— Research Letter —

Comparison of the Gene Expression Levels of Mevalonate Pyrophosphate Decarboxylase between Stroke-prone Spontaneously Hhypertensive and Wistar Kyoto Rats

Akihiro Michihara,* Mai Shimatani, and Kenji Akasaki

Faculty of Pharmacy and Pharmaceutical Sciences, Fukuyama University, Gakuencho, Fukuyama 729–0292, Japan

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The relationship between protein and mRNA levels of mevalonate pyrophosphate decarboxylase (MPD) in rat tissues remains to be clarified. In this study, we examined the distribution of the mRNA in Wistar rat tissues by real-time PCR. When the relative expression of MPD in 1 mg of tissue was quantified using glyceraldehyde-3-phosphate-dehydrogenase (GAPDH) as an internal control, the mRNA level was found to be markedly higher in the spleen and liver than in other organs. The correlation coefficient between protein and mRNA levels of MPD was 0.847, indicating that the protein level of MPD in Wistar rat is distributed in the tissues almost entirely dependent on the mRNA level of MPD. We previously reported that the protein levels of MPD in the liver and brain of spontaneously hypertensive rats, stroke-prone (SHRSP) are reduced. Thus, we compared mRNA levels of MPD in the liver and brain between Wistar Kyoto rats (WKY) and SHRSP. The levels in liver of SHRSP were significantly decreased, but not in brain, as compared with WKY. Also, mRNA levels of sterol regulatory element binding protein-2, which is transcription factor of cholesterol, in liver of SHRSP was similar to those of WKY. These findings indicate that the reduced protein level of MPD in the liver of SHRSP is caused by a decrease in mRNA level of MPD, and that in the brain of SHRSP is caused by increased degradation of the MPD protein.

*To whom correspondence should be addressed: Faculty of Pharmacy and Pharmaceutical Sciences, Fukuyama University, Gakuencho, Fukuyama 729–0292, Japan. Tel.: +81-849-36-2111; Fax: +81-849-36-2024; E-mail: mitihara@fupharm.fukuyama-u.ac.jp

Key words —— mevalonate pyrophosphate decarboxylase, distribution, tissue, expression, rat

INTRODUCTION

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 5-pyrophosphate and ATP to form isopentenyl pyrophosphate, inorganic phosphate, adenosine-5′-diphosphate, and CO_2.

Epidemiological studies have indicated a negative association between the serum cholesterol level and the incidence of cerebral hemorrhage in humans.[1] The spontaneously hypertensive rats, stroke-prone (SHRSP) are a widely used animal model of hypertension and stroke.[2] 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).[3] The low level may be attributable to reduced activity of MPD and not 3-hydroxy-3-methylglutaryl coenzyme A reductase.[4] Moreover, we found a decline in MPD activity caused by a reduction in the amount of the enzyme in liver of SHRSP.[5] It is important to understand the mechanism of this decrease. It was also known that SHRSP possess genetic factors related to hypertension, cerebral hemorrhage, and lower serum cholesterol despite normal chow treatment. Therefore, it was discussed that reduction of MPD protein level was caused by mutation of gene. However, there is little information on the gene expression of MPD in rats, although analyses of the MPD protein have been performed.[6–13] Thus, we examined the distribution of the mRNA levels in Wistar rat tissues by real-time PCR, to clarify the relationship between protein and mRNA levels of MPD. Next, we compared the mRNA levels of MPD in liver and brain between SHRSP and normotensive WKY.

MATERIALS AND METHODS

Materials —— The Biomasher was obtained from Assist (Tokyo, Japan), QuickGene RNA tissue kit SII from Fujifilm (Tokyo, Japan), and SYBR Ex Script RT-PCR kit from TaKaRa (Otsu, Japan). All

Table 1. Primers Used for PCR

Genes	Primer sequence	Size (bases)	Position	Gene Bank
MPD	F5′-AGGACCGCATCTGGCTGAAC R5′-TGACGCCACGTGCACCTTA	158	230– 387	NM-031062
SREBP-2	F5′-GGAAGGCCATTGATTACATCAAG R5′-TTGCCAGCTTCAGCACCAT	81	1228–1308	NM-001033694
GAPDH	F5′-GGCACAGTCAAGGCTGAGAATG R5′-ATGGTGGTGAAGACGCCAGTA	143	242– 384	NM-017008

other chemicals of reagent grade, and purchased from commercial sources.

Animals —— Male Wistar rats weighing 200 g were obtained from Shimizu Experimental Animals (Kyoto, Japan). Male (9–10 weeks old) SHRSP/Izm and WKY/Izm were obtained from the Disease Model Co-operative Research Association, Kyoto, Japan. Tissue samples were stored at −80°C.

Protein and Total RNA Determination —— Protein levels were measured by the method of Lowry *et al.* using bovine serum albumin (BSA) as the standard.[14] The concentration of total RNA was calculated using a Qubit™ fluorometer (Invitrogen, Tokyo, Japan).

Real-time Quantitative Reverse Transcription-polymerase Chain Reaction (Real-time PCR) —— Rat tissue (15 mg) was homogenized using the Biomasher. The homogenate was dissolved using the QuickGene RNA tissue kit SII, and total RNA (60 μl) was isolated using the same kit (total RNA extract kit) and QuickGene-810 (Nucleic Acid Isolation System; Fujifilm). One hundred nanogram sample of total RNA from each tissue was subjected to reverse transcription (RT) using reverse transcriptase in a 50 μl reaction volume. After the RT reaction, the cDNA template was amplified by polymerase chain reaction with a SYBR Ex Script RT-PCR Kit (TaKaRa, Otsu, Japan). SYBR Green was used for the real-time PCR analysis. Real-time PCR was performed using a ABI7500 system (Applied Biosystems Japan, Tokyo, Japan). Cycling conditions were 40 cycles of 96°C for 35 s, 64°C for 35 s and 72°C for 35 s. Relative gene expression was quantified using glyceraldehyde-3-phosphate-dehydrogenase (GAPDH) as an internal control. As shown in Table 1, the primer pairs of GAPDH, MPD and sterol regulatory element binding protein-2 (SREBP-2) were used in real time-PCR. The cDNA products obtained by RT-PCR were electrophoresed on a 2% agarose gel and visualized by ethidium bromide staining under UV light.

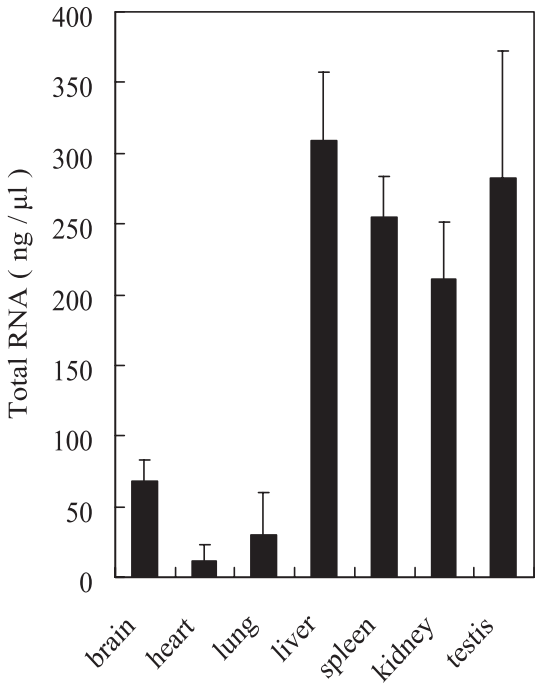

Fig. 1. Comparison of Total RNA in Rat Tissues

Sixty microliters of total RNA was extracted from 15 mg of rat tissue as described in Materials and Methods. Data are the mean for three identical experiments.

Statistical Analysis —— The statistical analysis was carried out using Student's *t*-test. Data are presented as the mean ± S.D.

RESULTS AND DISCUSSION

Extraction of Total RNA from Rat Tissues and RT-PCR

After 15 mg of rat tissue was homogenized using the Biomasher, total RNA was extracted with the QuickGene RNA tissue kit SII. As shown in Fig. 1, total RNA in the liver, spleen, kidney, and testis was well extracted, but that from the brain, heart, and lung was minimal.

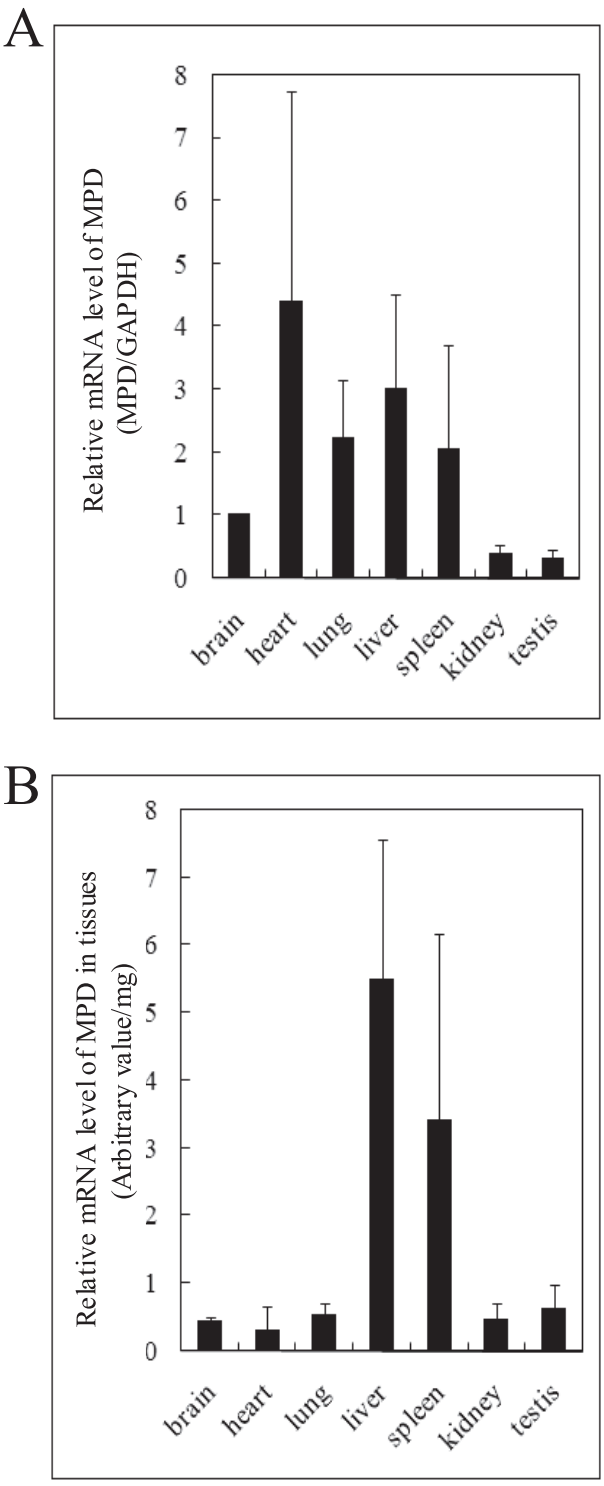

Fig. 2. Comparison of MPD mRNA Levels in Rat Tissues

A: The relative level of MPD in total RNA of tissues was quantified using GAPDH as an internal control. B: From the results of Figs. 1 and 2A, the relative MPD mRNA in 1 mg of tissue was estimated. Data are the mean for three identical experiments.

Comparison of the MPD mRNA Level in Rat Tissues

Real-time PCR was carried out using 100 ng of total RNA, and most of the MPD in 1 μg of the RNA was found to exist in the heart, lung, and liver, with minimal amounts in the kidney and testis (Fig. 2A). From the results in Figs. 1 and 2A, when the relative MPD mRNA level in 1 mg of tissue was measured, MPD was mostly found in the liver and spleen (Fig. 2B).

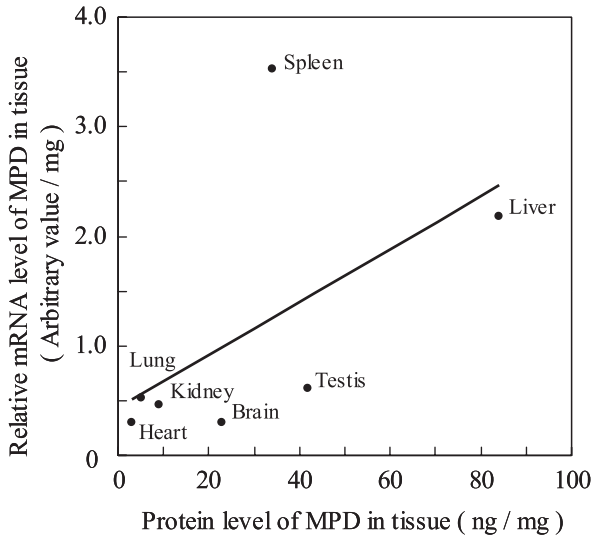

Fig. 3. Relationship between Expression and Protein Level of MPD in Tissue Distribution

The mRNA and protein level of MPD in 1 mg of tissues was determined simultaneously to obtain a regression line.

Correlation between Protein and mRNA Level of MPD in Tissue

When the correlation between the previously reported protein and mRNA level of MPD in the tissue of Wistar rat was estimated,[8] the correlation coefficient in the tissue distribution was 0.847 (Fig. 3). These findings indicated that the protein level of MPD in rat is distributed in the tissues almost entirely dependent on the mRNA level of MPD.

Analysis of the mRNA Levels of MPD in Liver and Brain Using Real-time PCR

We previously reported that the protein levels of MPD in the liver and brain of SHRSP are reduced.[5] However, it is not known whether the reduced amount of MPD in SHRSP is caused by a decrease in MPD mRNA levels or by an increase in degradation of the MPD protein. Therefore, we measured the mRNA levels of MPD in the liver and brain of SHRSP and WKY using real-time PCR. The levels in liver of SHRSP were significantly decreased as compared with WKY (Fig. 4A). The ratio of decrease of mRNA levels (52%) of MPD in liver were similar to previously reported protein levels

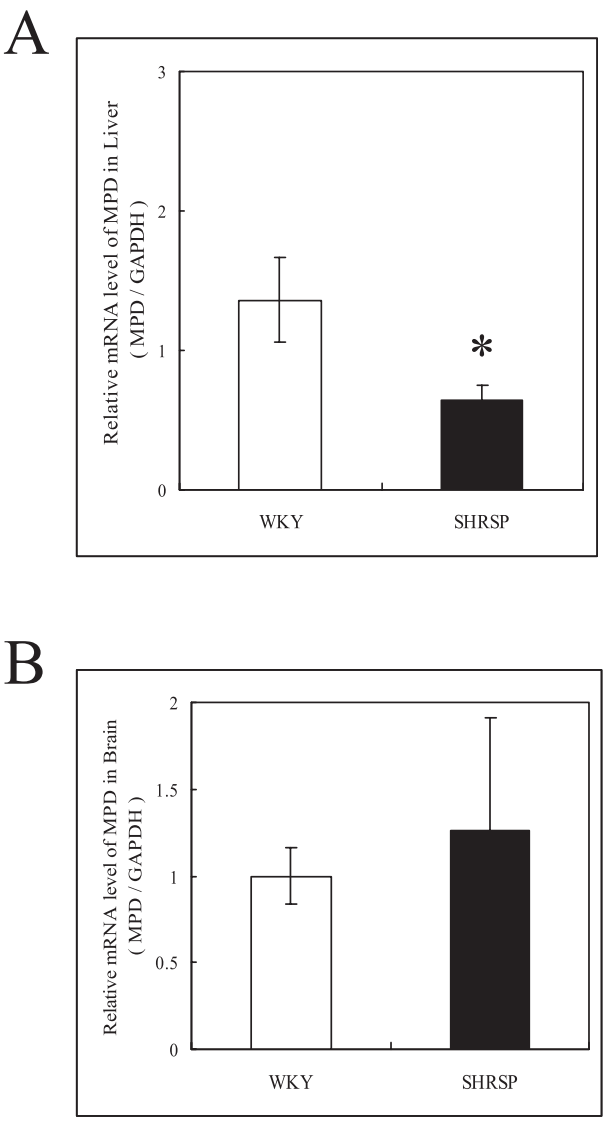

Fig. 4. mRNA Levels of MPD in the Liver and Brain of SHRSP and WKY

RT-PCR was performed using the primers listed in Table 1 with total RNA from the liver and brain of SHRSP and WKY, as described in Materials and Methods. cDNA products (1 μl) were electrophoresed on a 2% agarose gel and visualized with ethidium bromide staining under UV light. PCR resulted in a single band, and therefore real-time PCR was performed as described in Materials and Methods. Data are the mean for three identical experiments. Significant differences: $^{*}p < 0.05$.

(55%).[5] It was suggested that a decrease of MPD protein levels in liver reflects a reduction of MPD mRNA levels. The levels in brain of SHRSP were slightly increased (Fig. 4B). As the previously reported protein levels (48%) in brain of SHRSP were significantly decreased,[5] it was suggested that a decrease of MPD protein levels in brain were caused by an increase in degradation of the MPD protein. These findings indicate that the mechanisms of MPD protein decrease differed between liver and brain.

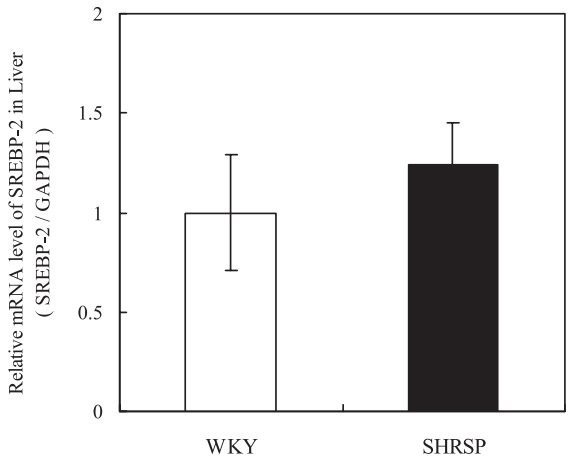

Fig. 5. mRNA Levels of SREBP-2 in the Liver of SHRSP and WKY

RT-PCR was performed using the primers listed in Table 1 with total RNA from the liver of SHRSP and WKY, as described in Materials and Methods. cDNA products (1 μl) were electrophoresed on a 2% agarose gel and visualized with ethidium bromide staining under UV light. PCR resulted in a single band, and therefore real-time PCR was performed as described in Materials and Methods. Data are the mean for three identical experiments.

Analysis of the mRNA Levels of SREBP-2 in Liver Using Real-time PCR

A number of cholesterol biosynthetic enzymes containing 3-hydroxy-3-methylglutaryl (HMG)-CoA reductase and MPD are regulated at the transcriptional level and their transcriptional regulation is controlled by a family of transcription factors, SREBP-2. To clarify whether the reduced MPD mRNA levels in liver of SHRSP were caused by a decrease of SREBP-2, we measured the mRNA levels of SREBP-2 in the liver of SHRSP and WKY using real-time PCR. The levels in SHRSP were similar to those in WKY (Fig. 5). Therefore, it was suggested that reduction of MPD mRNA levels in liver of SHRSP were caused by a mechanism specifically MPD decreasing. We previously reported that the levels of MPD protein in the liver and brain of SHRSP were reduced from as early as 2 weeks of age.[15] As described in the Introduction, SHRSP possess genetic factors related to lower serum cholesterol despite normal chow treatment. Therefore, we hypothesized that the reduced mRNA level of MPD in liver of SHRSP from 2 weeks of age is caused by a decrease in transcription factors (binding factors) for the promoter rather than a mutation or change in the promoter's sequence of MPD genes located on chromosomes 19. Also, we suggested that the reduced protein level of MPD in brain of SHRSP from 2 weeks of age is caused by an accelerated degradation mechanism or by

an increase of protease causing degradation of the MPD protein. Once the promoter sequences and transcriptional factors of MPD in liver or protease causing degradation of MPD in brain are clarified, experiments comparing the expression of MPD gene in liver and the protease causing degradation of MPD protein in brain between SHRSP and WKY will be necessary.

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