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Urea cycle disorder in C3H-H-2° mice with juvenile steatosis of viscera

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We determined the activities of urea cycle enzymes in the liver of C3H-H-2°-jsv mice. The activities of all urea cycle enzymes decreased in the latter period of lactation. The activities of carbamylphosphate synthetase and ornithine transcarbamylase in some affected mice were undetectable. On the other hand, the activities of enzymes other than urea cycle enzymes were normal. We consider that the decrease in the urea cycle enzymes is caused by an abnormality in the mechanism of gene expression.

Steatosis; Hyperammonemia; Urea cycle; Gene expression; Carbamylphosphate synthetase; (Animal model)

1. INTRODUCTION

Some clinical findings indicate an interrelationship between ammonia and lipid metabolism. Fatty liver occurs in hyperammonemic patients [1] and hyperammonemia is caused by a disorder of β-oxidation [2,3]. However, little basic research has been done into this problem. The C3H-H-2°-jsv mouse [4,5] is a useful animal model which suggests some interrelationships between ammonia and lipid metabolisms, because the mice exhibit several clinical features; hyperammonemia, fatty liver and hypoglycemia . In this study, we determined the activities of urea cycle enzymes to investigate the pathogenesis of hyperammonemia in C3H-H-2°-jsv mice.

2. EXPERIMENTAL

Homozygous mutants designated as *jsv/jsv* were identified by swollen fatty liver (yellow liver) at the age of 2–5 days. Apparently normal littermates, either normal homozygotes (+/+) or heterozygotes (+/*jsv*), served as control (+/?).

We determined the activities of urea cycle enzymes [6], aldolase with fructose-1-phosphate [7], lactate dehydrogenase (LDH) [8], glutamate dehydrogenase (GDH) [9], ornithine aminotransferase (OAT) [10], glucose-6-phosphatase (G6Pase) [11] and β-oxidation [12] by the previously described methods.

3. RESULTS AND DISCUSSION

The activities of urea cycle enzymes in the liver of *jsv/jsv* mice at the age of 25 days had fallen to 10–40% of the control ranges (table 1). In some *jsv/jsv* mice, carbamylphosphate synthetase (CPS) and ornithine transcarbamylase (OTC) activities were undetectable.

The amount of CPS protein in the liver was determined by sodium dodecyl sulphate-polyacrylamide gel electrophoresis (fig.1). The band corresponding to the CPS protein of a *jsv/jsv* mouse was reduced to about 1/9 of a control (see densitometric profiles) in accordance with the reduction of activity (15% of the control). The decrease of CPS protein was confirmed immunologically by Western blot analysis.

As shown in table 1, the activities of 5 enzymes and mitochondrial β-oxidation in the liver of *jsv/jsv* mice at 25 days did not exhibit particular decreases, suggesting that the depletion of urea cycle enzymes is not likely caused by the structural damage of some hepatocytes, or mitochondrial damage found in Reye's syndrome [3].

Though in theory one third of the controls (+/?) were +/+ and the remainder +/*jsv*, we could not distinguish them on the basis of the activities of their urea cycle enzymes. The absence of a dosage effect in heterozygote made it appear unlikely that the depletion of urea cycle enzymes is brought about by abnormalities in their own structural genes.

We have not yet determined the degradation rate of urea cycle enzymes in the liver of *jsv/jsv* mice. However, it is unlikely that the increase in the rate of degradation was unique to the urea cycle enzymes. We speculate that the depletion of urea cycle enzymes is caused by an abnormality in the mechanism of their gene expression.

The activities of urea cycle enzymes in the liver of *jsv/jsv* mice at 15–17 days were 56–86% of control ranges (table 1). The activities of CPS and OTC in the

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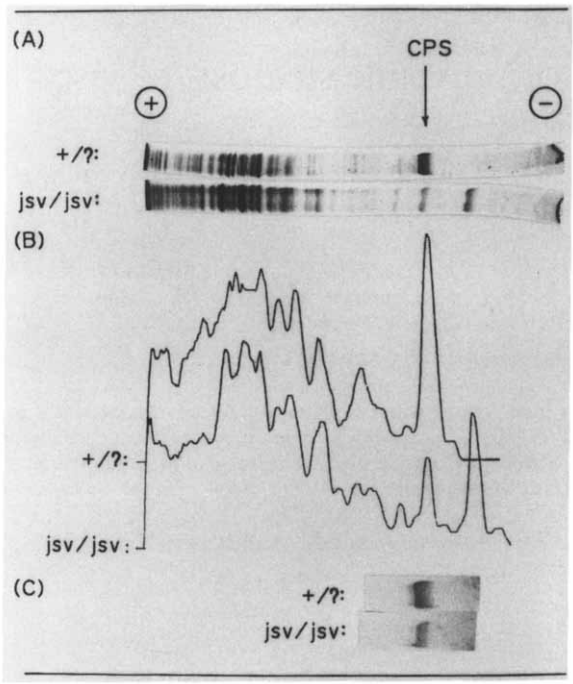

Fig.1. Detection of CPS protein in the liver homogenate with sodium dodecyl sulphate-polyacrylamide gel electrophoresis (A), its densitometric profile (B) and Western blot analysis (C). Liver homogenate protein (12.5 μg) obtained from a +/? and a *jsv/jsv* mouse were applied to a sodium dodecyl sulphate-polyacrylamide gel (5–20% gradient). After electrophoresis, protein bands were stained with Coomassie blue (A,B) or transferred electrophoretically onto nitrocellulose paper. Protein bands on nitrocellulose paper were detected with antiserum against rat CPS using the anti-rabbit IgG antibody-peroxidase method (C). CPS activities in the liver of +/? and *jsv/jsv* mice were 0.081 and 0.012 U/mg protein, respectively.

Table 1

Comparison between normal littermates (+/?) and homozygous mutants (*jsv/jsv*)

	+/?		*jsv/jsv*	
25 days				
CPS (U/mg)	0.074±0.024	(10)	0.0078±0.0076	(9)*
OTC (U/mg)	1.5±0.5	(9)	0.43±0.48	(9)*
ASS (U/mg)	0.043±0.005	(10)	0.010±0.005	(11)*
ASL (U/mg)	0.11±0.03	(10)	0.043±0.026	(11)
Arginase (U/mg)	45±12	(10)	19±8	(11)*
Aldolase (U/mg)	0.058±0.020	(10)	0.080±0.041	(11)
LDH (U/mg)	2.4±0.4	(6)	4.4±0.9	(7)*
OAT (U/mg)	0.0096±0.0012	(10)	0.014±0.007	(9)
GDH (U/mg)	0.92±0.24	(9)	0.62±0.30	(8)**
G6Pase (U/mg)	0.10±0.03	(6)	0.11±0.02	(6)
β-oxidation (nmol/min/mg)	0.39±0.11	(6)	0.67±0.13	(6)*
15–17 days				
CPS (U/mg)	0.11±0.03	(5)	0.063±0.051	(6)
OTC (U/mg)	1.4±0.6	(5)	1.2±0.2	(5)
ASS (U/mg)	0.032±0.009	(5)	0.018±0.006	(6)**
ASL (U/mg)	0.12±0.05	(5)	0.09±0.01	(6)
Arginase (U/mg)	41±13	(5)	29±4	(6)

Results are given as mean ± SD. The number of subjects is shown in parentheses. U, micromol of product/min. *$P<0.01$ and **$P<0.05$. ASS, argininosuccinate synthetase; ASL, argininosuccinate lyase.

liver of *jsv/jsv* mice at 5 days were also slightly lower than those of age-matched controls, as at 15–17 days (data not shown). We consider that the depletion of urea cycle enzymes in the liver of *jsv/jsv* mice occurs mostly in the latter period of lactation.

Mice homozygous for deletions overlapping the albino locus on chromosome 7 exhibit a decrease in urea cycle enzymes in the liver [13]. The deleted gene is postulated to encode a *trans*-acting regulatory factor that controls the liver-specific expression of urea cycle enzymes, gluconeogenic enzymes and so on. The affected mice lack the postneonatal developmental increase of the urea cycle enzymes and maintain a prenatal basal level of expression. In our C3H-H-2°-jsv mice, the activities of urea cycle enzymes are considered to decrease in the latter period of lactation and the activity of G6Pase, an enzyme of gluconeogenesis, stays in the normal range. We consider that the depletion of the activities of urea cycle enzymes found in C3H-H-2°-jsv mice is probably caused by an abnormality in the gene expression but at a stage other than that demonstrated in other mice.

Because the appearance of fatty liver preceded the depletion of urea cycle enzymes, we speculate that the depletion of urea cycle enzymes is caused by an abnormality of lipid metabolism. If this is so, C3H-H-2°-jsv mice prove to be a useful animal model for the study of the effect of lipid metabolism on the expression of urea cycle enzymes in the liver.

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