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Increased Expression of Carnitine Palmitoyltransferase I Gene Is Repressed by Administering L-Carnitine in the Hearts of Carnitine-Deficient Juvenile Visceral Steatosis Mice

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The juvenile visceral steatosis (JVS) mouse is a novel mutant animal for studying systemic carnitine deficiency. The importance of the model has been pointed out in carnitine-deficient cardiac hypertrophy, since cardiomyopathy has been often improved after oral carnitine therapy in human systemic carnitine deficiency. To understand the effects of carnitine deficiency on gene expression in the heart, we tried to find the genes regulated by carnitine by means of a modified differential display procedure. Carnitine palmitoyltransferase I (CPT I) was one of the isolated genes. The level of CPT I gene expression in the ventricles of the JVS mice was at least three- to sixfold that of normal mice as judged by reverse transcription-polymerase chain reaction (RT-PCR). When the JVS mice were treated with carnitine, CPT I gene expression was repressed to the level of normal mice. Therefore, the increased expression of the CPT I gene was associated with carnitine deficiency.

Key words: cardiac hypertrophy, carnitine palmitoyltransferase I, differential display, gene expression, systemic carnitine deficiency.

Juvenile visceral steatosis (JVS) in mouse is inherited in an autosomal recessive manner and associated with severe lipid accumulation in the liver, hypoglycemia, hyperammonenia, and cardiac hypertrophy (*1-3*). The affected mice are identified by a whitish liver within 3-4 days of age, which can be observed through the abdominal skin. Growth retardation appears 2 weeks after birth and most JVS mice die within 5 weeks. JVS mice are systemically deficient in L-carnitine (β-hydroxy-γ-trimethyl aminobutyric acid) (*4*), and Horiuchi *et al.* showed that the primary defect of JVS mice is in the reabsorption system for carnitine in the kidney (*5*). Carnitine administration improves growth retardation and cardiac hypertrophy (*6-9*). A decline in the expression of urea cycle enzyme genes in the liver of JVS mice causes hyperammonenia and is improved by administering carnitine (*6, 7*). It is suggested that the cardiac hypertrophy in JVS mice is caused by an increase in the noncollagen protein content, which increases the mass of cardiac myocytes (*9*). Miyagawa *et al.* have reported that the number of mitochondria increases at 4 weeks of age and further increases at 8 weeks in the myocytes of JVS mice, according to an electron microscopy study (*10*).

Carnitine is an essential cofactor in transferring long-chain fatty acids across the mitochondrial outer and inner membranes *via* carnitine palmitoyltransferase I (CPT I) and CPT II, respectively. Since fatty acids constitute a major source of fuel for cardiac muscle, a carnitine deficiency should impair energy production in the heart (*11*). Recently, it has been reported that carnitine-depletion by inhibitors enhances the CPT I enzymatic activity in rat liver, but it is unknown whether the gene expression itself is enhanced (*12*).

Many cases of human systemic carnitine deficiency have been reported (*13-21*), and one of the most common clinical manifestations in these patients is cardiomyopathy. In addition, intermittent haemodialysis causes a carnitine loss and expands cardiomyopathy in some patients with renal failure (*22*). From this aspect, it is important to know the pathophysiology of the cardiomyopathy caused by carnitine deficiency. To understand the molecular events involved in the effects of carnitine, we studied the gene expression in the hearts of JVS mice. We applied a modified differential display procedure (*23*) to isolate and identify genes that are differentially expressed in normal *versus* JVS hearts. We found that expression of the CPT I gene is specifically increased in the JVS ventricles and that administration of carnitine represses the increased expression of the CPT I gene.

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Abbreviations: CPT I, carnitine palmitoyltransferase I; CPT II, carnitine palmitoyltransferase II; GAPDH, glyceraldehyde 3-phosphate dehydrogenase; JVS, juvenile visceral steatosis; RT-PCR, reverse transcription-polymerase chain reaction.

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MATERIALS AND METHODS

Animals—All animals were maintained under specific pathogen-free conditions. Homozygous mutants designated jvs/jvs had a swollen fatty liver that was recognizable through the abdominal wall at 2-5 days after birth. These mutants were considered to be JVS mice. These mutant mice were produced by mating heterozygous mutant (jvs/+) males and females. Homozygous controls (+/+) were obtained by mating C3H-OH mice, the original strain of the JVS mice and these were used as wild-type (designated as normal) controls. Five micromoles of L-carnitine (Sigma) was administered intraperitoneally once each day. The mice were sacrificed by an intraperitoneal injection of pentobarbital (0.1 mg/g body weight). Isolated tissues were immediately frozen at −80°C until use.

Differential Display of mRNA—Total RNA was prepared from whole hearts using Isogen (Nippon Gene, Toyama), and poly(A)$^+$ RNA was purified from the total RNA fraction using Dynabeads^R Oligo(dT)$_{25}$ (Dynal A.S, Oslo, Norway). Twelve reverse-transcription reaction mixtures were prepared for poly(A)$^+$ RNA (0.1 μg) in a reverse-transcription buffer containing 5 mM dithiothreitol, 20 μM each dATP, dGTP, dCTP, and dTTP, and 0.2 μM oligo(dT)$_{12-14}$ of either (dT)$_{14}$AA, (dT)$_{13}$AG, (dT)$_{13}$AC, (dT)$_{14}$AT, (dT)$_{13}$GA, (dT)$_{12}$GG, (dT)$_{12}$GC, (dT)$_{13}$GT, (dT)$_{13}$CA, (dT)$_{12}$CG, (dT)$_{12}$CC, or (dT)$_{13}$CT oligonucleotides. The reaction mixture was heated to 65°C for 5 min, cooled to 37°C, then incubated at 37°C for 65 min with 100 units of RNase H minus reverse-transcriptase (Superscript II, Life Tech, Gaithersburg, MD, USA). After incubation, the mixture was heated to 95°C for 5 min to inactivate the enzymes prior to storage at 4°C and then the 12 kinds of cDNAs synthesized as above were mixed. The PCR reaction for mRNA display was performed in reaction mixtures containing 0.05 volume of the cDNA mixture, PCR buffer, 20 μM each dGTP, dCTP, dTTP, 2 μM dATP, 4 μCi (1 Ci=37 GBq) of [^{35}S]dATP, 0.5 unit of AmpliTaq DNA polymerase (Perkin Elmer Cetus, Norwalk, CT, USA) and 0.6 μM concentrations of two arbitrary 12-mer oligonucleotides. We used two random 12-mer oligonucleotides with arbitrary combinations from among 72 different nucleotide sequences, which were obtained from Wako Chemical (Kyoto). The PCR reactions were as follows: 3 cycles of 94°C for 30 s, 37°C for 2 min, and 72°C for 30 s; and 37 cycles of 94°C for 30 s, 43°C for 2 min, and 72°C for 30 s and finally at 72°C for 5 min. The PCR products were dissolved in a DNA loading solution (95% formamide, 10 mM EDTA, pH 8.0, 0.09% xylene cyanole FF, and 0.09% bromophenol blue) and heated to 80°C for 2 min prior to loading on 6% polyacrylamide sequencing gels. Gels were run at a 65 W constant current, dried without fixation, then exposed directly to Fuji Medical X-ray film for 2 days at room temperature followed by autoradiography. Electrophoresis was performed twice for each sample for different periods. The first run separated shorter fragments and the longer fragments were resolved by the second run.

Recovery of DNA Fragments and Sequencing—Bands that were more intense in samples from JVS mice than in those from normal mice were excised using a razor. The dried gel slices were soaked in distilled water for 10 min and boiled for 15 min. DNA fragments soaked in the supernatant were collected and precipitated with ethanol in the presence of glycogen, then washed with 85% ethanol. The DNA fragments were reamplified using the same primer sets and PCR conditions with an additional 20 μM dATP and without radioactive dATP. The amplified DNA fragments were isolated by 1% agarose gel electrophoresis and subcloned into the T-tailed *Eco*RV site of Bluescript II (Strategene, La Jolla, CA, USA). The nucleotide sequences of the inserts were determined with an automatic DNA sequencer (Applied Biosystems, Foster City, CA, USA) using a Taq Dye Deoxy Terminator Cycle Sequencing Kit (Applied Biosystems).

RT-PCR of mRNA—RNA was extracted from the ventricles, but not the total hearts, and from the total brain. About 50 ng of poly(A)$^+$ RNA purified as described was reverse-transcribed with random hexamers and RNase H minus reverse-transcriptase (Superscript II) in 20-μl reaction mixtures. PCR reactions were performed in 50-μl reaction mixtures containing PCR buffer, 200 μM each dATP, dGTP, dCTP, dTTP, and 1.5 μCi of [α-^{32}P]dCTP, 0.4 μM concentrations of primers, the pooled cDNA, and 1.2 units AmpliTaq DNA polymerase. The PCR reaction was performed with primers for the CPT I gene (forward primer for CPT I and reverse A primer for CPT I), then a primer set for the individual internal control was added after the indicated cycles of the PCR reaction for normalizing the semi-quantitative analysis. GAPDH (glyceraldehyde 3-phosphate dehydrogenase), CPT II, and cytochrome *c* mRNAs were used as the internal controls. To normalize the total cDNA synthesis, the volume of the cDNA pool was varied slightly to obtain constant values of internal controls co-amplified by PCR.

To avoid the effects of genomic DNA contaminating the cDNA solution, the same reaction mixture in the absence of reverse-transcriptase was used for negative controls. The absence of an amplified fragment confirmed the absence of contributions by genomic DNA.

Estimation of Radioactivity in RT-PCR—The PCR products were separated by 7.5% polyacrylamide gel electrophoresis. The dried gel was exposed to an imaging plate, and the radioactivity levels of each band were measured using a Bioimaging analyzer BAS 2000 (Fuji Photo Film Tokyo).

Primers Used in This Study Are Listed as Follows.

A_{51}: 5′-GGT GGT GGT ATC-3′

A_{71}: 5′-ACT CTT CTA CAA-3′

forward primer for CPT I: 5′-CCT GTG GAT ACT TGG GAC-3′

reverse A primer for CPT I: 5′-GAA ATG TGG AGT CAA ATG TG-3′

reverse B primer for CPT I: 5′-TCC AGG AAA TGT GGA GTC AAA TGT G-3′

CPT I-COD primer: 5′-ACT TCC ATA TTT CTT CCA AGT TCT C-3′

forward primer for GAPDH: 5′-AGT ATG TCG TGG AGT CTA C-3′

reverse primer for GAPDH: 5′-CAT ACT TGG CAG GTT TCT C-3′

forward primer for CPT II: 5′-TGC ATA CCA GCG GAT AAA C-3′

reverse primer for CPT II: 5′-ACC TTC AGT TGG GAT CTT C-3′

forward primer for cytochrome *c*: 5′-TCA ATA GTA AAA

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GGC AAC ATC C-3′
reverse primer for cytochrome *c*: 5′-GTC ATA AAG TGG GTA CGG TC-3′

RESULTS

Isolation and Identification of Genes Expressed Specifically in the JVS Hearts—Homozygous mutant mice are deficient in blood carnitine (*4*). To find the genes regulated by the carnitine deficiency, the mutant (jvs/jvs) mice were maintained without carnitine for 1 week and sacrificed at 4 weeks of age (carnitine had been administered intraperitoneally from 10 to 21 days after birth). The RNA display procedure showed that 204 samples were amplified with the oligonucleotide combinations, and each sample was resolved into about 200 bands in each lane. Finally, about 200×204 species of mRNA were revealed. The intensity of most bands derived from the JVS mice was similar to that of the normal mice as shown in Fig. 1. This observation indicates that there were no drastic changes in the expression of most genes. Of all the bands, 125 bands that were more intense in samples from JVS mice than in those from normal mice, were excised from the gels of the differential display method. The DNA fragments were isolated and sequenced as described in "MATERIALS AND METHODS." About half of them closely resembled or were identical to the reported sequences (GenBank™). For example, the following genes were identified: EF-1α, ribosome S3, heart fatty acid binding protein, ApoD protein, catechol-*O*-methyltransferase, non-receptor type Tyr phosphatase, ferritin heavy chain, cytochrome *c*, mitochondrial uncoupling protein, the subunit I of cytochrome oxidase, transaldolase, phosphofructokinase, and so on (GenBank™). However, their enhanced expression should be confirmed by an other method to substantiate any conclusions (to be published elsewhere). The nucleotide sequence of a more intense band than that from the normal mouse (Fig. 1) showed 84% similarity to the 3′-noncoding region of the rat liver-type CPT I [EC 2.3.1.21] gene (*24*) as shown in Fig. 2A. The nucleotide sequence of a template oligonucleotide (A_{51} primer) was found at the 5′ and 3′ ends of the cloned cDNA fragment. Since this sequence corresponds to the 3′-noncoding region and the mouse CPT I gene has not been cloned, a region further upstream was amplified, using an additional primer (CPT I-COD primer) according to the rat CPT I cDNA sequence and a reverse primer for this CPT I cDNA fragment (reverse B primer), then sequenced. The deduced amino acid sequence was identical to that of rat CPT I except for one amino acid residue, as shown in Fig. 2B. The marked expression of the CPT I gene was confirmed by RT-PCR with a different PCR primer set (a forward primer for CPT I and a reverse A primer for CPT I).

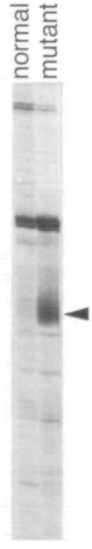

Fig 1 **Autoradiogram of the differential display of the heart mRNA from normal *versus* JVS mice at the age of 4 weeks.** JVS mice were treated with carnitine from 10 to 21 days and sacrificed at 4 weeks of age after being maintained without carnitine for 1 week and differential display studies were performed as described in "MATERIALS AND METHODS." An arrowhead indicates a more intense band derived from the hearts of the JVS mice. Nucleotide sequences of the 12-mer primers used in these lanes, A_{51} and A_{71} are 5′-GGT GGT GGT ATC-3′ and 5′-ACT CTT CTA CAA-3′, respectively.

Semi-Quantitative Analysis of Expressed mRNA by RT-PCR—Rat liver-type CPT I mRNA showed too faint a band to be analyzed quantitatively by Northern blotting using the total rat heart RNA (*25*). In addition, poly(A)$^+$ RNA was not sufficient from a single mouse ventricle. Since we wished to analyze many different DNAs using an RNA sample from a single ventricle, we used semi-quantitative RT-PCR to examine differences in the CPT I gene expression. We compared the rate of amplification of CPT I cDNA with that of the internal controls, which were the GAPDH, CPT II, and cytochrome *c* genes. GAPDH is widely used as internal control for gene expression, because GAPDH mRNA levels are unchanged in various tissues and under various conditions (*26, 27*). CPT II is an enzyme involved in long-chain acyl-CoA transport, and cytochrome *c* is a mitochondrial protein, as is CPT I. At first, PCR with a primer set for the CPT I gene was performed for the indicated cycles and continued with an additional primer set for one of the internal controls. As shown in Fig. 3, CPT I cDNA as well as the internal control cDNAs increased in a logarithmic manner. Therefore, we concluded that RT-PCR should be reliable under these conditions in terms of quantitation.

Figure 4 shows some examples of DNA fragments being amplified in a logarithmic manner. It is clear that JVS mutant mice express more CPT I gene as compared with the internal controls (GAPDH, CPT II, and cytochrome *c*). Figure 5 summarizes the average of the results normalized with the internal controls ($n=3$-4). The differences are statistically significant between samples from the control and mutant mice at 2 weeks old in panel (1) ($p<0.05$) and panels (2) and (3) ($p<0.01$), and at 4 weeks old in panels (1), (2), and (3) ($p<0.05$).

The Effect of Administration of Carnitine—We examined the effect of carnitine on CPT I gene expression. As shown in Figs. 4 and 6, CPT I expression was repressed by administration of carnitine. The differences between the levels of JVS(+) and JVS(−) are statistically significant in Fig. 6, panels (1) and (3) ($p<0.05$). In addition, it is notable that the standard deviation was very high in the experiment using JVS mice ventricles under carnitine-deficient conditions.

Expression of CPT I Gene in Brain—Whereas the heart

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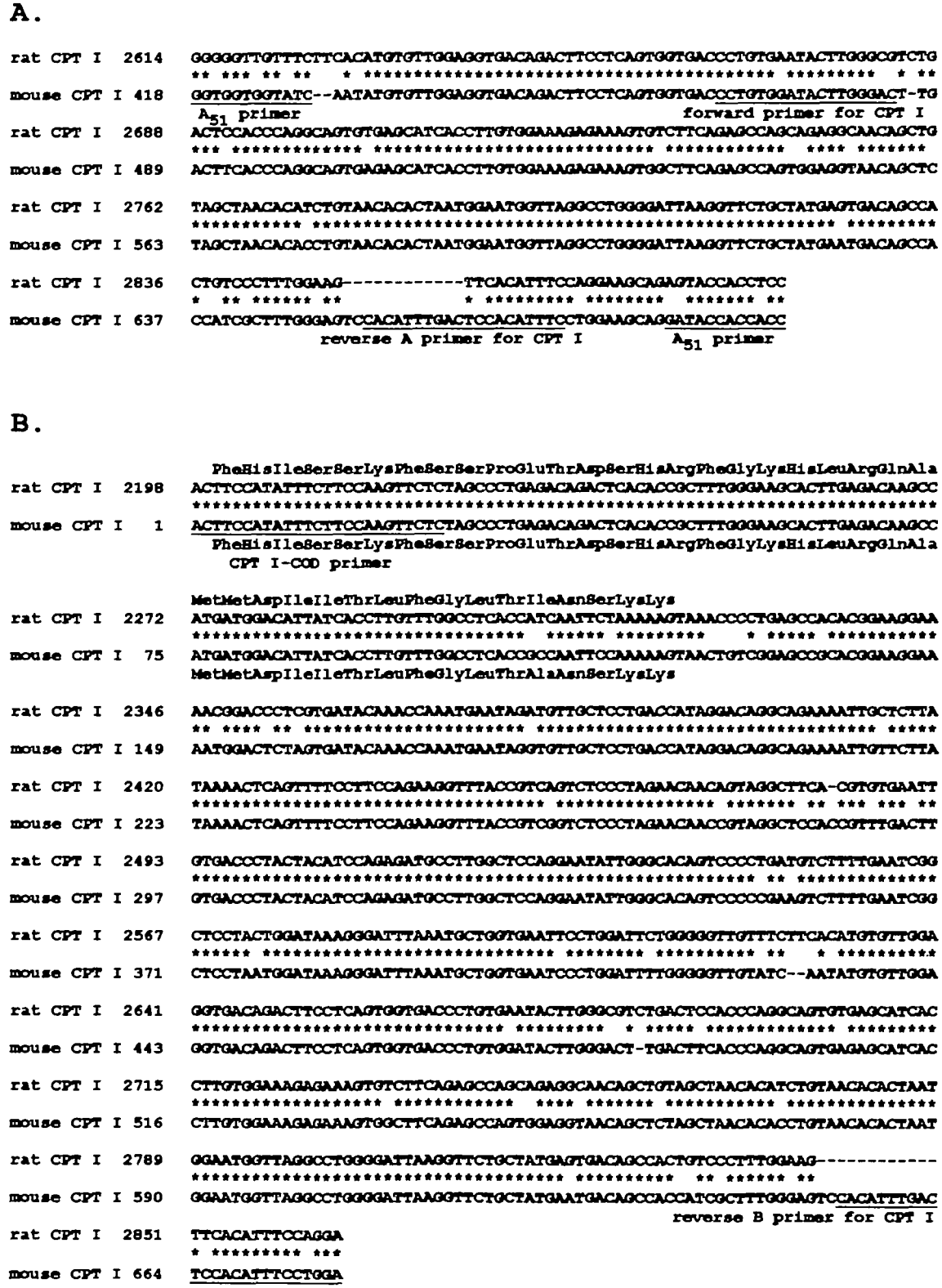

Fig. 2. **Nucleotide and amino acid sequence of a DNA fragment selected in the differential display experiment.** Panel A: A comparison of the nucleotide sequence of a part of rat CPT I cDNA with that of the DNA fragment isolated by differential display procedure. A_{51} primer sites were found in 5′ and 3′ ends of the cloned cDNA. Nucleotides identical to the rat CPT I (so-called liver type) are shown by stars. Panel B: Nucleotide sequences of a part including the coding region of CPT I cDNAs. A fragment including the coding region was amplified and cloned using an additional primer (CPT I-COD primer), which is synthesized according to the rat CPT I cDNA sequence, and a reverse primer for this mouse cDNA sequence (reverse B primer), and then sequenced. Sites of the primers are shown by underlines. Sequences of the primers are listed in "MATERIALS AND METHODS."

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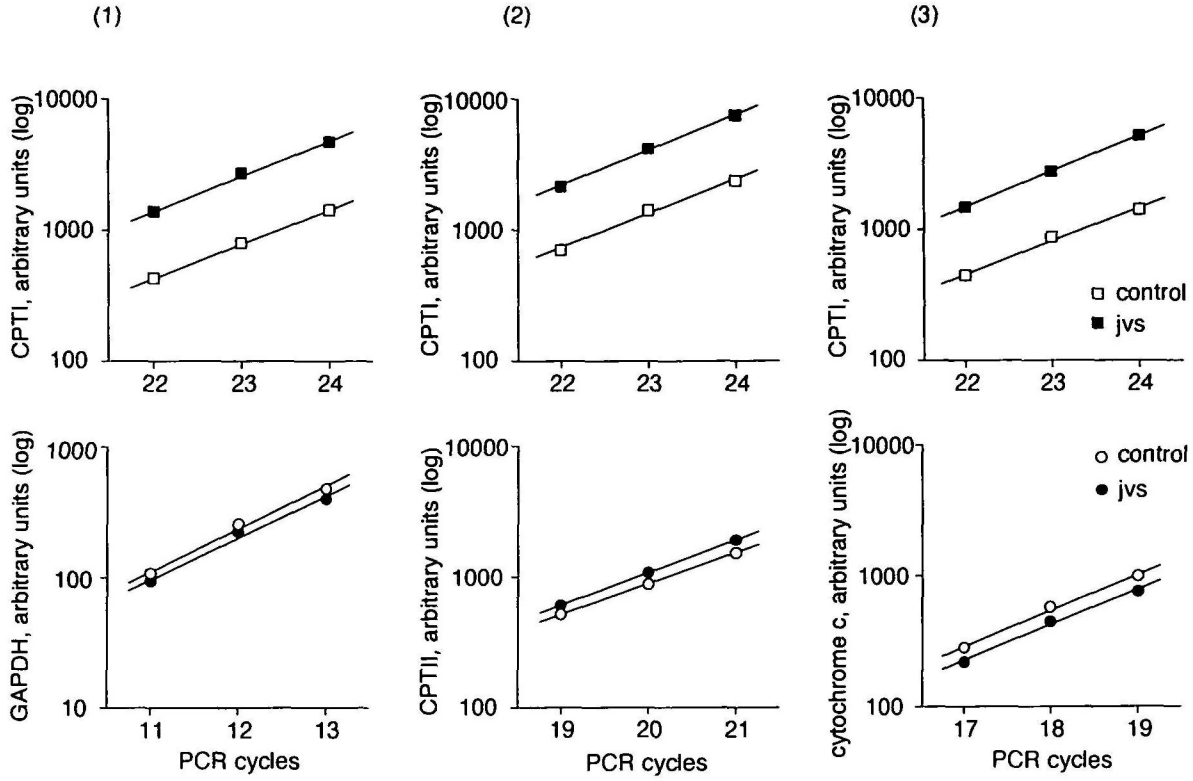

Fig. 3. **Rates of amplification of a CPT I cDNA fragment compared with those of internal controls.** A primer set of CPT I gene (the forward primer for CPT I and the reverse A primer for CPT I) was used for PCR, then a primer set for each internal control was added to continue the PCR to generate a suitable level of radioactivity in the PCR-products. The total number of PCR cycles is shown in the horizontal axis. One PCR cycle was as follows: 95°C for 30 s, 50°C for 45 s, and 72°C for 45 s. PCR products were separated by polyacrylamide gel electrophoresis followed by drying the gel, and the radioactivity of the each band was examined with a Bioimaging analyzer BAS 2000. Unfilled and filled circles indicate samples obtained from normal and JVS mice without carnitine treatment at the age of 4 weeks. Panels (1), (2), and (3) present GAPDH, CPT II, and cytochrome *c* as internal controls, respectively.

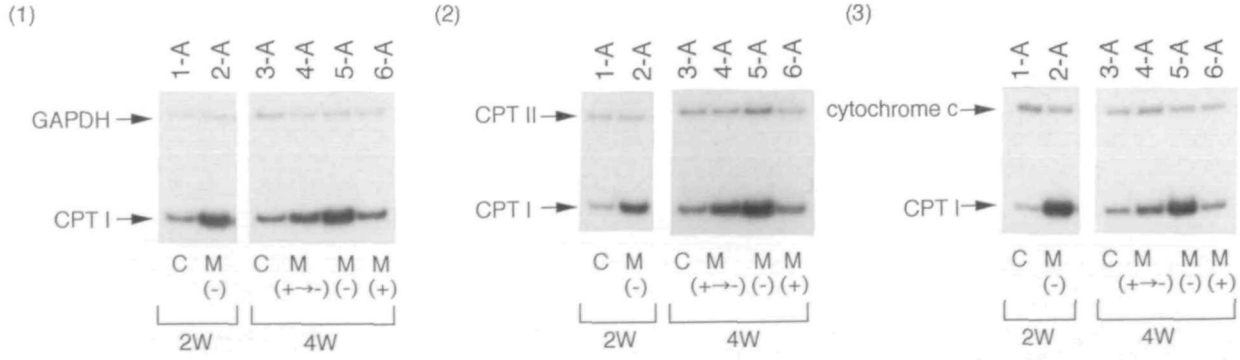

Fig. 4. **Autoradiograms showing RT-PCR analysis of the ventricles from normal and JVS mice at 2 and 4 weeks of age.** PCR reactions were performed as described below. Panel 1: After 11 cycles of PCR with only the primers for CPT I (the forward primer for CPT I and the reverse A primer for CPT I), 13 cycles were continued with primers for GAPDH. Panel 2: After 3 cycles with only the primers for CPT I, 21 cycles were continued with those for CPT II. Panel 3: After 5 cycles with only the primers for CPT I, 19 cycles were continued with those for cytochrome *c*. One cycle of PCR was 95°C for 30 s, 50°C for 45 s, and 72°C for 45 s. Radioactive bands were separated by polyacrylamide gel electrophoresis and exposed to an X-ray film with an intensifying screen for 2-7 days. Abbreviations are as follows: C, +/+ mice without treatment; M (−), jvs/jvs mice without carnitine treatment; M (+ → −), jvs/jvs mice sacrificed at 4 weeks of age after carnitine treatment from 10 to 21 days after birth; M (+), jvs/jvs mice with carnitine treatment from 10 days to the day of sacrifice at 4 weeks of age; W, weeks of age.

uses long-chain fatty acids as carbon sources, the brain uses glucose as a fuel. Therefore, we examined the expression of the CPT I gene by RT-PCR in the brain of the JVS mice as well as normal controls ($n=3$). The relative mRNA values of CPT I compared to GAPDH by RT-PCR are shown in Fig. 7. This expression in the JVS mice appeared higher than that of the normal mice with a statistical significance ($p<0.01$). Therefore the increase of the CPT I gene expression

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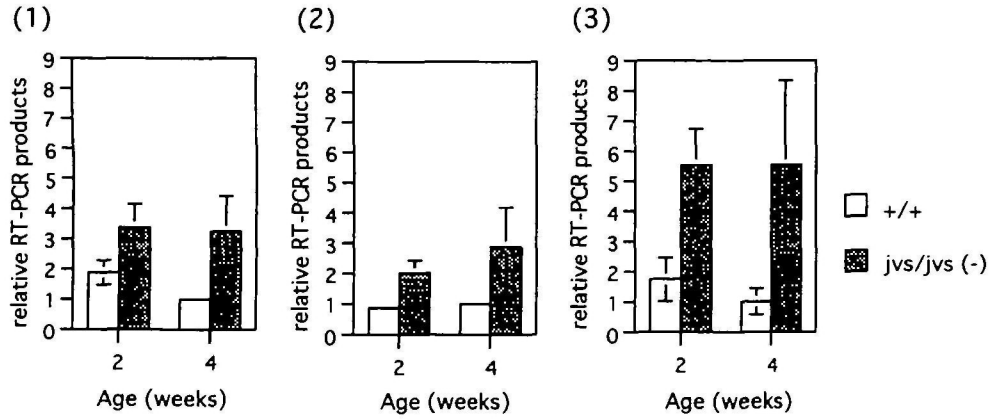

Fig. 5. **Relative values of the expression of the CPT I gene in the ventricle from the JVS and normal mice.** Unfilled and hatched bars indicate the values from the normal and JVS mice without carnitine treatment at 2 and 4 weeks of age, respectively. The average values from the normal mice (4 weeks) are taken as unity for normalization. Panels (1), (2), and (3) show the relative values of RT-PCR products using GAPDH, CPT II, and cytochrome *c* genes as internal controls, respectively. The differences are statistically significant between samples from the control and mutant mice at 2 weeks old in panel (1) ($p<0.05$), and panels (2) and (3) ($p<0.01$), and at 4 weeks old in panels (1), (2), and (3) ($p<0.05$), respectively. Each bar represents an average value of three or four samples with a standard deviation.

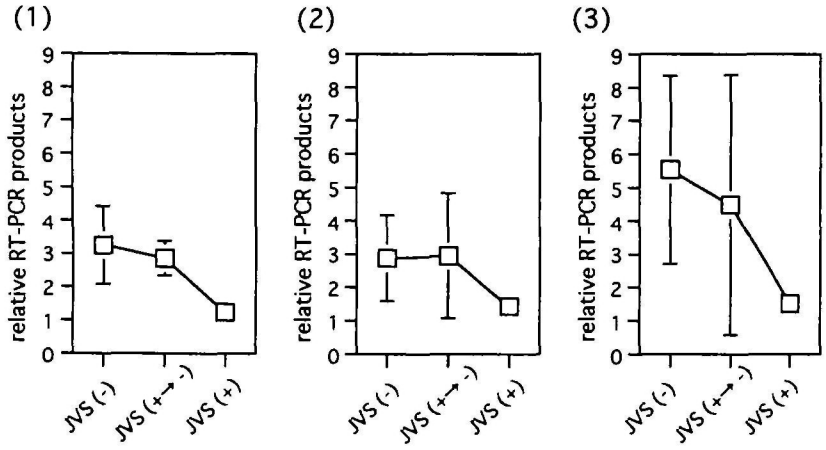

Fig. 6. **Effect of carnitine treatment on relative CPT I mRNA content of JVS mice ventricles at 4 weeks of age.** Relative values of CPT I mRNA were examined by RT-PCR as described in "MATERIALS AND METHODS." JVS (−), JVS (+ → −), and JVS (+) indicate the jvs/jvs mice without carnitine treatment, the jvs/jvs mice sacrificed at 4 weeks of age after administering carnitine from 10 to 21 days after birth, and the jvs/jvs mice after administering carnitine from 10 days to the day of sacrifice at 4 weeks of age, respectively. Relative values of the PCR-product of CPT I from the control mice (4 weeks) were taken as unity for normalization. Internal standards are: Panel 1, GAPDH; panel 2, CPT II; and panel 3, cytochrome *c*, respectively. Data are average values of three or four samples with standard deviations. The differences are statistically significant between JVS (−) and JVS (+) in panels (1) and (3) ($p<0.05$).

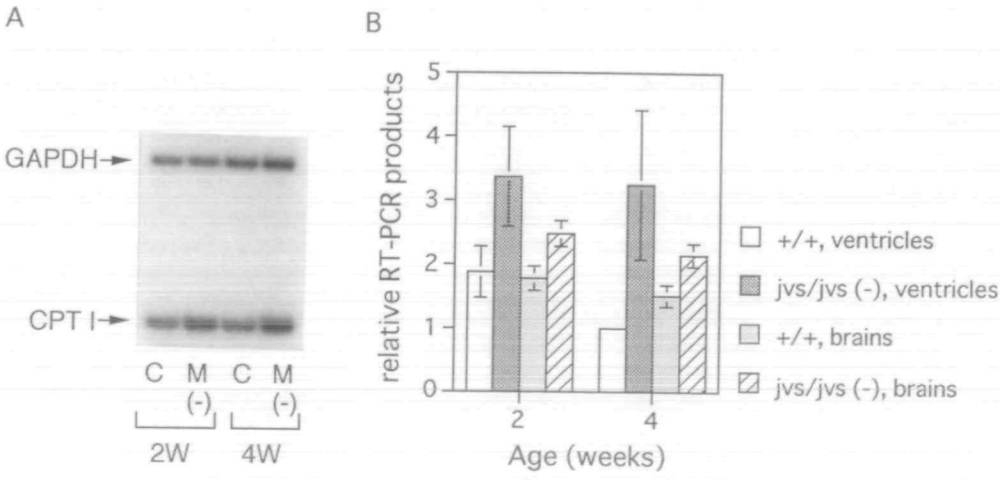

Fig. 7. **Analysis of the expression of CPT I in the brain.** Isolation of poly(A)$^+$ RNA and RT-PCR proceeded in the same manner as for those of the heart as described in "MATERIALS AND METHODS." Panel A is an autoradiogram showing the radioactive bands from the CPT I gene as well as the GAPDH gene as an internal control. PCR conditions were as follows. After 9 cycles of PCR with only the primers for CPT I (the forward primer for CPT I and the reverse A primer for CPT I), 15 cycles were continued with primers for GAPDH. PCR conditions were the same as those described for the cDNA from the ventricles. The abbreviations C and M (−) are samples from normal and jvs/jvs mice without carnitine treatment, respectively. 2W and 4W indicate the ages of the mice. Panel B shows the relative values of radioactivity of CPT I mRNA in the brains and ventricles. The average values of the brains were obtained from three mice in individual experiments. +/+ and jvs/jvs (−) indicate samples from the normal and the mutant mice without carnitine treatment as shown on the right. The relative values were obtained as the radioactivity levels of CPT I PCR-products divided by those of GAPDH. Note that the number of PCR cycles of the brain and the ventricle samples after adding the primer set for GAPDH were 15 and 13, respectively. Since the total PCR cycles with the primer set for CPT I is the same, 24 cycles, the relative levels of CPT I cDNA were corrected from the slope in Fig. 3 (1). Each bar represents an average value with a standard deviation ($n=3$-4). The differences are statistically significant between samples from age-matched control and mutant mice in brains ($p<0.01$).

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was induced by carnitine deficiency even in the brain.

DISCUSSION

JVS mice have been established as a model animal of systemic carnitine deficiency because they have a marked decrease of carnitine concentration in the serum, liver, and skeletal muscles (*4*). The carnitine concentration in the heart of JVS mice is also decreased, and the carnitine contents are about 10-20% of those in age-matched control mice (Ono, A., *et al.*, in preparation). The primary defect of JVS mice is in the reabsorption system for carnitine in the kidney (*5*). JVS mice suffer abdominal ascites at 3-4 months of age associated with congestion and enlargement of the heart, heart failure. An analysis of the hearts of JVS mice (even without abdominal ascites) at 2-3 months of age found that the remarkable increase in heart weight was associated with increases in noncollagen protein and DNA contents, although the number of cardiac cells did not seem to be increased according to a pathological examination. It is assumed that the increase in the heart weight is mainly caused by cardiac cell hypertrophy associated with an increase in cytosolic and mitochondrial proteins (*9*).

Many cases of human systemic carnitine deficiency have been reported (*13-21*). In these patients, one of the most common clinical manifestations is cardiomyopathy. Over 20 individuals have defective carnitine uptake into cultured fibroblasts (*18, 19, 21*) and are suspected to have impaired renal conservation of carnitine (*16, 18, 21*). An autopsy has revealed a remarkably decreased carnitine concentration in the heart (*15*). In clinical and pathological analyses, only congestive heart failure, the accumulation of triglycerides, and increases in mitochondria and/or ultrastructural mitochondrial changes have been described. Most patients undergo a rapid and dramatic improvement in cardiac function after oral carnitine administration (*14-16, 19, 21*). Therefore, the discovery of cardiac hypertrophy in carnitine-deficient JVS mice should help clarify the pathophysiology of cardiomyopathy in systemic carnitine deficiency in humans.

To describe the genes specifically expressed or overexpressed in the heart during the early phase of carnitine deficiency, we applied a differential display method using hearts from JVS mice. We sequenced 125 bands after isolation and identified 109 distinct genes. Among these, the sequences of 49 have been reported (data not shown, will be published elsewhere). CPT I (on the mitochondrial outer membrane), which is the most important enzyme for utilizing long-chain acyl-CoA for mitochondrial β-oxidation, was among them. CPT I is a carrier of long-chain acyl-CoA for transfer into mitochondria, and carnitine is an essential cofactor for this process.

As estimated by PCR-cycles for amplifying cDNA [in Fig. 3, panel (1)], mRNA of CPT I is approximately 1/1,000 of that of GAPDH. Thus, we judged it difficult to quantify the level of mRNA by Northern blotting. We, therefore, applied a RT-PCR method with internal standards to quantify the relative mRNA level. If the linear evaluation over a wide range is achieved by plotting the amplification curves, this PCR approach is one of the most sensitive methods for quantitative analysis, as reported (*28, 29*). In this study, CPT I cDNA as well as internal standards were amplified in a logarithmic manner using coupled primer sets. The level of CPT I mRNA expression in ventricles of JVS mice without carnitine administration increased compared with age-matched controls at 2 and 4 weeks, and carnitine administration corrected the CPT I mRNA level. CPT II, used as one of the standards, is located on the inner face of the inner mitochondrial membrane, and CPT II gene expression is unchanged in the hearts of JVS mice at 2 weeks of age (*30*). Phosphofructokinase, involved in the glycolysis pathway, and cytochrome *c* genes were identified as the genes more expressed in the JVS mice. Gene expression of phosphofructokinase was comparable to that of GAPDH by the RT-PCR methods (data not shown). Since the CPT I gene was more markedly expressed than those of the other enzymes involved in energy metabolism, the increased level of mRNA for CPT I in the carnitine-deficient ventricles might be more specific, but not result from compensation for the decreased fatty acid oxidation. The level of the mRNA for CPT I in the ventricle increased to the highest levels after 7 days without carnitine at 4 weeks of age. Further study is required to elucidate the mechanism that regulates CPT I gene expression.

The rat heart contains two isoforms of CPT I. One is a CPT I protein of ~88 kDa called the liver-type, of which the cDNA has been cloned. It is distributed widely including liver and heart (*31*). The other is a CPT I protein of ~82 kDa, of which the cDNA has not been isolated. It is distributed only in the heart (designate heart type). Since the DNA that we isolated was very similar in sequence in the coding and noncoding regions to rat liver-type CPT I, we concluded that the selected mRNA was derived from that of liver-type CPT I. Since the heart-type cDNA has not been sequenced, the enhanced expression of the heart-type CPT I is unknown. If different isoforms are generated by tissue-specific alternative RNA splicing, it is possible that both isoforms are regulated by carnitine. However, since polyclonal antibody against rat liver-type CPT I did not bind heart-type CPT I (*31*), these two isoforms are probably derived from two independent genes. A novel CPT I-like protein of 88.2 kDa that is expressed at high levels in the rat heart and brown adipose tissue has been isolated and its cDNA sequences determined (*25*). It is unknown whether this is a heart-type CPT I, but the DNA isolated in this study had no similarity to it.

The level of brain CPT I mRNA increased in carnitine deficiency, but the difference was not so marked as that between the normal and mutant ventricles. The deviations of the levels of CPT I mRNA in the carnitine-deficient ventricle were much larger than those in normal ventricle, the mutant ventricle with carnitine treatment, and in normal and mutant brains. It is not clear what causes the considerable differences among mutant mice ventricles. Further investigation is necessary to clarify the precise mechanism of the variation of CPT I mRNA by carnitine deficiency and whether or not it affects cardiomyopathy.

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