Change in the Protein Level of Mevalonate Pyrophosphate Decarboxylase in Tissues of Mouse by Pravastatin

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We previously reported that treatment of rats with a diet containing 0.1% pravastatin and 5% cholestyramine markedly increased mevalonate pyrophosphate decarboxylase (MPD) activity in liver crude extracts compared with nontreated rats. In this study, we examined the change in the protein level of MPD in the tissues of mice administered pravastatin. When MPD content in the tissues of nontreated mice was analyzed by quantitative immunoblotting, a single protein band with an apparent molecular weight of 46 kDa was detected in all tissues and the specific protein content of MPD in liver and kidney was markedly higher than that in other tissues. When MPD content in the tissues of pravastatin-treated mice was analyzed by immunoblotting, MPD was markedly increased (9-fold) only in the liver compared with nontreated mice. Next, when MPD activity was measured in the liver between nontreated and pravastatin-treated mice, MPD activity as well as protein levels were markedly increased (11-fold) in the liver of pravastatin-treated mice compared with nontreated mice. These data suggest that a marked induction of MPD in the liver by pravastatin is responsible for the tissue-specific effect of pravastatin.

Key words mevalonate pyrophosphate decarboxylase; tissue distribution; mouse

One of the first steps in the biosynthesis of cholesterol from acetic acid is catalyzed by mevalonate pyrophosphate decarboxylase (MPD). This decarboxylase catalyzes a bimolecular reaction between mevalonate pyrophosphate and ATP to form isopentenyl pyrophosphate, inorganic phosphate, ADP, and CO2. The enzyme has been purified from various sources, including yeast,1,2) latex of Hevea brasiliensis,3)pig liver,4,5) rat liver,6—8) mouse liver,9) and chicken liver.10) Toth and Huwyler reported the cDNA sequences of MPD from human liver and yeast.11) The recombinant human enzyme is a homodimer of a 43-kDa subunit with 400 amino acids. Epidemiological studies have indicated a negative association between the serum cholesterol level and the incidence of cerebral hemorrhage in humans.12) Spontaneously hypertensive rat stroke-prone (SHRSP) is a widely used animal model for hypertension and stroke.13) Iritani et al. reported that the serum cholesterol level in SHRSP was low when compared with that in normotensive age-matched Wistar Kyoto rats (WKY).14) We previously reported that the low serum cholesterol level in SHRSP as compared with WKY may be attributable to the reduced activity of MPD and not 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase.15) Moreover, we found that the decline in activity was caused by a reduction in the amount of enzyme in SHRSP.16) As described above, it is important to understand the mechanism of the decrease in MPD. We are also very interested in whether MPD-knockout or MPD-reduced mice experience cerebral hemorrhage. However, information on MPD in normal mice is relatively scarce, except for its characterization.

We previously reported that treatment of rats with a diet containing 0.1% pravastatin and 5% cholestyramine (CP diet) markedly increased MPD activity in a liver crude extract compared with nontreated rats.17) However, whether or not the expression of MPD by pravastatin is induced not only in the liver but also in other tissues remains unclear.

In the present study, we quantitatively measured the tissue distribution of MPD in mice and carried out a comparison of tissue distribution of MPD between nontreated and pravastatin-treated mice.

MATERIALS AND METHODS

Animals Male ddy mice (8 weeks old) administered pravastatin were used to examine the tissue distribution of MPD and change in the protein level of MPD in tissues. Male Wistar Kyoto rats (8 weeks old) were used in the induction of 37 kDa MPD experiment.

Materials Pravastatin was kindly provided by Sankyo Co., Ltd. and cholestyramine by Bristol Laboratories. All other chemicals were of reagent grade and purchased from commercial sources.

Purification of MPD from Mice The purification of MPD from mice was carried out as described by Michihara et al.9)

Purification of MPD Antibody The purification of MPD antibody was carried out as described by Michihara et al.8)

Radioactive Assay The enzyme activities of the crude extract were measured according to the method of Sawamura et al.15)

Preparation of Crude Extract Mouse or rat tissues were washed with 100 mM sodium phosphate (pH 7.0) containing 10 mM β-mercaptoethanol and 1 mM EDTA, and then homogenized in 3 volumes of ice cold homogenate buffer (100 mM sodium phosphate [pH 7.0] containing 1% Triton X-100, 10 mM β-mercaptoethanol, 1 mM EDTA, 0.5 mM PMSF, and protease inhibitors [1 mM leupeptin, 1 mM pepstatin A, 1 mM chymostatin, and 1 mM antipain]). The homogenate was centrifuged at 106000 × g for 1 h to obtain a crude extract.

Gel Electrophoresis SDS-PAGE was performed on 10% slab gels according to the method of Laemmli.17)

Immunoblot Procedures Proteins in SDS-slab gels were transferred to a nylon membrane (NEN) by electrophoresis, using a modified version of the procedure of Towbin et al.18) The positive bands were visualized using

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ECL Western blotting detection kits (Amersham Pharmacia, Amersham, U.K.) that contain a sensitive chemiluminescent substrate for horseradish peroxidase.

**Protein Determination** Proteins were determined by the method of Lowry et al.\(^1\) using bovine serum albumin as the standard.

**RESULTS AND DISCUSSION**

**Tissue Distribution of MPD in Male ddy Mice** We examined the expression of MPD in mouse tissues by Western blotting. As shown in Fig. 1, MPD appeared as a single band with a molecular weight of 46 kDa. When the amount of MPD in the crude extract was measured, a parallel experiment with known amounts (5—110 ng) of purified MPD from mouse liver was conducted to prepare a standard curve (Fig. 2). The signals were quantified with a Gel-Pro analyzer and the amount of MPD in the crude extract was quantitatively estimated from the standard curve. The specific content was determined by quantitative immunoblotting (Table 1). The crude extracts of brain, liver, kidney, and testis contained more MPD than other tissues, while those of heart and lung contained less MPD than other tissues. The highest level was detected in the liver, and the lowest level was detected in the heart. The brain contains more myelin than other tissues, and the testis is the major tissue in testosterone synthesis. As cholesterol is a major constituent of myelin and testosterone is synthesized from cholesterol, the brain and testis may contain more MPD than other tissues. A high level of MPD was observed in the kidney of mouse. These data suggest that a physiological role for MPD other than the synthesis of cholesterol and isoprenoid was present in the kidney.

**Change in Protein Level of MPD in Mouse Tissues by Pravastatin** Figure 3 shows a typical increase in the amount of MPD in the liver of the pravastatin-treated mice. The amount of MPD in the liver crude extract was markedly increased by treatment with pravastatin. When treated with 0.1% pravastatin for 15 d, a maximum amount up to 9-fold that in mice given normal chow was observed. Therefore, the tissue distribution of MPD was examined using tissues of mice treated with pravastatin for 15 d (Fig. 4). When the MPD content in tissues of pravastatin-treated mice was analyzed by immunoblotting, MPD was markedly increased only in the liver compared with nontreated mice (Fig. 5). Next, when the MPD activity was measured in the liver between nontreated and pravastatin-treated mice, MPD activity as well as protein levels were markedly increased (11-fold) in the liver of the treated mice compared with the nontreated mice (Fig. 6). It is known that the decreasing effect on serum cholesterol of pravastatin, an HMG-CoA reductase inhibitor, occurs only in the liver. Also, it was reported that HMG-CoA reductase and 14α-demethylase mRNA or protein levels involved in cholesterol biosynthesis were increased in rats fed a diet containing lovastatin, an HMG-CoA reductase inhibitor.\(^{2,21}\) These data indicate that HMG-CoA reductase inhibitors increase the protein level of enzyme involved in cholesterol biosynthesis by increasing mRNA. Therefore, it is suggested that the marked induction of MPD in the liver by pravastatin is responsible for the tissue-specific effect of pravastatin, and is caused by an increase in MPD mRNA.

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**Table 1. Amount of MPD in Mouse Tissues**

<table>
<thead>
<tr>
<th>Tissue</th>
<th>MPD in crude extract (ng/μg)</th>
<th>MPD in tissue (ng/mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Brain</td>
<td>1.5 ± 0.2</td>
<td>48 ± 4</td>
</tr>
<tr>
<td>Heart</td>
<td>0.1 ± 0.02</td>
<td>4 ± 0.5</td>
</tr>
<tr>
<td>Lung</td>
<td>0.5 ± 0.09</td>
<td>17 ± 0.8</td>
</tr>
<tr>
<td>Liver</td>
<td>3.1 ± 0.5</td>
<td>262 ± 12</td>
</tr>
<tr>
<td>Spleen</td>
<td>0.6 ± 0.15</td>
<td>35 ± 7</td>
</tr>
<tr>
<td>Kidney</td>
<td>2.0 ± 0.1</td>
<td>152 ± 20</td>
</tr>
<tr>
<td>Testis</td>
<td>3.1 ± 0.3</td>
<td>91 ± 11</td>
</tr>
</tbody>
</table>

Data are the means of four identical experiments.

**Induction of 38 kDa Protein in Mouse Liver by Pravastatin** We previously reported that a CP diet induced a new species, a 37 kDa subunit MPD, which is characteristically
and immunologically very similar to the well known 45 kDa subunit MPD in rat. In this study, pravastatin induced 38 kDa bands in the liver of mouse (Figs. 3, 4). As shown in Fig. 6, when treated with pravastatin for 15 d, a maximum activity and protein level up to 11- and 9-fold in mice given normal chow were observed, respectively. If the MPD activity was proportional to the protein level, the increase in MPD activity should be 9-fold. However, the increase in MPD activity (11-fold) was higher than that of the MPD protein level (9-fold). These data suggest the possibility that the 38 kDa protein in the liver of mice treated with pravastatin possessed the residual MPD activity.

Next, to examine the changes in the increases in MPD activity and protein level when protein retained MPD activity is induced, MPD activity and the protein level were measured in the liver of rats fed normal chow or the CP diet. As shown in Fig. 7, when treated with the CP diet for 12 d, maximum MPD activity or protein level of up to 17- or 10-fold in rats
given normal chow was observed, respectively. The increase in MPD activity (17-fold) in rats as well as mice was higher than that of the MPD protein level (9-fold). If the MPD activity was proportional to the protein level, the increase in MPD activity in rats given the CP diet should be 9-fold. Therefore, the additional MPD activity in the liver of rats given the CP diet was the activity of 37 kDa MPD. From these data, it was found that the increase in MPD activity was higher than that of the MPD protein level, if the induced protein has MPD activity. Therefore, it is highly likely that the 38 kDa protein in the liver of mice induced by pravastatin has MPD activity.

In conclusion, we have described quantitatively the tissue distribution of mouse MPD and the liver-specific induction of MPD by pravastatin. These data are useful for purifying MPD from mouse liver, preparing MPD-knockout mice, and investigating MPD-reduced mice.

REFERENCES