REDUCTION OF ACETOHEXAMIDE BY RABBIT HEART CYTOSOL

YORISHIGE IMAMURA, YUICHIRO KOJIMA, AND MASAKI OTAGIRI

Faculty of Pharmaceutical Sciences, Kumamoto University, 5-1, Oe-honmachi, Kumamoto, 862, Japan

(Received September 11, 1985)

The acetohehexamide reducing activity of cytosol of rabbit heart was compared with that of rabbit liver or kidney. The heart exhibited an approximately 2-fold higher activity than either the liver or kidney. Both aldehyde and ketone reductases may contribute to the reduction of acetohehexamide by cytosol of rabbit heart. It is noteworthy that the heart is an important organ reducing acetohehexamide.

**Keywords** — drug metabolism; acetohehexamide; metabolic reduction; rabbit heart cytosol; aldehyde reductase; ketone reductase

INTRODUCTION

In a previous paper,\(^1\) we reported that acetohehexamide, an oral antidiabetic drug, is reduced to a pharmacologically active metabolite\(^2\) in the cytosol of rabbit liver or kidney, and its reduction is catalyzed by ketone reductase. In this communication, we report the reduction of acetohehexamide by cytosol of rabbit heart. Little is known about the role of the heart in drug metabolism. Accordingly, present findings provide evidence that the heart plays an important role in drug metabolism.

MATERIALS AND METHODS

**Materials** — Acetohehexamide was supplied by Shionogi Pharmaceutical Co., Ltd. Nicotinamide adenine dinucleotide phosphate (NADPH) and Nicotinamide adenine dinucleotide (NADH) were purchased from Sigma Chemical Co., Ltd. All other chemicals used in this study were guaranteed reagents.

**Preparation of Cytosol** — Male albino rabbits (2.0–3.0 kg) were exsanguinated from the carotid artery. The tissues were removed and homogenized with 0.01 M phosphate buffer, pH 7.4, containing 1.15% KCl. The homogenate was centrifuged at 113000 × g for 60 min. The supernatant fraction (cytosolic fraction) was used to assay for enzyme activity.

**Assay of Acetohehexamide Reducing Activity** — Assay of acetohehexamide reducing activity was carried out according to the method described previously.\(^1\) Protein concentration in the cytosolic fraction was determined by the method of Lowry *et al.*\(^3\) with bovine serum albumin as a standard.

RESULTS AND DISCUSSION

The metabolic reduction of acetohehexamide was examined in rabbit heart, liver and kidney. The activities of heart, liver and kidney were 4.19 ± 0.56, 0.76 ± 0.04 and 2.12 ± 0.18 µmol/g tissue, respectively. In addition, the cytosol of rabbit heart exhibited an approximately 2-fold higher activity than that of the liver or kidney (heart; 11.36 ± 1.16, liver; 5.14 ± 0.25, kidney; 5.38 ± 0.46, nmol/min/mg protein). Since there is little information about the role of the heart in drug metabolism, it is interesting to note that the heart largely contributes to the reductive metabolism of acetohehexamide.

Carbonyl reductases (aldehyde and ketone reductases) and alcohol dehydrogenase are well known enzymes concerning the reductive metabolism of ketones. These enzymes can be distinguished by their specificity for NADPH or NADH.\(^4–6\) For acetohehexamide reducing activity by rabbit heart cytosol, NADPH was found to
Reduction of Acetohexamide by Heart Cytosol

TABLE I. Effect of Various Inhibitors on Acetohexamide Reducing Activity of the Cytosol of Rabbit Heart

<table>
<thead>
<tr>
<th>Inhibitor</th>
<th>Concentration (mM) (^a)</th>
<th>Relative activity (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pyrazole</td>
<td>10</td>
<td>98.9 ± 3.6</td>
</tr>
<tr>
<td>Phenobarbital</td>
<td>1</td>
<td>48.0 ± 0.5</td>
</tr>
<tr>
<td>Quercetin</td>
<td>0.1</td>
<td>60.2 ± 2.9</td>
</tr>
<tr>
<td>Quercitrin</td>
<td>0.1</td>
<td>70.6 ± 2.6</td>
</tr>
<tr>
<td>EDTA</td>
<td>1</td>
<td>98.1 ± 2.3</td>
</tr>
<tr>
<td>2,2'-Bipyridyl</td>
<td>1</td>
<td>96.4 ± 1.9</td>
</tr>
<tr>
<td>pCMB</td>
<td>0.1</td>
<td>65.4 ± 4.1</td>
</tr>
</tbody>
</table>

\(^a\) Concentration of inhibitors were determined according to ref. 5 and 7. Values represent means ± S.D. (n = 3).

be a better cofactor than NADH (NADPH; 7.06 ± 0.55, NADH; 0.11 ± 0.20, nmol/min/mg protein). This suggests that alcohol dehydrogenase is not an acetohexamide reducing enzyme.

Table I summarizes the effects of various inhibitors on the acetohexamide reducing activity of rabbit heart cytosol. Pyrazole and phenobarbital were potent inhibitors of alcohol dehydrogenase and aldehyde reductase, respectively. Quercetin and quercitrin were effective inhibitors which distinguish ketone reductase from alcohol dehydrogenase or aldehyde reductase. As shown in Table I, pyrazole and metal chelating reagents (ethylenediaminetetraacetic acid (EDTA) and 2,2'-bipyridyl) had little effect on the acetohexamide reducing activity, supporting the above assumption that alcohol dehydrogenase did not contribute to the reduction of acetohexamide. On the other hand, phenobarbital, quercetin, quercitrin and p-chloromercuribenzoic acid (pCMB) inhibited the acetohexamide reduction. This suggests that the reduction of acetohexamide in rabbit heart cytosol may be catalyzed by both aldehyde and ketone reductases.

Recently, we demonstrated that in the cytosol of rabbit liver or kidney, the reduction of acetohexamide is catalyzed only by ketone reductase. Consequently, the contribution of aldehyde reductase to the acetohexamide reduction is of interest. Further studies including the purification of these enzymes are in progress in our laboratory.

REFERENCES