Tissue Distribution of Mevalonate Pyrophosphate Decarboxylase in Guinea Pig

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We previously reported that mevalonate pyrophosphate decarboxylase (MPD) is located in the cytosol and that MPD level in the liver is higher than in other rat tissues. In the present study, we further investigated the tissue distribution of MPD in guinea pigs by immunoblotting using anti-rat MPD antiserum. When immunoblot analysis was carried out using guinea pig brain, the antiserum reacted with 46-kDa protein as well as a substance with the same molecular weight of MPD in mice. Protein of 46-kDa detected in guinea pig liver treated with 0.1% pravastatin, a 3-hydroxy-3-methylglutaryl-CoA reductase inhibitor indicating a liver-specific effect, was increased 3-fold as compared with nontreated guinea pigs; however, 46-kDa protein in the brain treated with pravastatin was similar to that treated without pravastatin. When the subcellular distribution of MPD in the brain, liver, kidney, and testis, was examined by cell fractionation, MPD was mostly detected in the cytosolic fraction of all tissues. From these data, the 46-kDa protein was identified as MPD. Next, when the tissue distribution of MPD was examined, MPD in the liver was higher than in other tissues. The relative amount of MPD in guinea pig kidney was higher than in rats and similar to in mice, as MPD in the liver of the same species was taken as 1.

Furthermore, the correlation coefficient between guinea pigs and rats or mice in the tissue distribution of MPD was 0.69 or 0.72, respectively. These data indicate a relationship in tissue distribution between guinea pigs and rats or mice, although the tissue-specific regulator of MPD between species somewhat differed.

Key words mevalonate pyrophosphate decarboxylase; guinea pig; tissue distribution

One of the first steps in the biosynthesis of cholesterol from acetic acid is catalyzed by mevalonate pyrophosphate decarboxylase (MPD). This decarboxylase catalyzes a bi- molecular reaction between mevalonate 5-phosphate (MVAPP) and ATP to form isopentenyl pyrophosphate, inorganic phosphate, adenosine-5'-diphosphate (ADP), and CO₂.

MPD 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) Its properties in rats and chickens have been examined in detail. Toth and Huwiler reported cDNA sequences of MPD from human liver and yeast.11) The recombinant human enzyme is a homodimer of 43-kDa subunits with 400 amino acids. We recently established a procedure for purifying MPD from the liver of rats fed a diet containing 5% cholestyramine and 0.1% pravastatin using chromatography and polyclonal antiserum raised against rat MPD.12) We also previously reported that a high level of MPD was observed in the kidney of other species or whether anti-rat MPD antiserum reacted with MPD of other species other than mice.

In the present study, we found the tissue distribution of MPD in guinea pigs, after confirming that anti-rat MPD antiserum reacted with MPD of guinea pigs.

MATERIALS AND METHODS

Materials Pravastatin was kindly provided by Sankyo (Tokyo, Japan). All other chemicals were of reagent grade and purchased from commercial sources.

Animals Male Guinea pigs, Wistar rats, and ddY mice weighing 250, 250, and 30 g, respectively, were obtained from Shimizu Experimental Animals (Kyoto, Japan). They were housed in a light-controlled room (lights on, 6:00—18:00). Induction of MPD in the liver of guinea pigs was performed by ingesting water containing 0.1% pravastatin or normal water. The guinea pigs were fed 20 ml/d on average. The animal experiment was carried out according to the guidelines for animal experimentation, Faculty of Pharmacy and Pharmaceutical Sciences, Fukuyama University.

Preparation of Crude Extract Tissues (100 mg) of guinea pigs were homogenized in 3 volumes of Buffer H (0.1 M phosphate buffer (pH 7.0), 1 mM EDTA, 10 mM leucine, 1% Triton X-100, 0.5 mM PMSF, 0.1 μM leupeptin, 0.1 μM pepstatin A, 0.1 μM antipain, and 0.1 μM chymostatin). The homogenates were centrifuged at 10600×g for 1 h. The supernatants (crude extract) were subjected to sodium dodecyl sulfate-polyacrylamide (SDS-PAGE), followed by Western blot analysis using anti-rat MPD antiserum raised against 45-kDa MPD of rats, as described previously.3) The signals were measured by Intergen Quantifier (Bio Image).

Cell Fractionation Isolation of post-nuclear supernatant (PNS), mitochondria, peroxisome, lysosome (MPL), and microsomal and cytosol fractions from various guinea pig tissues by cell fractionation was carried out as described by Michihara et al.14) Tissues were homogenized with 3 volumes of homogenate buffer without Triton X-100. The homogenate was centrifuged at 10000×g for 10 min. The supernatant (PNS) was centrifuged at 20000×g for 10 min. (i) The supernatant contained cytosol and microsomes. (ii) The pellet contained MPL fraction. The supernatant of (i) was centrifuged at 106000×g for 1 h. The supernatant contained cytosol and the pellet contained microsomes.

Protein Determination Proteins were determined by the method of Lowry et al.15) with bovine serum albumin as standard.

Statistics Statistical analysis was carried out using Student's t-test. Data are the means ± S.D.
RESULTS

Identification of MPD in Guinea Pig We previously reported that MPD was purified from rat liver, and anti-rat MPD antiserum (MPD antiserum) was produced in rabbits by multiple injections of purified MPD with a molecular weight of 45-kDa.8) The antiserum reacted with not only 45-kDa protein in various rat tissues (brain, heart, lung, liver, spleen, kidney, and testis) but also 46-kDa protein in various mouse tissues.9,13) In the present study, we examined whether antiserum reacted with MPD in various guinea pig tissues. When crude extract of the brain of rats, mice, and guinea pigs was subjected to immunoblot analysis using MPD antiserum, this antiserum reacted with the protein of 45-kDa in rats and 46-kDa in guinea pigs as well as mice (Fig. 1).

There is known to be a marked increase in the enzyme involved in cholesterol biosynthesis in only the liver by treatment with pravastatin, a 3-hydroxy-3-methylglutaryl-CoA reductase inhibitor, indicating a liver-specific effect. Further to establish whether the 46-kDa protein is MPD, immunoblot analysis of the brain and liver of guinea pigs treated with pravastatin was performed. When immunoblot analysis was carried out using crude extract of the brain and liver of guinea pigs treated with normal water or water containing 0.1% pravastatin for 4 d, the level of 46-kDa protein in the brain treated with pravastatin was similar to that treated without pravastatin (Fig. 2A), and that in the testis treated with pravastatin was similar to that treated without pravastatin (data not shown); however, that in the liver treated with pravastatin was significantly increased compared with that treated without pravastatin (Fig. 2B). From these data, the 46-kDa protein in guinea pigs detected by MPD antiserum was indicated to be MPD.

Subcellular Distribution of MPD by Cell Fractionation We previously reported that MPD in rats and mice was predominantly located in cytosol.13,16) Further to identify that the 46-kDa protein is MPD, immunoblot analysis of the brain and liver of guinea pigs by cell fractionation was performed as described in Materials and Methods. When the 46-kDa protein level was compared in each fraction of the brain, liver, kidney, and testis, that in the cytosol fraction was increased as compared with that in PNS; however, that in the MPL and microsomal fraction was markedly decreased (Fig. 3). Since 46-kDa protein in guinea pigs was mostly located in the cytosol fraction, it was further suggested that this 46-kDa protein is MPD.

Tissue Distribution of MPD in Guinea Pig We examined the protein level of MPD in guinea pig tissues by immunoblot analysis. MPD appeared in various tissues as a band with a molecular weight of 46 kDa (Fig. 4A). Crude extracts of the brain and testis contained more MPD than other tissues, while those of the heart contained less MPD than other tissues (Fig. 4B). The highest level of MPD in 1 mg of tissues was detected in the liver, and the lowest level was detected in the heart (Fig. 4C).

Protein Level of MPD in Guinea Pig, Rat, and Mouse Kidney We previously reported that a high level of MPD was observed in the kidney of mice as compared with rats.12,13) To establish whether the high level of MPD observed in the kidney is, a mouse-specific effect, the relative...
The protein level of MPD in rat, mouse, and guinea pig kidney was estimated, and the protein level of MPD in the liver of the same species was taken as 1. The relative protein level of MPD in rat kidney was lower than that in mice and guinea pigs (Fig. 5); in addition, that of guinea pigs was similar to that of mice. These data indicate that a high level of MPD is observed in the kidney of guinea pigs as well as mice.

**Relationship between Guinea Pigs and Rats or Mice in Tissue Distribution of MPD**

Since a difference in the tissue distribution of MPD among guinea pigs, mice, and rats was present, the relationship between guinea pigs and rats or mice in the tissue distribution of MPD was examined. As shown in Fig. 6, the correlation coefficient between guinea pigs and rats or mice in the tissue distribution of MPD was 0.69 or 0.72, respectively. These data indicated a relationship in the tissue distribution between guinea pigs (46-kDa) and rats (45-kDa) or mice (46-kDa).

**DISCUSSION**

In the present study, we indicated that MPD antiserum reacted with MPD in the various guinea pig tissues (Fig. 4). The antiserum findings will be useful in the purification and characterization of MPD from guinea pigs, analysis of detailed subcellular distribution of MPD from guinea pigs, and analysis of the protein level of guinea pig MPD treated with serum cholesterol-lowering drugs.

To correct for the difference of reactivity by MPD antiserum among species, the relative protein level of MPD in rat, mouse, and guinea pig kidney was estimated, with the protein level of MPD in the liver of the same species taken as 1, indicating that a high level of MPD was observed in the kidney of guinea pigs as well as mice (Fig. 5). If the physiological role for MPD is other than the synthesis of cholesterol, upregulation of isoprenoid or modification of the Ras, Rho, Rab family might occur in the kidney of mice and guinea pigs. It is known that the cholesterol biosynthetic enzyme is regulated by hormones such as insulin and glucagon; therefore suggesting the possibility that enzymes involved in the synthesis of isoprenoid containing MPD are controlled by hormones with a kidney-specific effect in mice and guinea pigs. MPD in rat kidney was significantly decreased as compared with guinea pigs and mice (Fig. 5). These data suggest that the tissue-specific regulator of MPD differs by species. Furthermore, there may be a relationship in the tissue distribution between guinea pigs and rats or...
mice. In conclusion, we found that tissue with a high and low level of MPD was similar between guinea pigs and rats or mice, although the tissue-specific regulator of MPD somewhat differed among species. Further study is required to understand the physiological role of MPD in each tissue, including the kidney.

REFERENCES