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Species Difference in the *in Vitro* Metabolic Reduction of Acetohexamide

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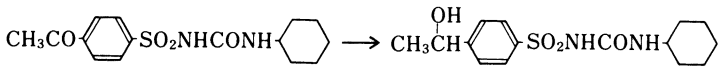
Species difference in the *in vitro* metabolic reduction of acetohexamide, an oral antidiabetic drug, was investigated using the rabbit, guinea pig, hamster, rat and mouse. The rabbit exhibited the highest acetohexamide reductase activity in cytosol of the liver and kidney among the species tested. The sensitivities to specific inhibitors of cytosolic acetohexamide reductase in the liver and kidney of the rabbit were different from those of the rat. Furthermore, species difference in acetohexamide reductase activity was found in the microsomes of the liver and kidney; only the rat and guinea pig showed significant activity.

Keywords——species difference; acetohexamide; metabolic reduction; cytosol; microsome; specific inhibitor

It is well-known that marked species differences exist in drug metabolism. In previous papers,[1,2] we demonstrated that acetohexamide, an oral antidiabetic drug, is reduced to a pharmacologically active metabolite (−)-hydroxyhexamide (Fig. 1), and this reduction is catalyzed by carbonyl reductases or aldehyde reductases. Little attempt, however, has been made to elucidate species differences in the metabolic reduction of acetohexamide. In this paper, we describe the differences in the *in vitro* metabolic reduction of acetohexamide among the rabbit, guinea pig, hamster, rat and mouse.



acetohexamide (−)-hydroxyhexamide

Fig. 1. Metabolic Reduction of Acetohexamide

Experimental

Materials——Acetohexamide was supplied by Shionogi & Co., Ltd. (Osaka, Japan). β-Nicotinamide adenine dinucleotide phosphate ($NADP^+$), glucose-6-phosphate and glucose-6-phosphate dehydrogenase were purchased from Sigma Chemical Co. (St. Louis, U.S.A.). Barbital, pyrazole and quercitrin were obtained from Wako Pure Chemical Industries, Ltd. (Osaka, Japan). All other chemicals used in this study were of reagent grade.

Preparation of Cytosol and Microsomes——Male rabbits (Japanese White, 2.0—3.0 kg), guinea pigs (Hartley, 280—380 g), hamsters (Syrian, 90—125 g), rats (Wistar-KY, 170—290 g) and mice (ddY, 28—31 g) were fasted for about 24 h prior to experiments, but drinking water was allowed *ad libitum*. The rabbits were exsanguinated from the carotid artery, and the other animals were killed by decapitation. The liver and kidneys were perfused with ice-cold 1.15% KCl solution and excised carefully. Subsequent procedures were carried out at 0—4°C. The tissues were homogenized in 3 volumes of 0.01 M phosphate buffer containing 1.15% KCl (pH 7.4) with a Potter-Elvehjem homogenizer. The homogenates were centrifuged at $10000 \times g$ for 20 min and the resulting supernatants were centrifuged at $105000 \times g$ for 60 min to obtain the microsomal pellets and the cytosolic fractions. The microsomal pellets were suspended in 0.01 M phosphate buffer containing 1.15% KCl (pH 7.4) and recentrifuged at $105000 \times g$ for 60 min. The microsomal pellets and cytosolic fractions obtained were assayed for enzyme activity.

Enzyme Assay——Acetohexamide reductase activity was assayed in an incubation mixture containing acetohexamide (1 mM), $NADP^+$ (0.25 mM), glucose-6-phosphate (6.25 mM), $MgCl_2$ (6.25 mM), glucose-6-phosphate dehydrogenase (0.25 units), the cytosolic or microsomal fraction of the liver or kidney homogenate, and 0.1 M phosphate buffer (pH 7.4) in a final volume of 2.0 ml. 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 1N HCl to the mixture. The reduction product, hydroxyhexamide, was determined by high-performance liquid chromatography (HPLC). Protein concentration was determined by the method of Lowry *et al.*[3] with bovine serum albumin as the standard.

Determination of Hydroxyhexamide[4]——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 rpm for 10 min, the organic phase (4.0 ml) was evaporated *in vacuo* and the residue was dissolved in acetonitrile (0.3 ml) and subjected to HPLC. HPLC was carried out using a Hitachi 655A-11 HPLC apparatus (Hitachi Ltd., Tokyo, Japan) equipped with a LiChrosorb RP-18 column (250 × 4 mm i.d., Cica-Merck, Kanto Kagaku Ltd., Tokyo, Japan) and a Hitachi 638-41 UV monitor (230 nm). Acetonitrile–0.2% acetic acid (47 : 53, v/v) was employed as a mobile phase at a flow rate of 1.0 ml/min.

Statistical Analysis——Statistical significance of differences between means was determined by using the unpaired Student's *t*-test.

Results

Species Difference of Acetohexamide Reductase Activity in Cytosol

Figure 2 shows the species difference of acetohexamide reductase activity in cytosols of the liver and kidney. The order of the activity was rabbit ≫ mouse > rat, hamster > guinea pig in the kidney cytosol, and rabbit ≫ mouse, hamster, guinea pig, rat in the liver cytosol. In both the liver and kidney cytosols, that is, the rabbit exhibited the highest acetohexamide reductase activity. Furthermore, acetohexamide reductase activity in the kidney cytosols of the hamster, rat and mouse was significantly higher than that in the corresponding liver cytosol.

Effect of Specific Inhibitor on Activity of Cytosolic Acetohexamide Reductase

The effects of specific inhibitors on the activity of cytosolic acetohexamide reductase in the liver and kidney of the rabbit and rat are summarized in Table I. Barbital, a well-known inhibitor of aldehyde reductase,[5] inhibited the acetohexamide reduction in the cytosols of rat liver and kidney. In contrast, quercitrin, a classical inhibitor of carbonyl reductase,[6,7] markedly inhibited the acetohexamide reduction in the cytosols or rabbit liver and kidney. Pyrazole, an effective inhibitor which distinguishes alcohol dehydrogenase from carbonyl or aldehyde reductase, did not affect any of the acetohexamide reductase activities.

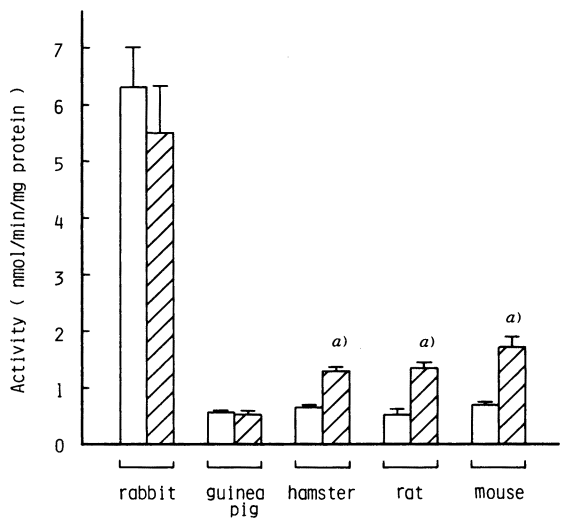


Fig. 2. Species Difference of Acetohexamide Reductase Activity in Cytosols of the Liver and Kidney

□, liver; ▨, kidney.
Each bar represents the mean ± S.D. of 5—6 experiments.
a) Significantly different from the activity in the liver cytosol of the corresponding species at $p < 0.001$.

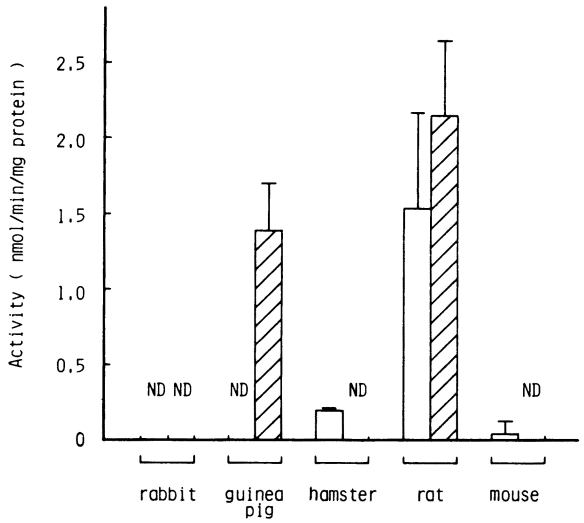


Fig. 3. Species Difference of Acetohexamide Reductase Activity in Microsomes of the Liver and Kidney

□, liver; ▨, kidney; ND, not detected. Each bar represents the mean ± S.D. of 3 experiments.

TABLE I. Effect of Specific Inhibitor on the Activity of Cytosolic Acetohexamide Reductase in the Liver and Kidney of Rabbit and Rat

Inhibitor	Concn. (mM)	Relative activity (%)			
		Rabbit		Rat	
		Liver	Kidney	Liver	Kidney
Barbital	1	97.0 ± 4.4	101.7 ± 3.8	67.8 ± 0.3	31.5 ± 1.7
Quercitrin	0.1	31.5 ± 1.3	43.7 ± 4.3	83.5 ± 1.0	89.0 ± 2.6
Pyrazole	10	100.0 ± 5.1	97.4 ± 4.9	97.0 ± 1.6	100.7 ± 1.0

Each value represents the mean ± S.D. of 3 experiments.

Species Difference of Acetohexamide Reductase Activity in Microsomes

Figure 3 shows the species difference of acetohexamide reductase activity in microsomes of the liver and kidney. In the rat, the microsomes of these two tissues displayed acetohexamide reductase activity. In the guinea pig, unlike the rat, only the microsomes of the kidney displayed the activity. In the other species, however, there was little or no activity in the microsomes of the liver and kidney.

Discussion

Species difference in acetohexamide reductase activity was found in the cytosols of the liver and kidney (Fig. 2). Among the species tested, the rabbit exhibited the highest acetohexamide reductase activity. Similar results have been obtained for the *in vitro* metabolic reduction of a variety of ketone-containing drugs.[7,8]

In an attempt to elucidate the properties of cytosolic acetohexamide reductase, the effects of specific inhibitors on the activity were compared between the rabbit and the rat. The sensitivities to specific inhibitors of acetohexamide reductases in the liver and kidney cytosols of the rabbit were evidently different from those of the rat (Table I). These findings suggest the heterogeneity of cytosolic acetohexamide reductases in the rabbit and rat; these enzymes may be classified as a carbonyl reductase and an aldehyde reductase, respectively.

Species difference in acetohexamide reductase activity was also observed in the microsomes of the liver and kidney, as shown in Fig. 3. In general, the enzymes which catalyze the reduction of ketone-containing drugs have been reported to be localized in the cytosolic fraction.[7-9] For example, loxoprofen reducing enzyme was localized only in the cytosolic fraction of the livers of rat, guinea pig and rabbit.[9] Thus, it is interesting that in the rat and guinea pig, acetohexamide reductase activity was observed in the microsomal fraction.

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