Mevalonate Pyrophosphate Decarboxylase in Stroke-Prone Spontaneously Hypertensive Rat Is Reduced from the Age of Two Weeks

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We carried out a comparison of tissue distribution of mevalonate pyrophosphate decarboxylase (MPD) between normotensive Wistar Kyoto rat (WKY) and stroke-prone spontaneously hypertensive rat (SHRSP) using Western blotting. However, there was no difference in tissue distribution of MPD between WKY and SHRSP, except in brain and liver. We then compared the MPD between WKY and SHRSP liver at several weeks of age. We found that MPD in the liver as well as brain of SHRSP was significantly reduced from two weeks of age. This data is useful to identify or understand the mechanism underlying the reduced amount of MPD in SHRSP.

Key words mevalonate pyrophosphate decarboxylase; WKY; SHRSP; liver; tissue distribution; weeks of age

One of the first steps in the biosynthesis of cholesterol from acetic acid is catalysed by mevalonate pyrophosphate decarboxylase (MPD). This decarboxylase catalyzes a bimolecular reaction between mevalonate pyrophosphate and ATP to form isopentenyl pyrophosphate, inorganic phosphate, ATP 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) and chicken liver.9) Toth and Huwyler reported the cDNA sequences of MPD from human liver and yeast.10) The recombinant human enzyme is a homodimer of a 43 kDa subunit with 400 amino acids.

Stroke-prone spontaneously hypertensive rat (SHRSP) is a rat that suffers from severe hypertension and cerebral hemorrhage.11,12) SHRSP has a lower serum cholesterol level than the normotensive Wistar Kyoto rat (WKY).13) We previously indicated that the lower activity of MPD caused by the reduced amount of this enzyme was responsible for the reduced cholesterol biosynthesis in the liver of SHRSP.14) However, the mechanism underlying the reduced amount of this enzyme remains unclear.

In this paper, we report a comparison of tissue distribution between WKY and SHRSP; the difference in MPD content recognized by tissue of varying weeks of age was also compared.

MATERIALS AND METHODS

Animals Male WKY and SHRSP (1, 2, 3, 5, 9 weeks old) were housed in a light-controlled room (light phase, 6:00–18:00). These rats were weaned at three weeks of age.

Immunoblot Procedures Various tissues were homogenized in 3 volumes of Buffer H (0.1 M phosphate buffer (pH 7.0), 1 mM EDTA, 10 mM 2-mercaptoethanol, 0.5 mM phenylmethylsulfonyl fluoride (PMSF), 0.1 mM leupeptin, 0.1 mM pepstatin A, 0.1 mM antipain, 0.1 mM chymostatin). The homogenates were centrifuged at 20000 × g for 30 min. The supernatants (crude extract) were subjected to sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) followed by Western blot analysis using anti-MPD antiserum raised against the purified 45 kDa MPD of WKY as described previously.8) The signals were measured with a Shimadzu Chromatoscanner (S-910) (Shimadzu, Tokyo).

Statistics Statistical analysis was carried out using Student’s t test. Data are presented as mean ± S.D.

RESULTS AND DISCUSSION

Comparison of Tissue Distribution of MPD between WKY and SHRSP We examined the expression of MPD in various tissues by Western blotting. As shown in Fig. 1, MPD protein in SHRSP as well as WKY showed a single band with a molecular weight of 45 kDa MPD. As shown in Fig. 2, this amount of 45 kDa MPD was reduced in the crude extract and tissue of the brain and liver of SHRSP, while no change was observed in the heart, lung, stomach, small intestine, large intestine, kidney, or testis. These data indicated that these two organs are very important tissues by which to

Fig. 1. Tissue Distribution of MPD in WKY and SHRSP

Slices (0.25 g) of various tissues in WKY and SHRSP fed on normal chow were homogenized in Buffer H (0.1 M phosphate buffer (pH 7.0), 1 mM EDTA, 10 mM 2-mercaptoethanol, 0.5 mM PMSF, 0.1 mM leupeptin, 0.1 mM pepstatin A, 0.1 mM antipain, 0.1 mM chymostatin). The homogenates were centrifuged at 20000 × g for 30 min. The supernatants (crude extract, 60 μg) were subjected to SDS-PAGE, followed by immunoblot analysis using anti-MPD antiserum raised against the purified 45 kDa MPD of WKY.

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understand the mechanism underlying the reduced amount of MPD in SHRSP. It is generally known that cholesterol in brain is synthesized by the brain itself.\textsuperscript{15,16} Recently, a part of the cholesterol in brain is suggested to be uptake by receptor-mediator from outside the brain.\textsuperscript{17} Therefore, we believe that the low level of serum cholesterol and cholesterol biosynthesis in brain caused by the reduced amount of MPD in brain and liver of SHRSP may cause the cerebral stroke in this rat.

The crude extract of brain, liver and testis in WKY contained 45 kDa MPD an amount higher than that in other tissues. However, no difference was observed in testis between WKY and SHRSP. That the amount of 45 kDa MPD between the two animal types is the same may suggest that MPD has an important roles other than cholesterol synthesis alone. The testis is the major organ of testosterone synthesis. If the main role of 45 kDa MPD in testis is the cholesterol synthesis involved in testosterone synthesis, testosterone synthesis may be performed by the cholesterol synthesized from the testis itself, independent of serum cholesterol. Comparison of the amount of testosterone between WKY and SHRSP is necessary to understand the main role of 45 kDa MPD in testis.

**COMPARISON OF MPD WEEKS OF AGE BETWEEN WKY AND SHRSP**

An experiment duplicating the conditions of Fig. 1 was performed three times. Signals of bands were measured by chromatoscanner. Each value represents the standard deviation of triplicate determinations. A: MPD in 1 \( \mu \)g of crude extract; B: MPD in 1 g of tissues. WKY (○) and SHRSP (■). Significantly different, \( p<0.05 \) (a).

**SHRSP** In WKY and SHRSP liver, both MPD proteins remained low during the two weeks after hatching, then increased sharply by three weeks of age, and remained practically unchanged thereafter (Fig. 3). As shown in Fig. 4, a reduced amount of MPD in the liver was observed at two weeks and later in SHRSP. We previously reported that the reduced amount of MPD in the brain was observed at two weeks of age in SHRSP.\textsuperscript{14}

In conclusion, we found for the first time that MPD in the liver as well as brain of SHRSP was significantly reduced from the age of two weeks. This data is useful in identifying the mechanism underlying the reduced amount of MPD in SHRSP.

**REFERENCES**


