Influence of Aging on Acetohexamide Reductase Activities in Liver Microsomes and Cytosol of Male Rats

Yorishige IMAMURA,† Yuri KOZONO, Hideyuki MURATA, and Masaki OTAGIRI

Faculty of Pharmaceutical Sciences, Kumamoto University, 5-1, Oe-honmachi, Kumamoto 862, Japan.
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The influence of aging on the reductase activity of acetohexamide, an oral antidiabetic drug with a ketone group, was examined in liver microsomes and cytosol of male rats. Acetohexamide reductase activities in liver microsomes of male rats at 26 and 31 months of age were much lower than that in liver microsomes of male rats at 9 weeks of age. Testectomy markedly decreased acetohexamide reductase activity in liver microsomes of the 9-week old rats and the decreased enzyme activity was significantly increased by testosterone administration. These results indicate, at least in part, that aging decreases the enzyme activity by decreasing the secretion of testosterone from the testes. On the other hand, aging (26 months of age) did not affect acetohexamide reductase activity in liver cytosol of male rats, although the enzyme activity at 31 months of age was slightly but significantly lower than that in liver cytosol of male rats at 9 weeks of age. Testectomy or testosterone administration had no effect on the enzyme activity in liver cytosol of 9-week old male rats.

Keywords acetohexamide reductase activity; aging; rat liver microsome; rat liver cytosol; testectomy

Activity of drug-metabolizing enzymes has been reported to be altered with aging.1–5) Several investigators propose that in male rats, age-related alteration in activity of drug-metabolizing enzyme is involved in feminization of the gonadal function.3–5) We have recently shown that the reductase activity of acetohexamide, an oral antidiabetic drug with a ketone group, in liver microsomes of male rats is androgen-dependent and is much higher than that in liver microsomes of female rats.6) These observations suggest that acetohexamide reductase activity in liver microsomes of male rats may be decreased with aging. The present study was undertaken to elucidate the influence of aging on this activity in liver microsomes of male rats. Influence of aging was also examined in liver cytosol of male rats, since its enzyme activity was detected in this fraction and the cytosolic enzyme appeared to be distinguishable from the microsomal enzyme.6)

MATERIALS AND METHODS

Materials Acetohexamide was supplied by Shionogi Co. (Osaka, Japan). Hydroxyhexamide was synthesized from acetohexamide according to the method of Girgis-Takla and Chronoes.7) NADP⁺, glucose-6-phosphate and glucose-6-phosphate dehydrogenase were purchased from Oriental Yeast Co. (Tokyo, Japan). Testosterone propionate was purchased from Sigma Chemical Co. (St. Louis, MO, U.S.A.). Other chemicals were of analytical grade.

Animals and Treatments Male rats (Fischer-344 strain, 9 weeks of age) and female rats (Fischer-344 strain, 9 weeks of age) were purchased from SLC Japan Inc. (Shizuoka, Japan) and male rats (Fischer-344 strain, 26 and 31 months of age) were supplied by Eisai Co. (Ibaraki, Japan). Testectomy of male rats was performed at 4 weeks of age. Testosterone propionate (5 mg/kg) dissolved in approximately 1 ml of corn oil was injected s.c. to the testectomized rats once every day for 7 d before they were sacrificed by decapitation at 9 weeks of age.

Assay of Enzyme Activity Acetohexamide reductase activity was assayed in the microsomal suspension or cytosolic fraction prepared from the liver of rats as reported previously.8) Protein concentration was determined by the method of Lowry et al.9) with bovine serum albumin as standard.

Data Analysis Data were analyzed statistically by Student's unpaired t-test. A p value of 0.05 or less was considered to be significant.

RESULTS

Table I shows acetohexamide reductase activities in liver microsomes and cytosol of male and female rats at 9 weeks of age and male rats at 26 and 31 months of age. A significant sex-related difference in the activity was observed between the two sexes at 9 weeks (p<0.001).

<table>
<thead>
<tr>
<th>Sex</th>
<th>Subcellular fraction</th>
<th>Activity (nmol/min/mg protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>9 weeks</td>
<td>26 months</td>
</tr>
<tr>
<td>Male</td>
<td>Microsomes</td>
<td>1.79±0.44</td>
</tr>
<tr>
<td>Cytosol</td>
<td>0.61±0.07</td>
<td>0.59±0.22</td>
</tr>
<tr>
<td>Female</td>
<td>Microsomes</td>
<td>0.04±0.06*</td>
</tr>
<tr>
<td>Cytosol</td>
<td>0.79±0.06*</td>
<td>—</td>
</tr>
</tbody>
</table>

Values are the mean ± S.D. of 4–9 rats. NS, not significant. a) Significantly different from the enzyme activity in liver microsomes of male rats at 9 weeks of age, \( p<0.001 \). b) Significantly different from the enzyme activity in liver cytosol of male rats at 9 weeks of age, \( p<0.001 \).

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<table>
<thead>
<tr>
<th>Subcellular fraction</th>
<th>Activity (nmol/min/mg protein)</th>
<th>Control</th>
<th>Testectomy</th>
<th>Testectomy + testosterone</th>
</tr>
</thead>
<tbody>
<tr>
<td>Microsomes</td>
<td>$p &lt; 0.001$</td>
<td>1.79 ± 0.44</td>
<td>0.19 ± 0.09</td>
<td>1.11 ± 0.34</td>
</tr>
<tr>
<td>Cytosol</td>
<td>NS</td>
<td>0.61 ± 0.07</td>
<td>0.65 ± 0.05</td>
<td>0.58 ± 0.02</td>
</tr>
</tbody>
</table>

Values are the mean ± S.D. of 4—9 rats. NS; not significant.

The enzyme activities in liver microsomes of male rats at 26 and 31 months were much lower than that in males at 9 weeks, with the activity at 31 months being decreased to the level of female rats at 9 weeks. The enzyme activity in liver cytosol of male rats at 31 months was slightly but significantly lower than that in liver cytosol of males at 9 weeks. We also examined the effects of testectomy and testosterone administration on acetoxyhexamide reductase activities in liver microsomes and cytosol of male rats (Table II). The enzyme activity in liver microsomes at 9 weeks was markedly decreased by testectomy. This decreased activity approximated the enzyme activity in liver microsomes of females at 9 weeks and was significantly increased by testosterone administration. In contrast, testectomy or testosterone administration had no effect on the enzyme activity in liver cytosol of male rats at 9 weeks of age.

**DISCUSSION**

This study provides evidence that aging causes a pronounced decrease of acetoxyhexamide reductase activity in liver microsomes of male rats. This is the first report of age-related alteration during older life in the metabolic reduction of drugs with a ketone group such as acetoxyhexamide, buprenorphine, and haloperidol. We have further shown that acetoxyhexamide reductase activity in liver microsomes of 9-week old male rats is markedly decreased by testectomy and that the decreased enzyme activity is significantly increased by testosterone administration. These results indicate, at least in part, that aging decreases the enzyme activity in liver microsomes of male rats by decreasing the secretion of testosterone from the testes. In fact, Kamataki et al. revealed that the serum level of testosterone in male rats at 25 months of age (1.09 ± 0.53 ng/ml) is significantly lower than that in these animals at 3 months (3.60 ± 1.49 ng/ml). It has been reported that testosterone exerts its action on drug-metabolizing enzymes such as cytochrome P 450 and glutathione S-transferases by changing the secretion pattern of growth hormone in the pituitary gland. In this manner, testosterone has an indirect effect on some drug-metabolizing enzymes through the hypothalamic-pituitary system. Further studies should be made to elucidate whether the influence of aging on acetoxyhexamide reductase activity in liver microsomes of male rats is mediated by the indirect effect of testosterone.

Acetoxyhexamide reductase activity in liver cytosol of male rats at 26 months of age was similar to that in liver cytosol of males at 9 weeks. However, the enzyme activity of males at 31 months was slightly but significantly lower than that of males at 9 weeks. The enzyme activity in liver cytosol of male rats was unaffected by testectomy. Thus, the decrease of acetoxyhexamide reductase activity in liver cytosol of males at 31 months may be independent of the decrease of testosterone secretion.

Our previous paper demonstrated postnatal development, sex-related differences and hormonal regulation of acetoxyhexamide reductase activities in liver microsomes and cytosol of Wistar rats. In this study, we used Fischer-344 strain rats. Interestingly, acetoxyhexamide reductase activity in liver microsomes of males was much higher in the Fischer-344 strain than in the Wistar strain. However, age-related alterations of the enzyme activity in the two strains seem to be based on a similar androgen-dependent mechanism. We are currently investigating details of the strain difference of acetoxyhexamide reductase activity in liver microsomes of male rats.

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**REFERENCES**