Biochemical Pharmacology, Vol. 39, No. 7, pp. 1193–1197, 1990.
Printed in Great Britain.

0006-2952/90 $3.00 + 0.00

THE EFFECT OF STREPTOZOTOCIN-INDUCED DIABETES ON THE *IN VIVO* ACETYLATION CAPACITY AND THE *IN VITRO* BLOOD *N*-ACETYLTRANSFERASE ACTIVITY OF THE ADULT MALE SPRAGUE–DAWLEY RAT

R. M. LINDSAY* and J. D. BATY
Department of Biochemical Medicine (University of Dundee), Ninewells Hospital and Medical School, Dundee DD1 9SY, U.K.

(*Received* 24 *July* 1989; *accepted* 2 *November* 1989)

Abstract—Induction of experimental diabetes using streptozotocin significantly reduced the extent of sulphamethazine acetylation by Sprague–Dawley rats. This treatment did not significantly change the total amount of sulphonomide excreted in the urine. The *in vitro* blood *N*-acetyltransferase activity of rats treated with streptozotocin was significantly higher than that of untreated animals. Increasing the *in vitro* glucose concentration of blood samples from both groups significantly increased the amount of acetylsulphamethazine produced.

The major metabolic route for drugs containing arylamine or hydrazino groups is acetylation, a reaction catalysed by the enzyme *N*-acetyltransferase (EC 2.3.1.5) and which requires acetyl-CoA as the acetyl group donor. The acetylation of certain compounds, including the antibacterial agent sulphamethazine, is subject to a genetic polymorphism, enabling classification of individuals as either rapid or slow acetylators. This feature may be clinically important with respect to both drug therapy and susceptibility to certain disorders [1].

The relationship between acetylator phenotype and diabetes remains controversial. The increased incidence of rapid acetylators amongst both Type 1 [2, 3] and Type 2 [4] diabetic patients has been disputed [5, 6]. A recent comprehensive review does suggest that the proportion of rapid acetylators in diabetic patients is significantly higher than that in a control population [7].

An alternative explanation for the increased incidence of rapid acetylators amongst diabetics is that the higher blood glucose levels of diabetics could result in increased levels of acetyl-CoA, the acetyl group donor for the reaction. This could result in misclassification of certain individuals and the change in phenotype distribution may therefore be a biochemical artefact. The increased acetylation of sulphamethazine by both Type 1 and Type 2 diabetic subjects *in vivo* irrespective of acetylator phenotype [2], and the decrease in isoniazid half-life in healthy volunteers given a glucose load [8] support this hypothesis. We have previously demonstrated that blood samples obtained from diabetic subjects have higher *in vitro* *N*-acetyltransferase activities than those from healthy volunteers and that the addition of glucose increases the acetylation capacity of samples from both groups [9]. Recent work from our laboratory involving the addition of acetyl-CoA to human blood samples donated from diabetic and non-diabetic volunteers suggests that diabetic subjects have higher blood levels of this cofactor or one of its precursors [10]. These *in vitro* results provide support for the hypothesis that the increased *in vivo* acetylation of sulphamethazine by diabetic subjects is due to their higher blood acetyl-CoA levels.

In this study, we have investigated the effect of streptozotocin-induced diabetes on the *in vivo* acetylation capacity of the rat. The effect of diabetogenesis on the *in vitro* blood *N*-acetyltransferase activity of the rat has also been studied to determine if the activities in diabetic and non-diabetic animals display a pattern similar to that observed with human blood samples.

MATERIALS AND METHODS

Materials. Streptozotocin, sodium sulphamethazine and sulphapyridine were supplied by the Sigma Chemical Co. (Poole, U.K.). Sulphamethazine and acetic anhydride were obtained from the Aldrich Chemical Co. (Gillingham, U.K.). D-Glucose, potassium dihydrogen phosphate and disodium hydrogen phosphate were obtained from the BDH Chemical Co. Ltd (Poole, U.K.). HPLC grade acetonitrile and ethyl acetate were supplied by Rathburn Chemicals (Peebleshire, U.K.) and May & Baker (Dagenham, U.K.), respectively. All other reagents were of the highest grade commercially available. Acetylsulphamethazine was synthesized from sulphamethazine by heating with acetic anhydride and was recrystallized from aqueous ethanol (m.p. 254–255°). The structure of the product was confirmed by mass spectrometry and its purity was 100% as judged by HPLC analysis.

Animals. Adult male Sprague–Dawley rats were purchased from Charles River Ltd (Margate, U.K.). These animals (approx. 425–575 g) were maintained at 20° on 12 hr light/dark cycles in individual cages

* To whom correspondence should be addressed at the Metabolic Unit, University Department of Medicine, Western General Hospital, Edinburgh EH4 2XU, U.K.

and were fed SDS rat and mouse No. 1 expanded feed (Special Diet Services Ltd., Witham, U.K.) and water *ad lib*.

Administration of streptozotocin. Streptozotocin was used to induce experimental diabetes in adult male Sprague–Dawley rats after anaesthetizing the animals with diethyl ether. A single intravenous injection of streptozotocin (50 mg/kg; 0.2 mL/kg body wt) in citrate buffered (pH 4.5) isotonic saline was made into the tail.

In vivo *studies: experimental design*. The object of this study was to investigate the effect of chemically induced diabetes on the *in vivo* acetylation of sulphamethazine by the rat. The initial experiment (Trial 1) involved oral administration of sulphamethazine to rats as described below. This experiment was then repeated 1 week later (Trial 2) to determine the extent of intra-individual variability in acetylation capacity. After a further week, streptozotocin was administered and one week later the animals were again orally dosed with sulphamethazine (Trial 3). Urine samples collected from the animals shortly before dosing with sulphamethazine indicated that the time period between each acetylation study was sufficient to ensure that the parent compound and its metabolite had been completely eliminated from the previous trial.

Administration of sulphamethazine. The sodium salt of sulphamethazine was used in these studies because of its increased solubility in aqueous solution relative to the free compound. Sodium sulphamethazine (dissolved in isotonic saline) was administered orally (via oral-gastric tube) at a dose of 40 mg/kg and in an amount of 1.0 mL/kg body weight. The rats (N = 10) had previously been fasted for approximately 12 hr and immediately after dosing they were transferred to metabolic cages and starved for a further 2 hr. The animals had free access to water throughout the experiment. Urine samples were collected between the following time intervals; 0–4 hr, 4–8 hr, 8–12 hr, 12–24 hr, 24–32 hr and 32–48 hr post administration. The volumes of these samples were measured and they were then frozen prior to analysis.

HPLC analysis. Urine samples were centrifuged at 400 *g* for 10 min to deposit any particulate matter. Samples from non-diuretic rats were diluted 10-fold with 33.3 mmol/L phosphate buffer (pH 7.4) before reverse phase HPLC analysis. Samples from diuretic rats were analysed directly without dilution. Standard solutions of sulphamethazine and acetylsulphamethazine covering the range 0 to 0.750 mmol/L were prepared in 33.3 mmol/L phosphate buffer (pH 7.4) and analysed by HPLC. The HPLC system used to determine sulphamethazine and the acetylated metabolite was as previously reported [11].

Urine analysis. Labstix reagent strips (Ames Division, Miles Laboratories Ltd, Slough, U.K.) were used to measure the pH of urine samples and to test for the presence of glucose, ketones and protein.

In vitro *blood* N-*acetyltransferase assay*. Blood samples were collected from rats (non-fasting) after stunning and cervical dislocation. These samples were collected into heparinized syringes and were dispensed into potassium–EDTA anticoagulant containers. Rats were classified as diabetic if the non-fasting plasma glucose was above 15 mmol/L and urinary glucose was 55 mmol/L or above. These animals were killed 7 days after administration of streptozotocin. The acetylation capacity of blood samples from non-diabetic (N = 8) and diabetic (N = 8) rats was determined by adding 0.2 mL of whole blood to 0.2 mL of 33.3 mmol/L phosphate buffer (pH 7.4) containing sulphamethazine (72 nmol) and D-glucose (0 or 20 μmol). The samples were incubated at 37° for 24 hr then extracted with ethyl acetate. The extracts were analysed by HPLC using sulphapyridine as internal standard as previously described [11].

Glucose analysis. A Beckman glucose analyser 2 (Beckman-RIIC, High Wycombe, U.K.) employing a glucose oxidase method was used to determine plasma glucose concentrations.

RESULTS

The effect of streptozotocin on the in vivo *acetylation capacity of the rat*

The mean (±SE) weights of the rats at Trials 1, 2 and 3 were 497 (±13) g, 503 (±14) g and 470 (±14) g, respectively. Administration of streptozotocin therefore caused significant ($P < 0.001$, paired *t*-test) weight loss. Urinary glucose levels before streptozotocin dosing did not exceed trace levels (<5.5 mmol/L). In contrast, streptozotocin treated rats exhibited significant glycosuria (urine glucose levels = 55–≥111 mmol/L). The mean (±SE) urine pH at Trials 1, 2 and 3 were 7.3 (±0.2), 7.2 (±0.2) and 7.4 (±0.3), respectively. There was no significant change in urine pH between the three trials ($P > 0.1$, paired *t*-test). Streptozotocin administration had no significant effect on the levels of protein and ketones excreted in the urine (levels detected never exceeded trace). Nine of the treated rats also became diuretic and showed a marked increase in water intake after injection of the drug. The plasma glucose concentrations (Non fasting) of streptozotocin treated rats (mean = 22.5 mmol/L, range = 16.6 to 32.3 mmol/L) were significantly higher ($P < 0.005$, one-tailed Student's *t*-test) than those of untreated animals (mean = 6.6 mmol/L, range = 4.5 to 7.8 mmol/L).

The percentage of sulphamethazine acetylated *in vivo* by 10 adult male Sprague–Dawley rats before and after administration of streptozotocin is shown in Table I. Intra-individual variation of the *in vivo* acetylation of sulphamethazine was insignificant ($P > 0.05$, paired *t*-test) and the coefficient of variation of the values obtained on Trials 1 and 2 was 7.0%. Induction of experimental diabetes reduced the extent of sulphamethazine acetylation significantly ($P < 0.001$, paired *t*-test).

The mean (±SE) amounts of total sulphamethazine (free and acetylated forms) recovered in urine samples during Trials 1, 2 and 3 expressed as a percentage of the original dose were 13.7 (±2.2)%, 15.3 (±2.6)% and 13.3 (±1.7)%, respectively. There was no significant difference ($P > 0.05$, paired *t*-test) between these values.

Table 1. The effect of streptozotocin on the *in vivo* acetylation of sulphamethazine by adult male Sprague–Dawley rats

Time interval of urine collection (hr)	Percentage acetylation of SMZ		
	Trial 1	Trial 2	Trial 3
0–4	47.6 ± 2.4	48.6 ± 2.7	43.0 ± 3.4
4–8	58.7 ± 2.3	57.3 ± 1.9	52.5 ± 1.6
8–12	59.0 ± 2.3	57.8 ± 2.4	49.7 ± 1.6
12–24	57.6 ± 2.8	58.3 ± 2.5	49.2 ± 2.3
24–32	51.8 ± 4.2	52.5 ± 3.8	41.8 ± 4.0
32–48	35.7 ± 3.4	34.3 ± 3.7	27.1 ± 4.0

Trials 1 and 2 were performed 2 weeks and 1 week, respectively, before treatment with streptozotocin. Trial 3 was performed 1 week after streptozotocin administration.
Number of animals in each trial = 10.
Each value represents the mean ± SE of duplicate analyses.

In vitro *acetylation of sulphamethazine by rat whole blood*

Blood samples from rats rendered diabetic had significantly higher (non-fasting) plasma glucose concentrations ($P < 0.0005$, one tailed Student's *t*-test) and *in vitro* acetylation capacities ($P < 0.02$, two tailed Student's *t*-test) than those from non-diabetic animals. Elevating the incubation glucose concentration of samples from diabetic and non-diabetic rats by 50 mmol/L increased the *in vitro* blood *N*-acetyltransferase activity significantly ($P < 0.01$, paired *t*-test; Table 2). In addition to a peak at the retention time of authentic acetylsulphamethazine, rat blood samples incubated with sulphamethazine also produced another compound which eluted before sulphamethazine and its acetylated metabolite.

DISCUSSION

The effect of streptozotocin on the in vivo *acetylation capacity of the rat*

This study demonstrates that streptozotocin-induced diabetes significantly reduces the *in vivo* acetylation capacity of adult male Sprague–Dawley rats. Although several groups have reported that chemically-induced diabetes significantly alters hepatic mixed function oxidase activity of rats [12–14], only two other groups have compared the acetylation capacities of diabetic and non-diabetic rats. Induction of diabetes in rats using alloxan and streptozotocin significantly reduces the acetylation of *p*-aminobenzoic acid [15] and procainamide ethobromide [16], respectively. It has also recently been reported that streptozotocin treated rats are less efficient than control animals in activating the arylamine 2-aminofluorene, a compound which is polymorphically acetylated [17], to mutagenic oxidized intermediates [18].

One particular advantage of the experimental design used in the current investigation compared to the above studies is that each animal serves as its own control. The insignificant intraindividual variation in acetylation capacity prior to streptozotocin administration demonstrates that induction or inhibition of the acetylating enzymes did not occur following the initial exposure to sulphamethazine. The percentage acetylation of sulphamethazine observed during the two day collection period after administration increased to a maximum value which remained constant for between 8 and 24 hr before decreasing. A previous study reported no significant change [19] in this parameter 0–24 hr post-administration.

Streptozotocin treatment did not significantly change the percentage of the original dose excreted in the urine despite the significantly increased urine volumes produced by the diabetic rats. One possible explanation to account for the relatively low amounts of sulphamethazine recovered in this study is that the drug was poorly absorbed. Alternatively, rats may metabolize sulphamethazine by additional routes, a hypothesis supported by *in vitro* studies using isolated rat liver cells [20] and rat whole blood (see discussion below).

The results obtained in this study do not suggest a relationship between the induction of diabetes and increased *in vivo* acetylation capacity. However, if rats metabolize sulphamethazine by additional pathways to acetylation, induction of diabetes may preferentially increase the activities of these other enzymes. In man, the mass balance between the amounts of sulphamethazine and acetylsulphamethazine recovered and the original dose of sulphamethazine is virtually 100% [21] and therefore in the absence of any alternative metabolic pathways in human subjects, acetylation may be stimulated by the onset of diabetes.

In vitro *acetylation of sulphamethazine by whole blood from healthy and diabetic rats*

The production of an additional (and more polar) metabolite of sulphamethazine observed in this study has previously been reported with isolated rat liver cells [20]. Blood samples from diabetic rats did not produce more of the unknown compound than samples from non-diabetic animals. Previous studies on the *in vitro* acetylation of sulphamethazine by rat blood have either failed to detect any activity [22] or have been unable to accurately quantify the acetylsulphamethazine produced [23]. The increased sensitivity and specificity of the HPLC method used in the current study allows measurement of the low

Table 2. The effect of added glucose on the *in vitro* acetylation of sulphamethazine by whole blood obtained from non-diabetic and streptozotocin-induced diabetic rats

Subjects (N)	Mean (range) initial plasma glucose concentration* (mmol/L)	Increase in incubation glucose concentration (mmol/L)	Mean (range) amount of acetylsulphamethazine produced† (nmol)
Non-diabetic rats (8)	6.6 (4.5–7.8)	0	2.13 (1.50–3.48)
		50	4.52 (3.17–8.35)‡
Diabetic rats (8)	26.4 (22.2–32.3)	0	3.55 (2.56–4.45)
		50	7.39 (5.38–9.74)‡

Initial sulphamethazine concentration = 0.18 mmol/L (72 nmol per sample).
Incubation time = 24 hr.
The respective values in the two groups are significantly different using Student's *t*-test, * $P < 0.0005$ (one tailed test); † $P < 0.02$ (two tailed test).
‡ The values were significantly different from the control samples (no glucose added) by paired *t*-test ($P < 0.01$).

N-acetyltransferase activity present in blood from this species.

Blood samples from diabetic rats acetylated significantly more sulphamethazine *in vitro* than those from untreated animals. Elevating the incubation glucose concentration by 50 mmol/L significantly increased the *N*-acetyltransferase activity of both groups. Similar results have been obtained using human blood [9] although the acetylation capacity of rat blood is significantly lower ($P < 0.05$, two tailed Student's *t*-test) than that of human blood, irrespective of whether the donors are diabetic or not. The enhanced blood *N*-acetyltransferase activities of samples from non-diabetic rats in the presence of added glucose has previously been reported [24, 25].

The contrasting results of our *in vivo* and *in vitro* studies with diabetic and non-diabetic rats could indicate that the contribution of blood *N*-acetyltransferase activity to the total *in vivo* acetylating ability is insignificant in this species. In the rabbit, the *in vitro* acetylating ability of blood is estimated to be less than 4% of the total *in vitro* acetylating capacity [26].

Clarification of whether rapid acetylators are more susceptible to development of diabetes may be assisted by the use of certain strains of rabbits and mice which display the acetylation polymorphism. There are clearly ethical limitations about conducting any of these studies using human subjects. However, since the occurrence of diabetes seems to be at least partially genetically linked [27], a prospective study of sulphamethazine acetylation in potential diabetics may indicate if acetylator status and any form of diabetes segregate together or if the *in vivo* acetylation capacity changes with the onset of diabetes.

REFERENCES

1. Weber WW and Hein DW, *N*-Acetylation pharmacogenetics. *Pharmacol Rev* **37**: 25–79, 1985.
2. Shenfield GM, McCann VJ and Tjokresetio R, Acetylator status and diabetic neuropathy. *Diabetologia* **22**: 441–444, 1982.
3. Pontirolli AE, Mosca A, de Pasqua A, Alcini D and Pozza G, The fast acetylator phenotype in diabetes mellitus: abnormal prevalence and association with ABO blood groups. *Diabetologia* **27**: 235–237, 1984.
4. Burrows AW, Hockaday TDR, Mann JI and Taylor JG, Diabetic dimorphism according to acetylator status. *Br Med J* **1**: 208–210, 1978.
5. Bodansky HJ, Drury PL, Cudworth AJ and Evans DAP, Acetylator phenotypes and Type 1 (insulin-dependent) diabetics with microvascular disease. *Diabetes* **30**: 907–910, 1981.
6. Ladero JM, Arrajo A, de Salamanca RE, Gomez M, Cano F and Alfonso M, Hepatic acetylator phenotype in diabetes mellitus. *Ann Clin Res* **14**: 187–189, 1982.
7. Price-Evans DA, Survey of the human acetylator polymorphism in spontaneous disorders. *J Med Genet* **21**: 243–253, 1984.
8. Thom S, Farrow PR, Santoso B, Alberti KGMM and Rawlins MD, Effects of oral glucose in lime juice on isoniazid kinetics. *Br J Clin Pharmacol* **11**: 423, 1981.
9. Lindsay RM, Baty JD and Waugh NR, *In vitro* studies on the acetylation of sulphamethazine by human whole blood from healthy and diabetic subjects. In: *Developments in Analytical Methods in Pharmaceutical,*

Biomedical and Forensic Sciences (Eds. Piemonte G, Tagliaro F, Marijo M and Frigerio A), pp. 303–312, Plenum Publishing Corporation, New York, 1987.
10. Lindsay RM and Baty JD, A novel method for the estimation of blood acetyl-CoA levels. *Biochem Soc Trans* **17**: 921–922, 1989.
11. Baty JD, Lindsay RM and Sharp S, Use of high-performance liquid chromatography in the measurement of *in vitro* acetylation in man. *J Chromatogr* **353**: 329–337, 1986.
12. Ackerman DM and Liebman KC, Effect of experimental diabetes on drug metabolism in the rat. *Drug Metab Dispos* **5**: 405–410, 1977.
13. Reinke LA, Stohs SJ and Rosenberg H, Altered activity of hepatic mixed-function monooxygenase enzymes in streptozotocin-induced diabetic rats. *Xenobiotica* **8**: 611–618, 1978.
14. Warren BL, Pak R, Finlayson M, Gontovnick L, Sunahara G and Bellward GD, Differential effects of diabetes on microsomal metabolism of various substrates: comparison of streptozotocin and spontaneously diabetic Wistar rats. *Biochem Pharmacol* **32**: 327–335, 1983.
15. Charalampous FC and Hegsted DM, Acetylation in the diabetic rat. *J Biol Chem* **180**: 623–634, 1949.
16. Watkins JB and Dykstra TP, Alterations in biliary excretory function by streptozotocin-induced diabetes. *Drug Metab Dispos* **15**: 177–183, 1987.
17. Glowinski IB and Weber WW, Evidence that arylhydroxamic acid *N,O*-acetyltransferase and the genetically polymorphic *N*-acetyltransferase are properties of the same enzyme in rabbit liver. *J Biol Chem* **255**: 7883–7890, 1980.
18. Flatt PR, Bass SL, Ayrton AD, Trinick J and Ioannides C, Metabolic activation of chemical carcinogens by hepatic preparations from streptozotocin-treated rats. *Diabetologia* **32**: 135–139, 1989.
19. Zidek Z and Janku I, Sex and genetic differences in the elimination of sulphadimidine in rats. *Pharmacology* **14**: 556–562, 1976.
20. Olsen H and Morland J, Sulfonamide acetylation in isolated rat liver cells. *Acta Pharmacol Toxicol* **49**: 102–109, 1981.
21. Hekster YA and Vree TB, Clinical pharmacokinetics of sulphonamides and their N4-acetyl derivatives. *Antibiot Chemother* **31**: 22–118, 1982.
22. Tannen RH and Weber WW, Rodent models of the human isoniazid acetylator polymorphism. *Drug Metab Dispos* **7**: 274–279, 1979.
23. Gollamudi R, Rackley RJ and Autian J, A new substrate for the measurement of *N*-acetyltransferase activity. *Enzyme* **30**: 155–161, 1983.
24. Blondheim SH, *In vitro* acetylation of drugs by human blood cells. *Arch Biochem Biophys* **55**: 365–372, 1955.
25. Subrahmanyam R, Quentin-Blackwell R and Fosdick LS, Factors that influence the acetylating activity of blood: effect of addition of certain metabolites and physiologically active substances. *J Periodontol* **32**: 139–143, 1961.
26. Hearse DJ and Weber WW, Multiple *N*-acetyltransferases and drug metabolism: tissue distribution, characterisation and significance of mammalian *N*-acetyltransferase. *Biochem J* **132**: 519–526, 1973.
27. Kobberling J and Bruggeboes B, Prevalence of diabetes among children of insulin dependent diabetic mothers. *Diabetologia* **18**: 459–462, 1980.