only 2–3 fold after UDP *N*-acetylglucosamine activation. These preliminary findings show that there is little difference between the V_{max} in control and diabetic animals after Triton X-100 activation, in contrast to the V_{max} values obtained after UDP *N*-acetylglucosamine activation, suggesting that the decreased glucuronidation is related to the extent of membrane constraint in diabetic animals. The K_m for UDP *N*-acetylglucosamine binding was similar in control and diabetic animals (0.070 ± 0.008 mM compared with 0.067 ± 0.004 mM in diabetic rats; $n = 4$), but the V_{max} for the reaction was significantly higher in control rats (5.15 ± 0.15 nmole/mg/min compared with 4.09 ± 0.28 nmole/mg/min in diabetic rats; $n = 4$, $P < 0.01$). These results suggest that microsomal preparations of glucuronyltransferase from diabetic rats have unchanged affinity for *N*-acetylglucosamine, but that the enzyme is unable to express maximal activation to this agent.

Kinetic studies on crude microsomal preparations of the transferase enzyme suggest that the mechanisms of its activation by UDP *N*-acetylglucosamine and perturbants such as Triton X-100 are different [11]. According to one view [12] *N*-acetylglucosamine, an endogenous intermediate, is thought to act as an allosteric effector of glucuronyltransferase in the presence of bivalent metal ions by enhancing the binding of UDP-glucuronic acid to the enzyme while reducing the affinity for UDP, so limiting end-product inhibition. Triton X-100, on the other hand, would act on the membrane environment surrounding the enzyme by removing phospholipid constraint [11].

Pretreatment of microsomes with Triton X-100 results in activation of both control and diabetic enzymes to statistically similar values. However, maximal UDP *N*-acetylglucosamine activation does not eliminate the differences in activity between control and diabetic rat enzyme preparations. These observations suggest that the decrease in glucuronidation of *p*-nitrophenol in the streptozotocin-treated male rat may be due to an alteration in the membrane environment of the enzyme rather than to a transferase deficiency.

Table 2. Effect of activators on the Michaelis–Menten constants for UDP-glucuronic acid (UDPGA)

	Control		Diabetic	
	K_m (mM)	V_{max} (nmole glucuronide formed/mg/min)	K_m (mM)	V_{max} (nmole glucuronide formed/mg/min)
"Native" enzyme activity	0.591	1.81	0.599	1.23
Triton X-100 activation (0.25% v/v)	0.380	8.16	0.361	8.00
UDP *N*-acetylglucosamine activation (4 mM)	0.350	4.56	0.370	3.43
Triton X-100 (0.25% v/v) + UDP *N*-acetylglucosamine (4 mM)	0.064	7.75	0.116	7.52

All values are the mean of 2 or 4 determinations.

Previous workers [13, 14] have attributed the decreases observed in glucuronidation of *o*-aminophenol and testosterone by liver homogenates and liver slices from diabetic animals to a deficiency of UDP-glucuronic acid. It is possible that both mechanisms may contribute to the decreased glucuronidation of *p*-nitrophenol observed in the whole liver.*

Acknowledgements—We should like to thank the Drug Synthesis and Chemistry Branch, Division of Cancer Treatment, National Cancer Institute for the gift of streptozotocin.

Department of Pharmacology
University Medical Buildings
Foresterhill
Aberdeen AB9 2ZD, U.K.

M. HELEN MORRISON
GABRIELLE M. HAWKSWORTH†

REFERENCES

1. G. M. Hawksworth and M. H. Morrison, *Br. J. Pharmac.* **72**, 539P (1981).

* M. H. Morrison and G. M. Hawksworth, manuscript in preparation.

† Author to whom correspondence should be addressed.

2. L. C. Erickson, J. W. DePierre and G. Dallner, *Pharmac. Ther. A.* **2**, 281 (1978).
3. Y. Ichikawa and T. Yamano, *Biochim. biophys. Acta.* **131**, 490, (1967).
4. R. S. Chhabra and J. R. Fouts, *Drug Metab. Dispos.* **2**, 375 (1974).
5. A. Karakaya and D. E. Carter, *J. Chromatog.* **195**, 431 (1980).
6. M. Ralston, in *BMDP Biomedical Computer Programs* (Eds W. J. Dixon and M. B. Brown), p. 484. University of California Press, Berkley (1979).
7. D. Zakim, J. Goldenberg and D. A. Vessey, *Biochemistry* **12**, 4068 (1973).
8. A. Winsnes, *Biochim. biophys. Acta.* **284**, 394 (1971).
9. D. A. Vessey and D. Zakim, *J. biol. Chem.* **246**, 4649 (1971).
10. D. A. Vessey, J. Goldenberg and D. Zakim, *Biochim. biophys. Acta.* **309**, 58 (1973).
11. J. G. Dutton and B. Burchell, *Prog. Drug Metab.* **2**, 1 (1977).
12. D. Zakim and D. A. Vessey, *Biochem. Soc. Trans.* **2**, 1165 (1974).
13. B. Müller-Oerlinghausen, A. Hasselblatt and R. Jahns, *Life Sci.* **6**, 1529 (1968).
14. H. Schriefers, R. Ghraf and F. Pohl, *Hoppe-Seyler's Z. Physiol. Chem.* **344**, 25 (1966).

Biochemical Pharmacology, Vol. 31, No. 10, pp. 1946–1948, 1982
Printed in Great Britain.

0006-2952/82/101946-03 $03.00/0

1,4-Dithiothreitol non-specifically potentiates spasmogen actions on the guinea-pig ileum

(*Received* 1 *December* 1981; *accepted* 13 *January* 1982)

1,4-Dithiothreitol (DTT)* is commonly used to prevent the oxidation of biologically active materials (e.g. [1]) and to reduce disulphide bonds to sulfhydryl groups (e.g. [2]). This latter action has received application in the cleavage of a disulphide bond located on the nicotinic cholinoceptor at muscle end-plates, enabling the development of active site directed affinity labels [2]. This cleavage results in a decrease in the potency of acetylcholine, which on the frog rectus abdominus muscle is manifested as a 4–5 fold parallel shift to the right in the dose–response curve [3]. The disulphide bond can be reformed through treatment with dithio bis(2-nitrobenzoic acid) (DTNB) [2].

The action of DTT on other receptor systems has also received attention. Thus, it has been reported that treatment of the guinea-pig trachea with DTT increased the sensitivity of the tissue to all agonists tested, while in contrast, DTT treatment of the rabbit aorta produced a selective increase in sensitivity to histamine [4]. Similarly, Glover [5] observed a 4–6 fold increase in sensitivity to histamine in guinea-pig ileum and rabbit colon following treatment with DTT, but found no change in the potency of acetylcholine. Jordan and Owen [6] recently reported that DTT increased the depolarising potency of substance P on frog spinal cord *in vitro*. However, it is unclear whether these observations represent an action of DTT on the receptor recognition site analagous to that at the nicotinic receptor. Therefore, the present study was undertaken to characterise further the action of DTT, using the guinea-pig ileum as the model.

Male or female guinea-pigs (250–400 g) were stunned by a blow to the head and decapitated. The terminal ileum was dissected out and suspended in a 2 ml organ bath containing a modified Krebs bicarbonate solution (127 mM NaCl, 2.5 mM KCl, 1.8 mM $CaCl_2$, 1.2 mM $MgSO_4$, 25 mM $NaHCO_3$, 1.2 mM NaH_2PO_4, and 10 mM glucose), gassed with a 95% O_2/5% CO_2 mixture and maintained at a temperature of 37°. Contractions were recorded isotonically under a resting tension of 800 mg, and a 3 min drug cycle was employed with a 30 sec contact time. Following an initial 30 min equilibration period, the tissue was thrice challenged with a maximally effective dose of acetylcholine (1 mM) and left for a further 15 min. A dose–response curve to the agonist in question was then determined, followed by a 10 min incubation with DTT (1 mM). A second dose–response curve was then determined, and the tissue afterwards treated with DTNB (1 mM) for 30 min. Subsequently, a third dose–response curve was determined. Shifts in the curves were analysed using the EC_{50} value as the point of reference.

Stock solutions of agonists were made up in distilled water and stored at −20°. Dilutions were made on the day of the experiment in Krebs bicarbonate solution, which for the peptides had 0.1% bovine serum albumin added to

* Abbreviations used: DTT, 1,4-dithiothreitol; DTNB, dithio bis(2-nitrobenzoic acid).