Altered methionine metabolism in streptozotocin-diabetic rats

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Summary. We examined the origin of hypermethioninaemia in streptozotocin-diabetic rats. In rats administered streptozotocin over a range from 55 to 75 mg/kg, the dose of drug injected correlated directly with the plasma methionine concentration and inversely with the plasma insulin level. Although insulin administration prevented hypermethioninaemia in streptozotocin-diabetic rats, discontinuing insulin treatment resulted in a time-dependent increase in the plasma methionine level. Plasma methionine concentration was, however, normal in insulin-deprived BB Wistar rats despite severe hyperglycaemia. Thus, although insulin deficiency may be a contributing factor, it does not cause hypermethioninaemia

independent of other drug-related effects. Administering a loading dose of methionine (100 mg/kg) indicated that streptozotocin-diabetic rats have a reduced metabolic capacity. Since dietary intake is the primary source of methionine, it is likely that hyperphagia combined with limited disposal produces hypermethioninaemia. Methionine is the most toxic amino-acid; therefore, metabolic studies using the streptozotocin model of insulin deficiency must be interpreted with caution.

Key words: Streptozotocin diabetes, insulin deficiency, methionine, hypermethioninaemia, drug toxicity.

Streptozotocin (STZ) is a β cell-cytotoxic agent commonly used to induce experimental diabetes in rodents [1]. Using this model, the effect of insulin deficiency on the metabolism of branched chain and gluconeogenic amino-acids has been extensively studied [2, 3]. Other amino-acids have received less attention. We observed a sevenfold increase in the plasma methionine concentration of STZ-diabetic rats [4], although a marked increase in the plasma level of this amino-acid is not usually associated with insulin deficiency [2, 3]. The object of this study, therefore, was to determine the origin of hypermethioninaemia in STZ-diabetic rats.

Materials and methods

Experimental design

Five experiments were conducted. (1) The relationship between the dose of STZ administered and plasma methionine concentration was established by injecting animals with 55, 65 or 75 mg/kg of STZ. (2) The effect of insulin administration on plasma methionine concentration in STZ-diabetic rats (75 mg/kg) was determined by treating rats with 1 U/day of protamine zinc insulin (Connaught Laboratories, Willowdale, Ontario) and withdrawing therapy for 12 h, 36 h, 60 h, 5 days or 8 days before sacrifice. (3) The effect of insulin deficiency on plasma methionine concentration was examined independent of STZ

exposure by depriving spontaneously diabetic BB Wistar rats (278 ± 14 g; pre-diabetic control rats 312 ± 11 g) (Banting and Best Research Institute, Toronto, Ontario) of maintenance injections of insulin (2.5 U/day) for 36 h or 60 h before sacrifice; and by administering a sub-maintenance dose of insulin (0.5 U/day) for 5 days before sacrifice. (4) The ability of rats to clear a methionine load was assessed by administering methionine (100 mg/kg, intraperitoneally) (Grand Island Biological Company, Grand Island, New York) to (a) food-deprived normal and STZ-diabetic rats (75 mg/kg) and (b) food-deprived pre-diabetic (347 ± 5 g) and diabetic (296 ± 10 g) BB Wistar rats treated with 0.5 U/day of insulin. In part (a), rats were sacrificed between 16.00 and 19.30 h at 0, 30, 90 and 210 min intervals. In part (b), tail vein blood samples were obtained at 0 and 90 min. (5) The effect of STZ administration (100 mg/kg) on food intake in large rats (300 g) was evaluated. Rats were initially treated with insulin (2.5 U/day). Following withdrawal of insulin therapy, food intake was monitored at 24-h intervals.

All STZ-diabetic rats were killed on day 10 post-induction. All animals were fed a semi-purified diet containing 20% protein (casein) formulated as described previously [5]. Food intake and weight gain were measured every 3 days.

Production of diabetes

Male Wistar rats (Woodlyn Farms, Guelph, Ontario) with an initial weight of 80–100 g (experiments 1, 2, 4) or 300 g (experiment 5) were housed individually in wire meshed hanging, stainless steel cages in a room kept at 23 °C and lit for 12 h daily (08.00–20.00 h). Streptozotocin (Sigma Chemicals, St. Louis, Missouri, USA) was individually weighed for each rat, dissolved in 0.25 ml of 150 mmol/l saline, ad-

Table 1. Relationship of streptozotocin dose to weight gain, plasma glucose, insulin, methionine, half-cystine and hepatic cystathionase activity in diabetic rats

	Weight gain (g)	Plasma				Hepatic cystathionase activity (nmol · mg protein ⁻¹ · h ⁻¹)
		Glucose (mmol/l)	Insulin (mU/l)	Methionine (μmol/l)	Half-cystine (μmol/l)	
Control rats (<i>n</i> = 5)	78 ± 5 ^a	9.1 ± 0.2 ^a	51 ± 7 ^a	85 ± 4 ^a	24 ± 4 ^a	689 ± 30 ^a
STZ-diabetic rats						
55 mg/kg (<i>n</i> = 6)	64 ± 3 ^b	30.7 ± 4.0 ^b	19 ± 6 ^b	144 ± 57 ^a	24 ± 3 ^a	888 ± 83 ^{ab}
65 mg/kg (<i>n</i> = 5)	46 ± 6 ^c	36.7 ± 4.4 ^b	5 ± 0.6 ^c	393 ± 98 ^b	28 ± 9 ^a	1118 ± 74 ^{bc}
75 mg/kg (<i>n</i> = 6)	45 ± 3 ^c	36.9 ± 3.2 ^b	4 ± 0.6 ^c	607 ± 63 ^b	30 ± 4 ^a	1176 ± 109 ^c

Results are expressed as mean ± SEM. ^{a, b, c} superscript notation for Tukey's multiple range *t*-test. Values sharing a common superscript are not significantly different (*p* > 0.05).

justed to pH 4.4 with citric acid (0.05 mol/l) and immediately administered intraperitoneally. Control animals received vehicle only. All injections were given between 16.30 and 17.30 h following a 16-h fast. The presence of diabetes was verified by estimates of urinary glucose (Clinitest Reagent, Ames Divison, Miles Laboratories, Rexdale, Ontario) and blood glucose (GOD-PAP, Boehringer Mannheim, Mannheim, FRG). Animals with urinary glucose excretion <100 mmol/l and blood glucose levels <16 mmol/l were discarded. Diabetic animals exhibited polydipsia, polyuria and hyperphagia. Ketouria (Acetest Reagent, Ames Division, Miles Laboratories) was not generally observed.

Tissue analysis

All animals were allowed access to food overnight and killed by decapitation between 09.00 and 11.00 h unless indicated otherwise. Blood from the cervical stump was collected into chilled, heparinized tubes and centrifuged at 1000 *g* for 10 min. Plasma was deproteinized immediately with an equal volume of 15% sulphosalicylic acid containing norleucine as internal standard and stored at -70 °C until analyzed for amino-acids using an amino-acid analyzer (model 116/119, Beckman, Palo Alto, California). In experiment 4b, plasma methionine was determined by high performance liquid chromatography (Waters Associates, Milford, Massachusetts) using pre-column derivatization with fluorescence detection [6]. Insulin was determined using a single antibody with dextran coated charcoal separation [7] and calibrated using a rat insulin standard. Hepatic cystathionase activity was assessed by the method of Gaull et al. [8] and Gaitonde [9] using L-cystathionine (Cal Biochem-Boering, San Diego, California) as substrate. Protein was determined according to the method of Lowry et al. [10].

Statistical methods

All values are expressed as mean ± SEM. Tukey's multiple range *T*-test, two way analysis of variance, correlational analysis and linear regression were used to analyze the data where appropriate [11]. Significance was established at the 0.05 level of probability.

Results and Discussion

Hypermethioninaemia in STZ-treated Wistar rats was related to the dose of drug administered. When animals were injected with STZ over a range from 55 to 75 mg/kg, rats administered either 65 or 75 mg/kg exhibited a five- to sixfold increase in plasma methionine concentration, while that of the 55 mg/kg group was not differ-

Table 2. Plasma methionine and glucose in STZ-diabetic and BB Wistar rats deprived of insulin

	Methionine (μmol/l)	Glucose (mmol/l)
Control rats (<i>n</i> = 9)	57 ± 9 ^a	8.9 ± 0.2 ^a
STZ-diabetic rats ^a		
12 h post-insulin (<i>n</i> = 7)	48 ± 10 ^a	19.7 ± 3.2 ^b
36 h post-insulin (<i>n</i> = 9)	78 ± 11 ^a	20.5 ± 3.8 ^b
60 h post-insulin (<i>n</i> = 9)	162 ± 39 ^{ab}	31.2 ± 0.9 ^c
5 days post-insulin (<i>n</i> = 8)	447 ± 114 ^b	30.6 ± 1.7 ^c
8 days post-insulin (<i>n</i> = 8)	312 ± 104 ^{ab}	33.9 ± 1.6 ^c
no insulin (<i>n</i> = 8)	441 ± 123 ^b	39.1 ± 0.9 ^c
Non-diabetic BB Wistar rats (<i>n</i> = 6)	30 ± 5 ¹	11.0 ± 1.8 ¹
Diabetic BB Wistar rats ^b		
36 h post-insulin (<i>n</i> = 6)	35 ± 6 ¹	31.1 ± 2.1 ²
60 h post-insulin (<i>n</i> = 5)	37 ± 6 ¹	28.9 ± 2.5 ²

^a Animals were treated with insulin (1 U/day).

^b Animals were treated with insulin (2.5 U/day).

Results are expressed as mean ± SEM. ^{a,b,c,1,2} Superscript notation for Tukey's multiple range *t*-test. Within each treatment group, values sharing a common superscript are not significantly different (*p* > 0.05).

ent from normal (Table 1). In these rats, the dose of STZ injected correlated directly with the plasma methionine concentration (*r* = 0.65, *df* = 20, *p* ≤ 0.01) and inversely with the plasma insulin level (*r* = -0.88, *df* = 20, *p* ≤ 0.01).

Manipulating the dose of STZ administered altered both the degree of insulin deficiency and the potential for the toxin to have other effects. To isolate the origin of elevated plasma methionine in STZ-diabetic rats, we first examined the effect of insulin replacement. Although insulin administration prevented hypermethioninaemia, plasma methionine concentration increased after insulin withdrawal (Table 2). However, when spontaneously diabetic BB Wistar rats, a model where insulin deficiency can be examined independent of STZ exposure, were completely deprived of insulin for 60 h, plasma methionine levels remained normal (Table 2). Moreover, in contrast to the eightfold increase observed in STZ-diabetic rats, plasma methionine levels in BB Wistar rats, treated chronically with 0.5 U/day of insu-

Fig. 1. Methionine clearance in STZ-diabetic (○-----○) and normal rats (●—●) administered methionine at a dose of 100 mg/kg. Results are expressed as mean $\pm$ SEM ($n=6$). Statistical comparison was by two way analysis of variance: treatment effect (1, 10) $F=14.09$, $p<0.01$; time effect (3,30) $F=33.64$, $p<0.01$

lin and similarly hyperphagic to the STZ-diabetic rats, were elevated by only 20% above levels in food-deprived control rats. These observations suggest that although insulin deficiency contributed to hypermethioninaemia in STZ-diabetic rats, it did not cause altered methionine metabolism independent of other unknown effects of the drug.

To examine further the origin of hypermethioninaemia, STZ-diabetic rats were administered a dose of methionine (100 mg/kg) equivalent to that contained in a 3 g meal of a 20% casein diet. Although the basal level and the increase at 30 min were similar to that of normal animals, plasma methionine levels in STZ-diabetic rats remained elevated at 90 and 210 min (Fig. 1). Because the basal insulin level in normal rats (25 ± 2 mU/l) was threefold higher than in STZ-diabetic rats (7 ± 1 mU/l), it is possible that the difference in methionine clearance rate was due to increased incorporation into protein by normal rats rather than reduced catabolism by STZ-diabetic rats. To answer this question, we conducted a similar experiment using diabetic BB Wistar rats with symptoms of insulin deficiency similar to that exhibited by STZ-diabetic rats. That is, they were non-ketotic but had significant hyperphagia (25 ± 2 g/day; pre-diabetic control rats 17 ± 0.5 g/day) and glycosuria (9 ± 2 g/24 h). At 90 min, the point of maximum difference between normal and STZ-diabetic rats, the plasma methionine levels in diabetic BB Wistar rats (431 ± 14 μ mol/l, $n=6$) and pre-diabetic control rats (431 ± 12 μ mol/l, $n=6$) were identical. These results indicate that factors other than insulin deficiency contributed to the limited capacity of STZ-diabetic rats to metabolize methionine.

A limited capacity to catabolize methionine might be predicted from the hepatocellular damage caused by STZ [12, 13], since methionine is metabolized primarily in liver tissue. To assess the capacity for methionine catabolism, we measured hepatic cystathionase activity as an index of transsulphuration. In STZ-diabetic rats, cystathionase activity was increased over the control value while plasma half-cystine levels remained unchanged (Table 1). Probably because of increased age [14], cystathionase activity was 40% higher in the BB Wistar rats compared with the STZ-diabetic rats. However, the level in BB Wistar diabetic rats (1841 ± 146 nmol $\cdot$ mg protein $^{-1} \cdot$ h $^{-1}$) was similar to that of prediabetic control rats (1685 ± 67 nmol $\cdot$ mg protein $^{-1} \cdot$ h $^{-1}$). It is possible, therefore, that the increased transsulphuration activity observed in STZ-diabetic rats arises from decreased transamination activity, the other major catabolic pathway [15]. However, the metabolic origin of the reduced capacity to catabolize methionine remains undetermined.

Because methionine cannot be synthesized *de novo* within the body, our results indicate that hypermethioninaemia in STZ-diabetic rats results from the combined effects of persistent hyperphagia throughout both the light and dark periods [16] and reduced capacity to metabolize methionine. The observed hyperphagia and hypermethioninaemia coinciding with insulin withdrawal are consistent with this interpretation. Moreover, in contrast to the fed state, plasma methionine concentration in food-deprived STZ-diabetic rats was normal despite equivalent low ambient insulin levels. Thus, normalization of plasma methionine in insulin-treated and food-deprived STZ-diabetic rats may be attributed to decreased intake rather than a reversal of tissue damage.

Although we have consistently observed hypermethioninaemia in young (120 g) Wistar rats, our results appear to be in contrast with the normal plasma methionine levels reported in older (300 g) Sprague-Dawley rats administered 100 mg/kg STZ and sacrificed 90 h post-insulin withdrawal [3]. Therefore, we administered 100 mg/kg STZ to 300 g Wistar rats and monitored food intake during the period of insulin withdrawal. In this experiment, food intake was decreased by 50% at 84 h post-insulin with five of the seven rats studied dying within the next 24 h. This suggests that the severe metabolic decompensation induced by this dose of STZ precluded the development of hyperphagia and hypermethioninaemia. Whether altered methionine metabolism would be manifested in old rats administered a smaller dose of STZ remains to be determined. Food intake per unit of body weight is greater in young rats, however, suggesting that hypermethioninaemia may be confined to STZ-induced diabetes in young, developing, hyperphagic animals.

In conclusion, the metabolism of methionine in STZ-diabetic rats is related not only to insulin deficiency but also to other unknown effects of drug exposure.

Methionine is the most toxic amino-acid. When hypermethioninaemia is induced by feeding diets containing excess quantities of this amino-acid, animals exhibit growth failure [17, 18], renal tubular hypertrophy [19], pancreatic acinar cell damage [19, 20], atrophy of liver cells [21], and anaemia with iron accumulation in the liver and spleen [19, 21]. Thus, the results of metabolic studies employing the STZ model of insulin deficiency must be interpreted with caution.

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