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COMMUNICATIONS

Influence of streptozocin-induced diabetes on reductive metabolism of acetohexamide in rat liver

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Abstract—Streptozocin-induced diabetes significantly decreased acetohexamide reductase activity of 10 000 g supernatant of liver homogenates from both male and female rats. However, the decrease in activity from female rats was smaller than that from male rats, thereby eliminating the sex difference in the activity of the 10 000 g supernatant. In male rats, the diabetes markedly decreased acetohexamide reductase activity only in the microsomal fraction of liver homogenate, whereas in female rats, it decreased the activity only in the cytosolic fraction. These results indicate that the mechanism for the decreasing effect of the diabetes on reductive metabolism of acetohexamide in 10 000 g supernatant differs between male and female rats.

Experimentally induced diabetes has been shown to affect oxidative metabolism of a variety of drugs in rats (Dixon et al 1961; Kato & Gillette 1965; Reinke et al 1978; Cook & Past 1979). For example, Dixon et al (1961) reported that alloxan-induced diabetes decreased the oxidative metabolism of hexobarbitone and prolonged its sleeping effect in rats. However, little attempt has been made to elucidate the influence of diabetes on reductive metabolism of drugs containing a ketone group. Recently, we have demonstrated that acetohexamide, a typical ketone-containing drug, is reduced to hydroxyhexamide in the rat liver and a sex difference is observed for the reduction (Imamura et al 1987). The present study was designed to elucidate the influence of streptozocin-induced diabetes on acetohexamide reduction in the rat liver. Since acetohexamide is widely used as an oral antidiabetic drug and hydroxyhexamide is a pharmacologically active metabolite, it was of interest to study the effect of diabetes on acetohexamide metabolism.

Materials and methods

Chemicals. Acetohexamide was supplied by Shionogi & Co., Ltd (Osaka, Japan). β-Nicotinamide adenine dinucleotide phosphate ($NADP^+$), glucose-6-phosphate, glucose-6-phosphate dehydrogenase and streptozocin (streptozotocin) were purchased from Sigma Chemical Co. (St. Louis, USA). Hydroxyhexamide was synthesized from acetohexamide according to the method of Girgis-Takla & Chroneos (1979). Fenbufen was supplied by Lederle Labs. (Pearl River, USA). All other chemicals were of reagent grade.

Preparation of subcellular fractions. Male (8–10 weeks, 210–290 g) and female (8–10 weeks, 160–260 g) Wistar rats were fasted for 24 h before experiments, but drinking water was freely available. The animals were decapitated and the liver excised, perfused with ice-cold 1·15% KCl solution, and then homogenized in a Potter-Elvenhjem homogenizer with 3 volumes of 0·01 M phosphate buffer containing 1·15% KCl (pH 7·4). The homogenate was centrifuged at 10 000 *g* for 20 min and the resulting supernatant centrifuged at 105 000 *g* for 60 min to obtain the microsomal pellets and cytosolic fraction. The microsomal pellets were suspended in 0·01 M phosphate buffer containing 1·15% KCl (pH 7·4) and recentrifuged at 105 000 *g* for 60 min.

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All these procedures were at 0–4°C. The 10 000 *g* supernatant, microsomal pellets and cytosolic fraction were used in the assay for enzyme activity.

Assay of acetohexamide reductase activity. In assaying the acetohexamide reductase activity, the typical mixture consisted of acetohexamide (1·0 mM), $NADP^+$ (0·25 mM), glucose-6-phosphate (6·25 mM), glucose-6-phosphate dehydrogenase (0·25 units), $MgCl_2$ (6·25 mM), enzyme preparation (10 000 *g* supernatant, microsomal pellets or cytosolic fraction of liver homogenate) and 0·1 M phosphate buffer (pH 7·4) in a final volume of 2·0 mL. The protein concentration in the incubations was about 5 mg mL^{-1}. The reaction was started by the addition of the cofactor, and the mixture was incubated at 37°C for 10 min under aerobic conditions. The reaction was stopped by the addition of 0·5 mL of 1 M HCl to the mixture. The reduction product (hydroxyhexamide) was determined by HPLC (Takagishi et al 1979). Each reaction mixture was extracted with 5 mL of benzene–ethyl acetate (1:1, v/v) containing fenbufen as the internal standard. After centrifugation at 3000 rev min^{-1} for 10 min, the organic phase (4·0 mL) was evaporated under vacuum and the residue was dissolved in acetonitrile (0·3 mL) and subjected to HPLC with a Hitachi 655A-11 HPLC apparatus (Hitachi Ltd., Tokyo, Japan) equipped with a LiChrosorb PR-18 column (250 × 4 mm i.d., Kanto Kagaku Ltd., Tokyo, Japan) and Hitachi 638-41 UV monitor (230 nm). Acetonitrile-0·2% acetic acid (47 : 53, v/v) was used as a mobile phase at a flow rate of 1·0 mL min^{-1}. Protein concentration was determined by the method of Lowry et al (1951) with bovine serum albumin as the standard.

Streptozocin treatment. Diabetes was induced by an intraperitoneal injection of streptozocin (70 mg kg^{-1}) prepared by freshly dissolving in 0·05 M citrate buffer (pH 4·5). Control animals were injected with an equivalent volume of 0·05 M citrate buffer (pH 4·5). Experiments were performed 12 days after the induction of diabetes. Only diabetic rats showing a serum glucose concentration of at least 400 mg 100 mL^{-1} were used. Serum glucose was measured enzymatically (glucose oxidase kit, Mizuho Medy Laboratories, Saga, Japan). Physical and biological characteristics in normal and streptozocin-induced diabetic rats are summarized in Table 1.

Statistics. The results were analysed statistically with Student's *t*-test or Cochran-Cox test. A P value of 0·05 or less was considered to be significant.

Results and discussion

The effect of streptozocin-induced diabetes on the metabolic reduction of acetohexamide was examined using 10 000 *g* supernatant of liver homogenates in male and female rats. As shown in Table 2, the diabetes significantly decreased the acetohexamide reductase activities in both male and female rats. Interestingly, the diabetes negated the sex difference in acetohexamide reductase activity of the 10 000 *g* supernatant by decreasing the activity of the enzyme from the male more than that from

Table 1. Physical and biological characteristics in normal and streptozocin-induced diabetic rats

	Sex	Normal	Diabetic
Serum glucose (mg 100 mL^{-1})	Male	130 ± 13	525 ± 55***,b
	Female	130 ± 10	459 ± 22***,a
Body weight (g)	Male	206 ± 11	191 ± 23
	Female	210 ± 43	149 ± 25**,a
Microsomal prot. cont. (mg g^{-1} wet liver wt)	Male	5·1 ± 1·3	6·8 ± 4·4
	Female	5·6 ± 0·5	7·3 ± 1·0*,a
Cytosolic prot. cont. (mg g^{-1} wet liver wt)	Male	39·4 ± 6·2	39·6 ± 12·2
	Female	42·5 ± 5·9	39·5 ± 8·5

Each value represents the mean ± s.d. of 3–6 rats.
* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, significantly different from normal.
[a] Student's t-test, [b] Cochran-Cox test.

Table 2. Acetohexamide reductase activity of 10 000 g supernatant, microsomal and cytosolic fraction of liver homogenate in normal and streptozocin-induced diabetic rats

		Activity (nmol 10 min^{-1} mg^{-1} protein)	
Fraction	Sex	Normal	Diabetic
10 000 *g* supernatant	Male	8·27 ± 2·21	4·31 ± 0·70*,a
	Female	5·28 ± 0·21	3·74 ± 0·76*,b
Microsomal	Male	9·03 ± 6·73	0·27 ± 0·39*,b
	Female	0·27 ± 0·28	0·36 ± 0·24
Cytosolic	Male	6·44 ± 0·43	6·43 ± 1·06
	Female	7·41 ± 0·79	5·25 ± 0·70**,a

Each value represents the mean ± s.d. of 4–7 rats.
* $P < 0.05$, ** $P0.01$, significantly different from normal.
[a] Student's t-test, [b] Cochran-Cox test.

the female; in normal rats, a sex difference was observed for the activity ($P < 0.05$, Student's t-test).

In an attempt to elucidate further the influence of streptozocin-induced diabetes on acetohexamide reductase activity, the activities in microsomal and cytosolic fractions of liver homogenate were compared between normal and diabetic rats. As is evident from Table 2, the diabetes markedly decreased acetohexamide reductase activity of the microsomal fraction from male rats and significantly decreased the activity of the cytosolic fraction from female rats. On the other hand, the diabetes had no effect on the activity of the cytosolic fraction from male rats or the microsomal fraction from female rats. These observations led us to conclude that the mechanism for the decreasing effect of diabetes on reductive metabolism of acetohexamide in 10 000 *g* supernatant of liver homogenate is different between male and female rats.

It has been reported that streptozocin-induced diabetes has a sex-dependent effect on the oxidative metabolism of a variety of drugs in rats (Kato & Gillette 1965; Kato 1974; Skett & Joels 1984). In the present study, we provide evidence that the effect of streptozocin-induced diabetes on the reductive metabolism of acetohexamide in rat liver is also sex-dependent. This appeared to be related to steroid metabolism in rat liver.

Recently, we have demonstrated that a microsomal enzyme largely contributes to the sex difference of acetohexamide reduction in normal rats (Imamura et al 1987). This fact suggests that enzyme may play a role in steroid metabolism in rats. Skett (1986) examined the difference in 17-oxosteroid reductase activity in the microsomal fraction of liver homogenate from male and female rats and the effect of streptozocin-induced diabetes on that activity. The results were in fair agreement with those in this study on acetohexamide reductase activity of the microsomal fraction of liver homogenate in male and female rats. Thus, the microsomal enzyme that can catalyse acetohexamide reduction may correspond to 17-oxosteroid reductase.

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