SEX DIFFERENCE OF ACETOHEXAMIDE REDUCTION IN RAT LIVER

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The acetohexamide reducing activity in hepatic $10000 \times g$ supernatant was significantly higher in male than in female rats. Evidence obtained in this study suggests that the microsomal carbonyl reductase may contribute to the sex difference in the reductive metabolism of acetohexamide in rats.

Keywords — acetohexamide; sex difference; reductive metabolism; rat liver; microsome; cytosol

INTRODUCTION

Although sex differences in oxidative metabolism of many drugs have been reported in rats, little is known about sex differences in reductive metabolism of drugs containing a ketone group such as warfarin and daunorubicin. In previous papers,^{1,2)} we demonstrated that acetohexamide, an oral antidiabetic drug, is reduced to hydroxyhexamide, a pharmacologically active metabolite. The reduction is catalyzed by carbonyl reductases in cytosols of rabbit tissues. A study concerning the sex difference of acetohexamide reduction was attempted in rabbits but the sex difference was not observed. Thus, in the present study, we examined the sex difference in the reductive metabolism of acetohexamide, using rat livers.

MATERIALS AND METHODS

Materials — Acetohexamide was supplied by Shionogi Pharmaceutical Co., Ltd. All other chemicals used in this study were reagent grade.

Preparation of $10000 \times g$ Supernatant, Microsomes and Cytosol — Male and female Wistar rats (230–290 g) were killed by decapitation. The livers were removed and homogenized with 0.01 M phosphate buffer, pH 7.4, containing 1.15% KCl. The homogenate was centrifuged at $10000 \times g$ for 20 min, and the resulting supernatant was centrifuged at $105000 \times g$ for 60 min to obtain microsomes and cytosol.

Assay of Acetohexamide Reducing Activity — Assay of acetohexamide reducing activity in the $10000 \times g$ supernatant, microsomes and cytosol were carried out according to the method described previously.¹⁾ Protein concentration was determined by the method of Lowry *et al.*³⁾

with bovine serum albumin as the standard.

RESULTS AND DISCUSSION

Acetohexamide reducing activity in hepatic $10000 \times g$ supernatant was examined in male and female rats. The activity was found to be significantly higher in male than in female rats (male; 0.76 ± 0.24 , female; 0.47 ± 0.08 , nmol/min/mg protein, mean $\pm$ S.D., $n = 4$, $p < 0.05$, Student's *t*-test). Since there is little information concerning sex difference in reductive metabolism of drugs containing a ketone group, it was interesting to note that such a sex difference was observed in acetohexamide reduction.

To obtain additional information about sex difference, acetohexamide reducing activities in

FIG. 1. Sex Difference of Acetohexamide Reducing Activity in Microsomes and Cytosol of Rat Liver
Ms, microsomes; C, cytosol. Each value represents mean $\pm$ S.D. The number of rats is given in parentheses.

TABLE I. *Effects of Various Inhibitors on the Acetohexamide Reducing Activity in Hepatic Microsomes and Cytosol of Male and Female Rats*

Inhibitor ^{a)}	Relative activity (%)		
	Male		Female
	Microsomes	Cytosol	Cytosol
None	100.0	100.0	100.0
Barbital	97.0 ± 1.0	67.8 ± 0.3	79.1 ± 3.5
Quercitrin	39.6 ± 6.0	83.5 ± 1.0	88.0 ± 2.9
Pyrazole	104.1 ± 7.7	97.0 ± 1.6	97.9 ± 1.6
Indomethacin	24.8 ± 4.5	46.1 ± 1.2	27.8 ± 4.4
SKF 525-A	87.4 ± 5.6	95.0 ± 2.6	94.2 ± 3.7

a) Concentration of inhibitors: barbital (1 mM), quercitrin (0.1 mM), pyrazole (10 mM), indomethacin (1 mM), SKF 525-A (0.1 mM). Each value represents mean ± S.D. (n = 3).

hepatic microsomes and cytosol were examined in male and female rats. In male rats, as shown in Fig. 1, both microsomes and cytosol exhibited acetohexamide reducing activity. However, in the case of females, only the cytosol exhibited the activity. Moreover, nicotinamide adenine dinucleotide phosphate (NADPH) was found to be a better cofactor than nicotinamide adenine dinucleotide (NADH) for all acetohexamide reducing activities in microsomes and cytosol of male and female rats (results not shown). These findings suggest that microsomal carbonyl reductase plays an important role in the sex difference of acetohexamide reduction in rats.

Table I summarizes the effects of various inhibitors on the acetohexamide reducing activities in hepatic microsomes and cytosol of male and female rats. Although barbital, an inhibitor of aldehyde reductase, inhibited acetohexamide reduction in the cytosol, it had no effect on its reduction in the microsomes. In contrast, quercitrin, an effective inhibitor which distinguishes ketone reductase from alcohol dehydrogenase or aldehyde reductase, markedly inhibited acetohexamide reduction in the microsomes. Pyrazole, a potent inhibitor of alcohol dehydrogenase, did not affect acetohexamide reduction in the microsomes and cytosol. Indomethacin, an inhibi-

tor of prostaglandin-9-ketoreductase⁴⁾ and human brain carbonyl reductase,⁵⁾ markedly inhibited acetohexamide reduction in the microsomes and cytosol but SKF 525-A did not. These findings indicate that the acetohexamide reducing enzyme in the microsomes is different from that in the cytosol.⁶⁾

Additional studies are in progress to elucidate the mechanism of sex difference in the reductive metabolism of acetohexamide in rat liver.

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