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Biochemical Pharmacology, Vol. 40, No. 9, pp. 2180–2183, 1990.
Printed in Great Britain.

0006-2952/90 $3.00 + 0.00

Cytosolic phenol and steroid sulphotransferase activities are decreased in a sex-dependent manner in streptozotocin-induced diabetic rats

(*Received* 3 *July* 1990; *accepted* 30 *July* 1990)

Sulphation is an important pathway of deactivation and elimination of potentially toxic compounds from the body, serving both xenobiotics and endogenous compounds [1, 2]. The reactions are performed by a large family of sulphotransferase (ST*) isoenzymes, subdivided according to the class of substrate metabolized, which transfer the sulphate group from the donor molecular 3′-phosphoadenosine 5′-phosphosulphate to the acceptor substrate (see Ref. 2 for review).

The effects of treatment of animals with the diabetogenic antibiotic streptozotocin (STZ) on the activities of many drug metabolizing enzymes have been extensively studied, and such experimental diabetes is known to induce alterations in the levels of activity of many of these enzyme systems, including the cytochrome P450s, the UDP-glucuronosyltransferases, the sulphotransferases and glutathione-*S*-transferases (e.g. Refs 3–12).

Most investigations of the effects of experimental diabetes on conjugation reactions have focused on the metabolism of xenobiotic substrates for the enzymes. However, the STs are also involved in the metabolism of important and potentially toxic endogenous compounds such as steroid hormones, bile salts and catecholamines [12–16], and indeed this may be seen as their primary function. The disruption, in diabetes, of key biochemical processes as a result of alterations in the metabolism of such endogenous molecules may have important consequences for the individual, and this may be particularly true for the sulphation of steroids, since dehydroepiandrosterone (DHEA), dehydroepiandrosterone sulphate (DHEA-S) and oestrogens have been shown to have potent anti-diabetic effects in experimental and genetically diabetic animals [15, 16]. Therefore, in this work we have investigated the effect of experimental diabetes on the sulphation of xenobiotic and endogenous steroid substrates selective for four different ST isoenzymes in STZ-induced diabetic rats. These enzyme activities exhibit well known sexual dimorphisms, so we have examined the effects of diabetogenesis on the activities in both male and female animals.

* Abbreviations: STZ, streptozotocin; ST, sulphotransferase; DHEA, dehydroepiandrosterone, DHEA-S, dehydroepiandrosterone sulphate; PAPS, 3′-phosphoadenosine 5′-phosphosulphate; Hepes, *N*-2-hydroxyethylpiperazine-*N*′-2-ethanesulphonic acid.

Materials and Methods

1-[1-^{14}C]Naphthol (59 mCi/mmol) and [2,4,6,7-^{3}H]oestrone (107 Ci/mmol) were purchased from Amersham, Aylesbury, U.K. and *p*-[^{3}H(G)hydroxyacetanilide (paracetamol, 1.2 Ci/mmol) and [1,2,6,7-^{3}H]dehydroepiandrosterone (78 Ci/mmol) were from DuPont/NEN (Stevenage, U.K.). BM-Test-Glycemie strips were from Boehringer Mannheim (Lewes, U.K.). Histone 2A, 3′-phosphoadenosine 5′-phosphosulphate, oestrone, paracetamol and dehydroepiandrosterone were purchased from the Sigma Chemical Co. (Poole, U.K.). Glucose-6-phosphate (monosodium salt), 1-naphthol and Scintran Cocktail T (scintillant) were from BDH Ltd (Glasgow, U.K.). All other reagents were of analytical grade and obtained from frequently used local suppliers.

Adult Wistar rats, approximately 12 weeks of age from the colony maintained in the Medical School animal facility, were used throughout, and had access to food and water *ad lib*. Diabetes was induced by a single tail vein injection of streptozotocin (75 mg/kg body wt) in buffered citrate (pH 4.5). Animals were killed 48 hr later, and blood glucose levels determined using BM-Test-Glycemie strips. Control animals received the vehicle only. Liver microsomes and cytosol (from the same livers) were prepared from 25% homogenates in 0.25 M sucrose, 5 mM Hepes, pH 7.4 by differential centrifugation. Briefly, homogenates were centrifuged at 10,000 *g* for 15 min and the resultant supernatant centrifuged at 105,000 *g* for 1 hr. The cytosolic fraction (105,000 *g* supernatant) was harvested avoiding the lipid layer at the surface, and aliquoted and frozen at −70°. The microsomal pellets were resuspended in sucrose/Hepes buffer to a protein concentration of approximately 20 mg/mL, aliquoted and stored frozen at −70°. All samples were used within 2 months of preparation, and microsome samples were frozen and thawed only once before assay in order to maintain the intactness of the microsomal membrane.

Glucose-6-phosphatase activities were assayed and calculated according to the method of Burchell *et al.* [17] following disruption of microsomes by histone 2A, and are expressed as μmol P_i released/min/mg microsomal protein. ST activity was determined with 1-naphthol [18], paracetamol [19], dehydroepiandrosterone [20] and oestrone as substrates. Assay conditions were as follows: 1-naphthol—pH 6.6, 8 μM 1-naphthol, 13 μM PAPS; paracet-

amol—pH 9.0, 500 μM paracetamol, 320 μM PAPS; DHEA—pH 6.5, 75 μM DHEA, 200 μM PAPS. Oestrone ST was assayed radiometrically, as follows: reaction mixtures (100 μL final volume) contained 500 μg total cytosolic protein, 5 μM [^{3}H]oestrone (100,000 cpm) and 20 mM potassium phosphate buffer, pH 5.0. Reactions were initiated by the addition of PAPS (final concentration 200 μM) and, after 20 min incubation at 37°, were terminated by adding 2 mL water-saturated dichloroethane. Water (300 μL) was added, and the tubes shaken for 2 min, followed by centrifugation for 2 min at 3,000 rpm to separate the phases. The aqueous phase (200 μL) was subjected to liquid scintillation counting to determine the amount of oestrone sulphate produced. Blank incubations contained no PAPS. All ST enzyme assays were optimized for pH, substrate concentration, PAPS concentration, incubation time and protein concentration. Statistical analysis was performed using Lord's range test [21] at a significance level of $P = 0.05$.

Protein content was estimated by the method of Lowry *et al.* [22], on samples which had been frozen and thawed only once.

Results and Discussion

Blood glucose levels were measured on the STZ treated rats, and all were found to have concentrations >17 mM. In order to confirm that the rats treated with STZ were diabetic, the effect of the treatment on glucose-6-phosphatase activity was determined in fully disrupted liver microsomes (Table 1), as it has been demonstrated previously that there is a significant increase in this enzyme activity in diabetic rats [23]. Glucose-6-phosphatase activity was increased approximately four-fold in the diabetic animals, with no significant difference in the effects on males and females. This agrees well with previously reported data [23] and confirms that the STZ-treated animals are diabetic. To determine whether this stimulation of enzyme activity was the result of the presence of increased enzyme protein in the diabetic animals, we performed immunoblot analysis of liver microsomes from control and diabetic rats with an antibody raised against the catalytic component of purified rat hepatic microsomal glucose-6-phosphatase, and found that induction of diabetes with STZ resulted in a substantial increase in the immunoreactive glucose-6-phosphatase protein in the diabetic animals, corresponding to the increase in enzyme activity (not shown).

Table 1. Glucose-6-phosphatase activities in disrupted liver microsomes preapred from control and STZ-treated rats

Treatment group	V_{max} (μmol/min/mg)
Control male	0.18 ± 0.02
Diabetic male	0.73 ± 0.06*
Control female	0.19 ± 0.02
Diabetic female	0.68 ± 0.17*

Data are the mean ± SD derived from preparations of liver microsomes from three different animals.

* Indicates significantly different from control animals at the significance level $P = 0.05$.

The sulphoconjugation of substrates respresentative of four different ST sub-families [2, 24] was determined in cytosol fractions prepared from male and female rats with STZ-induced diabetes and from control animals (Table 2). Activities towards all four substrates, 1-naphthol, paracetamol, DHEA and oestrone were reduced, to varying extents, in diabetic male liver, by 27%, 12%, 68% and 85%, respectively. Conversely, in female liver cytosol only the sulphation of DHEA and oestrone was reduced significantly (by 35% and 52%, respectively) in the diabetic rats. These data demonstrate that there are isoenzyme-dependent, sex-specific effects on sulphotransferases in the diabetic animals, with the sulphation of DHEA and oestrone being significantly more susceptible than the sulphation of the xenobiotic substrates. When immunoblot analysis of cytosol from control and diabetic rat livers was performed using an antibody raised against purified rat liver paracetamol ST [24], no major differences in the immunoreactive protein between the control and diabetic animals was observed (data not shown), a result which is consistent with the small (12%) decrease in the ST activity towards paracetamol in the diabetic male rats (Table 2).

Compared to the effects of STZ-induced diabetes on steroid sulphation, the decreases in ST activity towards the xenobiotic substrates 1-naphthol and paracetamol were fairly insignificant. However, the fact that we have found decreases in these activities in cytosol contradicts data from experiments performed on isolated hepatocytes [10] and *in vivo* [13], where the diabetic state has been shown to moderately increase the sulphation of substrates for the phenol STs. This could be due to the increased availability of PAPS resulting from the diabetes, as suggested by Price and Jollow [13]. Another explanation could be that substrates which are also glucuronidated may, as a result of the decrease in glucuronidation, be more extensively sulphated in the diabetic animal, even though the actual ST activity was decreased. Overall, however, these findings suggest that effects of diabetes on the sulphation of xenobiotics *in vivo* are likely to be negligible.

The dramatic decrease in the ability of the diabetic animals, males in particular, to sulphate important sex steroids such as DHEA and oestrone has far-reaching implications, however. The 68% and 85% decreases observed in the sulphation of DHEA and oestrone in diabetic male rats, respectively, and the similar but lower decreases in

Table 2. Sulphotransferase activities in fed and diabetic male and female rat liver cytosols

Substrate	Fed male	Diabetic male	Fed female	Diabetic female
		(pmol/min/mg)		
1-Naphthol	1628 ± 85	1183 ± 34*	641 ± 42	612 ± 35
Paracetamol	340 ± 14	299 ± 12*	214 ± 10	205 ± 10
DHEA	17.7 ± 1.1	5.7 ± 1.0*	176 ± 15	115 ± 7*
Oestrone	17.5 ± 1.1	2.7 ± 2.5*	3.9 ± 0.6	1.9 ± 0.2*

Data represent the mean ± SD of determinations on three different cytosol samples for each treatment group.

* Indicates significantly different from control animals at the significance level $P = 0.05$.

females, are likely to result in significantly increased levels of circulating, receptor active steroids (the sulphated forms are not receptor active) in the diabetic animals. This may have beneficial effects: for example, it has been reported that ovarian oestrogens have an important role in the maintenance of B-cell function [25], and therefore may have an anti-diabetic role. Similarly, DHEA has been shown to have potent anti-diabetic effects when administered orally [14], although it is postulated that this is the result of the metabolism of DHEA to oestrogens (oestrone, β-oestradiol) and etiocholanolones [16, 26] which are the active anti-diabetic agents. Interestingly, DHEA-S also has potent anti-diabetic effects [27], although it is believed that these effects are mediated through conversion of DHEA sulphate to oestrogens, in particular oestrone, as DHEA-S is more directly convertible to oestrone and oestrone sulphate than free, unconjugated DHEA [28]. The marked sex specificity of these decreases in steroid sulphation indicates that the regulation of the expression of the genes encoding DHEA and oestrone STs in diabetes is also under the control of sex hormones. Female diabetic rats appeared more refractory to the detrimental effect of STZ-induced diabetes on the sulphation of these steroids, although the sexual dimorphism in normal rats was not the same for DHEA ST (female specific) and oestrone ST (male specific). For the sulphation of the xenobiotic substrates 1-naphthol and paracetamol, the sex-dependence of the decrease in male diabetic rats was absolute, with STZ treatment having no effect on these enzyme activities. Kirkpatrick and Kraft [12] reported that STZ treatment resulted in an increase in bile acid ST activity in male rats. However, this increase, while large, was only observed over long periods (6–29 days post treatment). Obviously this conflicts with our findings, but this may be the result of the route of administration of the STZ (i.p. in Ref. 12, i.v. in our study). The data presented here show marked changes in ST activity within 48 h post-treatment, and it is possible that the effects reported by Kirkpatrick and Kraft [12] are the result of a different (long term) effect of the hyperglyceamic state. Further investigation of mechanism of these effects would require the use of molecular probes for the ST isoenzymes involved.

In conclusion, we have demonstrated marked decreases in the ability of diabetic rats to sulphate xenobiotic and, particularly, endobiotic substrates for four separate hepatic sulphotransferase isoenzymes, the effects being much more pronounced in male rats than in females. These data illustrate not only the high degree of complexity governing the response of an organism to the diabetic state, but also the multitude of factors governing the regulation of sulphotransferase gene expression in normal and diabetic animals.

Acknowledgements—This work was supported by grants from the Sir Jules Thorn Charitable Trust (to M.W.H.C.) and the Scottish Hospitals Endowment Research Trust (to A.B.). A.B. is a Lister Institute Research Fellow, and J.P. is a Novo Diabetic Clinical Research Fellow.

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