Differential Induction of Cystathionine $\gamma$-Lyase in the Livers and Kidneys of Mouse Dams during Gestation and Lactation

Noriyuki Akahoshi,$^a$ Takashi Izumi,$^b$ Yasuki Ishizaki,$^a$ and Isao Ishii*,$^a$

$^a$Department of Molecular and Cellular Neurobiology, Gunma University Graduate School of Medicine; and $^b$ Department of Biochemistry, Gunma University Graduate School of Medicine; 3–39–22 Showa-machi, Maebashi, Gunma 371–8511, Japan. Received March 21, 2006; accepted June 29, 2006; published online July 4, 2006

Cystathionine $\gamma$-lyase (CSE) is the last key enzyme in the transsulfuration pathway for the biosynthesis of cysteine from methionine in mammals, and catalyzes the hydrolysis of cystathionine into cysteine. Cysteine can be provided through diet; however, several investigators have suggested that infants may require dietary supplements of cysteine because of very low or undetectable CSE activity in their livers. We have previously shown that CSE levels are much lower in the livers and kidneys of fetal and infant mice than in those of adult mice, suggesting that the maternal supply of cysteine is important for the early development of mice. Here we examined changes of CSE expression in the livers and kidneys of dams during gestation and lactation. Hepatic enlargement was observed as early as gestational day 12.5 (G12.5) and thereafter became more prominent, whereas expression of CSE in the livers was found after postpartum day 1 (P1) and reached a peak at P14. The maintenance of lactation was essential for both hepatic expression and CSE expression in contrast, kidneys gained weight only slightly during lactation while CSE expression in kidneys was markedly induced at G15.5 and then gradually declined through to P28. Serum concentrations of homocysteine (the precursor of cystathionine) were significantly lower in G18.5 dams than in virgins or G15.5 dams, suggesting that the expression of CSE in the kidneys contributes to the effective clearance of homocysteine during the late gestational stage.

Key words  cystathionine $\gamma$-lyase; transsulfuration; cysteine; homocysteine; gestation; lactation

Mammalian cells can synthesize cysteine from methionine via a sulfur-transfer reaction called transsulfuration. Two pyridoxal 5'-phosphate-dependent enzymes, cystathionine $\beta$-synthase (CBS; EC 4.2.1.22) and cystathionine $\gamma$-lyase (CSE, $\gamma$-cystathionase; EC 4.4.1.1), play essential roles in transsulfuration; the former catalyzes the condensation of homocysteine and serine to form cystathionine, while the latter catalyzes the hydrolysis of cystathionine into cysteine. Several investigators have suggested that infants, especially premature infants, may require a diet supplemented with cysteine because of a very low or undetectable level of CSE activity in their livers, and for this reason cysteine is often called a conditionally essential amino acid. Biosynthesized cysteine could be a component of polypeptide chains, or be utilized to synthesize two major intracellular antioxidants, glutathione and taurine, both of which could be deficient in premature infants. Therefore, an adequate supply of cysteine from dam milk or a supplemental diet is considered to be important for the development of (premature) infants.

We have previously reported that CSE levels were very low in the livers and kidneys of mouse embryos or neonates. In this study, we examined the expression of CSE in dams during gestation and lactation.

MATERIALS AND METHODS

Chemicals and Animals All chemical reagents were of analytical grade, and were purchased from Sigma or Wako (Osaka, Japan) unless otherwise noted. Inbred mice (C57BL/6J Jcl) were purchased from Clea Japan (Tokyo, Japan). Eight- to ten-week-old mice were bred to achieve pregnancy. Pregnant females that had 7—10 embryos in their uteri were used for analyses. Gestational day was calculated from the mating date as identified by the presence of copulation plugs. Dams that had delivered $\geq 7$ pups were chosen for analyses, and the litter size was set at 7 within a day after the delivery; the extra pups were removed. The use of animals was in compliance with the guidelines established by the Animal Care and Experimentation Committee, Gunma University, Showa Campus.

Measurement of CSE Activity Female mice at various gestational or postpartum days were anesthetized with diethyl ether. The whole livers and pairs of kidneys were quickly removed, weighed, and homogenized with a Teflon tissue grinder in ice-cold phosphate buffer (100 mM sodium phosphate (pH 7.8), 1 mM phenylmethylsulfonyl fluoride, and 1×Complete® protease inhibitor cocktail (Roche Diagnostics, Mannheim, Germany)). The homogenates were sonicated with a Sonifier 450 (Branson Ultrasonics, Danbury, CT, U.S.A.) and centrifuged at 10900 $\times$ g for 5 min at 4 °C, and the supernatants were further centrifuged at 17400 $\times$ g for 20 min at 4 °C. The resulting supernatants were quickly frozen in liquid nitrogen and stored at $-80$ °C prior to use. Measurement of CSE activity was performed as described previously. This method was based on colorimetry for determination of the amount of pyruvate produced from $\beta$-chloro-L-alanine by a CSE-catalyzed $\gamma$-elimination reaction, coupling a color enzymatic reaction with pyruvate oxidase and peroxidase. Specific CSE activity was expressed as picomoles of pyruvate produced per min per $\mu$g of the cytosolic protein.

Western-Blot Analysis Tissue samples (5 $\mu$g protein) were solubilized in the sample buffer, boiled for 5 min, separated on a 10% SDS/polyacrylamide gel, and transferred onto PVDF transfer membranes. The 44-kDa CSE protein was detected with anti-CSE amino-terminal serum (1:3000 dilution), horseradish peroxidase-conjugated anti-rabbit IgG antibody, and the ECL® system (Amersham Biosciences), as described previously.

Measurement of Total Homocysteine Concentrations in
Serum concentrations of total homocysteine (homocysteine and all its derivatives that will give rise to the thiol homocysteine after reductive cleavage of disulfide bonds), were measured using a Homocysteine Microtiter Plate Assay kit (Diazyme Laboratories, San Diego, CA, U.S.A.).

Statistical Analysis Data are the means±S.D. of 3—10 independent samples from independent mice. Statistical analysis was done with Student’s t-test or One-way ANOVA using Tukey’s test, and the difference at \( p<0.05 \) (*) was considered to be statistically significant (\( **, p<0.01 \)).

RESULTS

Liver Weights and Hepatic CSE Expression Body weight, wet liver weight and hepatic CSE expression were examined on various gestational or postpartum days (Fig. 1). The average body and wet liver weights of 8 to 10-week-old virgin females were 21.7±2.3 g and 1.08±0.17 g (\( n=9 \), respectively (Figs. 1A, B). Both weights were significantly increased during gestation (Figs. 1A, B). The body weights of dams dropped at the moment of delivery but recovered gradually from P3 through P14, and then decreased at P21, the weaning age (Fig. 1A). The weight of the livers also increased after the delivery, reaching a peak at P14, and then decreased (Fig. 1B). The liver weight/body weight ratio was highest at P14 (7.30±0.40%, \( n=4 \)), and was significantly higher than that of the virgins (4.97±0.37%, \( n=9 \)) or the P1 dams (6.06±0.73%, \( n=6 \)) (\( p<0.001 \) and \( p<0.01 \), respectively). Hepatic enlargement was not accompanied by CSE induction during gestation; specific liver activity decreased and whole liver activity remained constant during G12.5—18.5 (Figs. 1C, D, respectively). Western blot analysis using anti-CSE antibody (Fig. 1C, the inset) indicated that the induction of CSE expression showed a good correlation with that of specific CSE activity (Fig. 1C, the bars). Both liver weight and specific hepatic CSE activity were markedly increased after the delivery, and thus, the whole liver CSE activity was remarkably increased; it reached a peak value at P14 (261.6±52.6% of the virgins, \( n=4 \)) and then declined by the weaning ages, P21 and P28 (Fig. 1D).

To examine whether the maintenance of lactation affects the expression of CSE, all offspring were removed at P1 from dams that had delivered ≥7 pups, and the dams were analyzed at P14 (Fig. 2). Within 13 d after removal of the pups, both liver weight and specific/whole liver CSE activity had returned to levels found in virgin females (Fig. 2), demonstrating that the maintenance of lactation was critical for hepatic enlargement and the induction of CSE expression.

Kidney Weights and Renal CSE Expression Alterations in kidney weights and renal CSE activity were investigated (Fig. 3). During gestation, kidney weights were unchanged (Fig. 3A) despite the gains in body weight (Fig. 1A). After the delivery, however, dam kidneys were slightly heavier after P3 and reached a maximum weight at P14 (131.0±16.5% of the virgins, \( n=4 \)) (Fig. 3A). In contrast,
specific renal CSE activity was increased remarkably between G12.5 and G15.5 with a peak at G15.5 (236.3±15.4% of the virgins, n=3), and then gradually decreased (Fig. 3B, the bars); alterations in specific activity were correlated with those in CSE protein levels (Fig. 3B, the inset). As a result, whole kidney CSE activity was significantly elevated during gestation, and then gradually declined through to P28 (Fig. 3C). Previous studies reported that serum homocysteine levels are significantly lower in normal pregnant women in the first, second, and third trimester, compared with non-pregnant controls.

DISCUSSION

We have previously shown that the CSE mRNA and protein are mainly and highly expressed in the livers and kidneys of adult mice. CBS is also mainly and highly expressed in those tissues, suggesting that these two organs are the major loci for transsulfuration. In rat embryos, specific liver CSE activity is very weak around G14.5 but increased dramatically during the subsequent days of gestation. In rat dams, specific liver CSE activity increases rapidly with the onset of lactation, reaching a maximal level at P14. In contrast, specific CSE activities in the livers and kidneys are very low in both embryos and neonates. These lines of evidence suggest that the supply of cysteine from milk (ca. 10 μM in whey) plays a more important role in mouse dams than rat dams during lactation. Here, we examined CSE expression in mouse dams during gestation and lactation.

Hepatic enlargement during gestation and lactation (Fig. 1B) has been previously described in mice; it is achieved by hypertrophy (increased cell size) but not by hyperplasia (increased cell numbers). During gestation, specific liver CSE activity was decreased (Fig. 1C) while whole liver activity was unchanged (Fig. 1D). Instead, a remarkable CSE induction was observed in the livers during lactation (Fig. 1D). These results indicate that CSE expression is accompanied by hepatic hypertrophy during lactation but not during gestation. The increased liver weight and whole liver CSE activity were both maintained by the lactation (Fig. 2). Barber et al. have reported that the inhibition of CSE activity in rat dams by a specific inhibitor, propargylglycine, is followed by a significant decrease in lactation associated with apoptosis of the lactating mammary gland. This phenomenon is also seen in weaned lactating rats. The mechanisms by which liver CSE maintains the lactation or vice versa are currently unknown. Increased food (or methionine) uptake could contribute to the elevated liver CSE activity during lactation, but not during gestation (Fig. 1D).

The alteration of CSE expression in the kidney during gestation/lactation was first described in this study (Fig. 3); the physiological roles of CSE in renal functions are poorly understood. The kidney is presumed to be a major site for the removal and subsequent metabolism of homocysteine, and hyperhomocysteinemia is a common finding in dialysis-dependent end-stage renal disease (ESRD) patients. Plasma (or serum) cystathionine levels are elevated in ESRD or renal transplant patients. In adult mouse kidneys, CBS protein is expressed in both the cortex and medulla (Ishii et al., unpublished observation) while CSE is expressed only in the cortex (especially cortical tubules), suggesting that these two organs are the major loci for transsulfuration enzymes might cooperate in the renal clearance of homocysteine. Serum homocysteine levels are significantly low during pregnancy in human, which could be caused by increased renal clearance of homocysteine via transulfuration. In pregnant mice, both specific and whole kidney CSE activities were markedly elevated at G15.5 (Figs. 3B, C), which may contribute to the subsequent reduction in homocysteine levels.
serum homocysteine concentrations (Fig. 4). Serum homocysteine levels were up-regulated after the delivery (Fig. 4) while whole liver and kidney CSE activities were up-regulated (Fig. 1D) and down-regulated (Fig. 3C), respectively, suggesting possible roles of renal CSE (rather than hepatic CSE) in regulating blood homocysteine levels during perinatal periods. Reduction in homocysteine levels is not observed in preeclampsia patients,\(^{25,26}\) and dysregulation of CSE (or transsulfuration) may lead to severe renal diseases during pregnancy. In conclusion, we found that CSE expression was differentially regulated in the livers and kidneys of mouse dams during gestation and lactation.

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