Carnitine Administration to Juvenile Visceral Steatosis Mice Corrects the Suppressed Expression of Urea Cycle Enzymes by Normalizing Their Transcription*

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Previous studies in our laboratories have revealed that juvenile visceral steatosis mice show suppressed transcription of urea cycle enzyme genes during development and are systemically deficient in carnitine. It has not yet been explained, however, how this carnitine deficiency relates to the abnormal gene expression. We investigated the effect of carnitine on normal gene expression, growth retardation, and fatty liver. Carnitine administration relieved the suppression of the developmental induction of two urea cycle enzymes examined, carbamoyl-phosphate synthetase and argininosuccinate synthase, and kept the activities of enzymes normal. However, the liver in the normal level. These results suggest that carnitine deficiency plays an important role in the abnormal expression of urea cycle enzyme genes and that the abnormal expression of the genes is not directly caused by lipid accumulation in the liver.

In 1988, Koizumi et al. (1) reported a CSH-H-2° strain of mouse autosomal recessively associated with microvesicular fatty infiltration of viscera. This mouse was later named juvenile visceral steatosis (jvs) mouse (2). jvs mice have severe lipid accumulation in the liver, hypoglycemia, hyperammonemia, and growth retardation. The lipid accumulation is found within 5 days of birth, but the hypoglycemia, hyperammonemia, and growth retardation appear 2 weeks after birth, usually during the weaning period (1).

Imamura et al. (3) reported that hyperammonemia in jvs mice is caused by a decrease of all the urea cycle enzyme activities and is not associated with general liver damage. The decrease was shown to be due to suppressed transcription of urea cycle enzyme genes during development (4). The five urea cycle enzyme activities are expressed in a coordinated manner following the course of development from the fetal to the infantile period (5), which seems to be regulated at the transcriptional levels, as judged from the changes in mRNA amounts (6-9). Glucagon, glucocorticoid, and/or insulin are suspected to be the factors which regulate the expression of urea cycle enzyme genes (10, 11).

On the other hand, Kuwajima et al. (12) discovered general carnitine deficiency in jvs mice, although the primary genetic defect of this disease is still not known.

In this study, we examined the effect of carnitine administration on jvs mice in order to clarify the relationship between carnitine deficiency and the suppressed expression of urea cycle enzyme genes. It is hoped that this will throw light on the pathophysiology and pathogenesis of human systemic carnitine deficiency (13, 14).

EXPERIMENTAL PROCEDURES

Animals—jvs mice were accidentally found in CSH-H-2° strain mice in Kanazawa University, Japan, in 1985 (1). Usually swollen whitish fatty liver is recognized through the abdominal wall 5 days after birth, at the latest, in homozygous mutants (jvs/jvs). Littermates without fatty liver served as controls (+/-) and heterozygotes (+/jvs).

Liver was taken from mice under pentobarbital anesthesia (50 mg/kg of body weight) and frozen at -80°C until used. Body weight was measured at about 5 p.m.

Carnitine Administration—One mg of L-carnitine-HCl (5 µmol) was dissolved in 50 µl of saline, neutralized with 25 µl of 0.2 N NaOH, and then injected intraperitoneally once a day at 5 p.m. Control mice were injected with 75 µl of saline alone. The carnitine-HCl was purchased from Sigma.

Enzyme Activities—The activities of carbamoyl-phosphate synthetase (EC 6.3.4.16, CPS) (15), ornithine carbamoyltransferase (EC 2.1.3.3) (16), and argininosuccinate synthase (EC 6.3.4.5) (17, 18) were measured by the published procedures. The activity of argininosuccinate synthase, the reaction was carried out at 25°C. Protein concentration was assayed by a modified method of Lowry et al. (19). Briefly, the sample solution was spotted on a Whatman 3MM filter, and protein was fixed on the filter with 5% trichloroacetic acid. After drying, protein on the filter was measured by Lowry's method. The above procedure was taken because the high concentration of lipid interfered with the determination of protein concentration.

Isolation and Analysis of RNA—Total RNA was isolated according to the method of Chomczynski and Sacchi (20). For Northern blotting, total RNA was denatured with formaldehyde, fractionated by 1% agarose gel electrophoresis, and transferred to a nitrocellulose filter (21). RNA slot blotting was carried out using a Schleicher and Schuell apparatus (Dassel, Germany). 32P-Labeled DNA probes were prepared using a random oligonucleotide primer (22). The following cDNA fragments were taken from cDNA clones: rat CPS (23), human argininosuccinate synthase (24), and human aldolase B (25). Hybridization was performed at 42°C, and all filters were washed four times in a solution (300 mM NaCl and 30 mM sodium citrate, pH 7.0) at 25°C for 10 min and three times in a solution (15 mM NaCl and 1.5 mM sodium citrate, pH 7.0) at 60°C for 30 min. Autoradiography was performed with different exposure times for each mRNA species. For the analysis of RNA dot blot, densitometric measurements were made.

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‡ The abbreviations used are: jvs, juvenile visceral steatosis; CPS, carbamoyl-phosphate synthetase.
carried out in the linear range of film density with an LKB Ultrascan XL laser densitometer (LKB, Bromma, Sweden).

Lipid Extraction—Lipid was extracted from liver with 20 volumes of 2:1 and 1:2 chloroform/methanol solutions (by volume) (26). The residue was weighed after drying.

Statistical Analysis—Data were analyzed statistically by the Student’s t test with the level of significance set at p < 0.05.

RESULTS

Effect of Carnitine on Body Weight—jvs/jvs mice show retarded growth from around 14 days after birth and weigh significantly less than control mice at 22 days (Fig. 1). From 10 days after birth, we injected 5 μmol of L-carnitine intraperitoneally. The body weight of the carnitine-administered jvs/jvs mice increased as normally as that of the control (+/?) mice.

Effect of Carnitine on Activities of CPS, Ornithine Carbamoyltransferase, and Argininosuccinate Synthase—We injected L-carnitine into the mice from 10 to 28 days after birth. The mice were sacrificed at 29 days after overnight fasting. The activities of CPS, ornithine carbamoyltransferase, and argininosuccinate synthase in the liver of the saline-administered jvs/jvs mice were lower than those of the saline- and carnitine-administered controls (Table I). The activities in the carnitine-administered jvs/jvs mice did not differ statistically from those of saline- and carnitine-administered controls. It thus seems that carnitine kept the activities of CPS, ornithine carbamoyltransferase, and argininosuccinate synthase to the normal level in jvs/jvs mice. We would like to emphasize that this effect was found not only in mitochondrial enzymes, CPS and ornithine carbamoyltransferase, but also in the cytoplasmic enzyme, argininosuccinate synthase.

Effect of Carnitine on mRNA Levels of CPS, Argininosuccinate Synthase, and Aldolase B and Lipid Concentration in the Liver—Fig. 2 shows the effect of carnitine on mRNA levels of CPS and aldolase B. Carnitine administration for both 5 and 9 days from 10 days after birth raised CPS mRNA levels much more in jvs/jvs mice than did saline administration to jvs/jvs mice. Especially in the case of 9-day carnitine administration, jvs/jvs mice had CPS mRNA in almost the same abundance as carnitine-administered controls. We used aldolase B mRNA as an internal control because there is no significant difference in this mRNA between jvs/jvs and control mice during this period (4). As shown in Fig. 2, there is no significant difference in the mRNA levels of aldolase B between carnitine- and saline-administered controls and jvs/jvs mice. Lipid concentration in the liver is shown in the bottom of Fig. 2. We observed that the lipid concentration in carnitine-administered jvs/jvs mice was slightly lower than in saline-administered jvs/jvs mice but still much higher than in control mice. Fig. 3 shows the changes of mRNA levels of CPS, argininosuccinate synthase, and aldolase B during development. Carnitine administration started at the 10th day after birth. Messenger RNA levels of CPS and argininosuccinate synthase in saline-administered jvs/jvs mice did not increase following development, but those in carnitine-administered jvs/jvs mice increased as much as in the controls.

DISCUSSION

Homozygous jvs/jvs mice have a low carnitine content in the organs examined, including blood, liver, and muscle (12). In the present study, we clarified the relationship of carnitine deficiency to the pathological features, such as growth retardation, fatty liver, and suppressed expression of urea cycle enzymes, CPS, ornithine carbamoyltransferase, and argininosuccinate synthase in the liver and the metabolic changes induced by carnitine administration.
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Fig. 3. Changes in the hepatic mRNA contents of CPS, argininomuccinate synthase, and aldolase B during development in carnitine- or saline-administered mice. Carnitine or saline was administered to the mice from 10 days after birth. Relative mRNA levels were calculated from the intensity of the spots on x-ray film after dot blot analysis. The intensity of the control mouse at 10 days after birth was set at 1.0 as the standard. Dot blot analyses were carried out with CDNA probes of CPS (A), argininomuccinate synthase (B), and aldolase B (C). Each point represents the mean ± S.D., and the numbers of animals used for the experiments are as follows: for carnitine-administered jvs/jvs (○) at 11, 15, and 19 days after birth, 4, 3, and 3 animals, respectively; for saline-administered jvs/jvs (□) at 11, 15, and 19 days after birth, 4, 3, and 3 animals, respectively; for carnitine-administered control (●) at 11, 15, and 19 days after birth, 5, 4, and 3 animals, respectively; for saline-administered control (□) at 11, 15, and 19 days after birth, 5, 4, and 3 animals, respectively. Δ and γ indicate 3 control and 4 jvs/jvs mice, respectively, without treatment at 10 days. Statistically significant differences between carnitine- and saline-administered jvs/jvs mice are given as: *, p < 0.05; and **, p < 0.01.

function of mitochondrial membranes. The other possible mechanism is that accumulated substances associated with carnitine deficiency, such as triglyceride (12), fatty acid, and acyl-CoA, may interfere with the hormonal effects on gene expression. Histological analysis revealed that lipid accumulates in the liver and kidney (1). This accumulated lipid is mainly triglyceride (12). Our results (shown in Fig. 2) suggest that triglyceride accumulation in the liver cannot account for the abnormal expression of urea cycle enzyme genes, because carnitine administration caused only a slight decrease in lipid content in spite of its powerful therapeutic effect on gene expression. Some reports have described the relationship between hormonal action and lipid-related compounds which are metabolized by carnitine; Li et al. and Inoue et al. (28,29) reported that fatty acyl-CoAs and fatty acids are potent inhibitors of the nuclear thyroid hormone receptor in vitro. These compounds inhibit the binding of 3,5,3'-triiodothyronine to the receptor. More detailed analyses of the effect of lipid-related compounds accumulated due to carnitine deficiency on hormonal action are necessary to clarify the mechanism of abnormal expression of genes in jvs mice. We are now analyzing the hormonal condition and the response of some hormones to the suppressed urea cycle enzyme genes in jvs mice.

In the present study, carnitine administration did not completely eliminate the accumulated lipid in the liver. If carnitine deficiency is the cause of the defect of jvs mice, carnitine administration alone would relieve the pathological features. The weak effect of carnitine administration on accumulated lipid in the liver may be due to the low doses or the low frequency (once a day) in the present experiment. Carnitine content returned to a low state in the liver of jvs mice 24 h after administration of 5 μmol once a day. Administration of almost the same amount used in the present study twice a day has been much more effective in relieving enlargement and lipid accumulation of the liver. From these results we assume that the amount used in the present study is insufficient to reduce lipid accumulation in jvs mice.

We think it is important that such a small dose of carnitine has a large effect on the abnormal expression of urea cycle enzyme genes and that the abnormal expression of the genes is not directly caused by lipid accumulation in the liver.

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