Homocysteine Hypothesis on the Impaired Peripheral but Not Central Nervous System Oxytocin Responses in Cystathionine γ-Lyase-Deficient Dam Mice

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An elevated plasma homocysteine level is an independent risk factor for cardiovascular diseases, neurological disorders, and pregnancy complications. We recently demonstrated partial lactation failure in cystathionine γ-lyase-deficient (Cth−−) dam mice and their defective oxytocin responses in peripheral tissues: uterine (ex vivo) and mammary gland (in vivo). We reasoned that elevated levels of circulatory homocysteine in Cth−− dam mice counteract with oxytocin-dependent milk ejection from the mammary gland. Based on our observation that those mice displayed normal maternal behaviors against their pups and adult Cth−− male mice exhibited normal social behaviors against adult wild-type female mice, both of which are regulated by oxytocin in the central nervous system (CNS), we conducted the present study to investigate the amino acid profiles, including total homocysteine, in both blood and cerebrospinal fluid (CSF) of wild-type and Cth−− female mice before pregnancy and at day 1 of lactation (L1). Serum levels of total homocysteine in wild-type and Cth−− L1 dam mice were 9.44 and 188 μmol/L, respectively, whereas their CSF levels were below 0.21 (limit of quantification) and 3.62 μmol/L, respectively. Their CSF/serum level ratio was the lowest (1/51.9) among all 20 proteinogenic amino acids, sulfur-containing amino acids, and citrulline/ornithine in Cth−− mice. Therefore, we hypothesize that the blood–brain barrier protects the CNS from high levels of circulatory homocysteine in Cth−− dam mice, thereby conferring normal oxytocin-dependent maternal behaviors.

Key words blood–brain barrier; cerebrospinal fluid; cystathionine γ-lyase; homocysteine; maternal behavior; oxytocin

INTRODUCTION

Elevated levels of plasma homocysteine (Hcy: HS-(CH₂)₂CH(NH₂)-COOH), an amino acid intermediate in methionine metabolism, are widely known as an independent risk factor for cardiovascular diseases such as myocardial infarction, stroke, and venous thromboembolism. They have also been implicated in the onset or progression of various neurological disorders, including autism spectrum disorder, attention deficit hyperactivity disorder, amyotrophic lateral sclerosis (ALS), Parkinson’s disease, and Alzheimer-type dementia in adults as well as neural tube defects in neonates. Moreover, increased plasma Hcy levels are attributed to pregnancy complications, including preeclampsia, spontaneous (recurrent) abortion, and premature/low-body-weight infants. However, the molecular mechanisms through which Hcy exerts its pleiotropic effects in vivo yet remain unexplored.

In the present study, we have addressed this issue using the following two lines of homocysteinemic mutant mice: cystathionine β-synthase (Cbs)-deficient mice (Cbs−−), a model of homocystinuria [OMIM 236200] and cystathionine γ-lyase (Cth)−− mice (Cth−−), a model of cystathioninuria [OMIM 219500]. The majority of Cbs−− mice died at 2–4 weeks of age perhaps due to hepatic dysfunction, whereas Cth−− mice appeared normal, just as observed in patients with cystathioninuria. However, we found that Cth−− mice exhibited increased susceptibility to oxidative injury and exposures to acetaminophen or various environmental electrophiles, as well as partial lactation failure.

The lactation failure observed in Cth−− dam mice was attributed to reduced oxytocin responses in the mammary gland (i.e., milk ejection) perhaps due to their homocysteinemia. Oxytocin is a hormone/neuropeptide that consists of nine amino acids with an intramolecular disulfide bond between two cysteine (Cys) and is normally produced in the hypothalamus and released by the posterior pituitary into the circulation to mainly regulate uterine contraction and milk ejection. It acts on Gq-coupled oxytocin receptor (Oxtr) to activate phospholipase C and intracellular calcium signaling. Previous studies conducted using oxytocin-deficient (Oxtr−−) mice and Oxtr-deficient (Oxtr−−) female mice have shown that either Oxtr−− or Oxtr−− female mice displayed normal fertility, pregnancy, and parturition, but failed to eject milk; therefore, all pups born to these mice died within 24 h after birth. Moreover, adult Oxtr−− male mice exhibited deficits in social discrimination and elevated aggressive behaviors.

Furthermore, Cth−− female mice displayed normal fertility, pregnancy, parturition, and maternal behaviors but insufficient milk ejection, and Cth−− male mice exhibited normal social behaviors; only their central nervous system (CNS) phenotypes were contrasted with those of Oxtr−− and Oxtr−− mice. Therefore, in the present study, we investigated the amino acid profiles in the blood and cerebrospinal fluid (CSF) of wild-type and Cth−− female mice before pregnancy and at day 1 of lactation (L1). We observed very low levels of Hcy in the CSF of homocysteinemic Cth−− mice compared to their circulation levels.

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MATERIALS AND METHODS

Cth-heterozygous (Cth+/−) mice were generated and backcrossed for 10 generations to C57BL/6J inbred strain (CLEA Japan, Tokyo, Japan), and thereafter, Cth+/+ males and females were bred to obtain Cth−/− mice. Blood and CSF samples were collected from both wild-type and Cth−/− mice (at 8–12 weeks of age) before pregnancy (virgin) and at L1 after anesthesia with isoflurane inhalation. Blood was collected by cardiocentesis (approx. 200 µL/mouse) and used for serum preparation. CSF was collected by ventricular puncture with a glass capillary tube (5–10 µL/mouse). Serum and CSF levels of amino acids were measured as described previously. Briefly, thiol-containing amino acids (Hcy, Cys and glutathione (GSH)) were reductively cleaved at their intramolecular disulfide bonds and then derivatized with the thiol-labeling reagent SBD-F (Dojindo, Kumamoto, Japan). All other amino acids (except tryptophan (Trp)) were derivatized with the amino acid-labeling reagent NBD-F (Dojindo). Trp was detected using its own fluorescence without labeling. The levels of all amino acids were determined using HPLC. Because a minimum volume of 10 µL was required for all these assays, equal volumes of CSF samples from 2–3 mice were often pooled. All animal procedures were conducted according to the Guide for the Care and Use of Laboratory Animals, 8th Edition published by the US National Research Council, and were approved by the Animal Care Committees of Showa Pharmaceutical University (No. P-2018-07).

RESULTS AND DISCUSSION

Serum levels of total Hcy in virgin and L1 Cth−/− mice were 153 and 188 µmol/L, and those of cystathionine were 98.9 and 137 µmol/L, respectively; all these levels were much higher than those of the respective wild-type mice (Table 1), as we had previously observed in adult Cth−/− male mice. Except for lower total Cys/total GSH and higher citrulline levels in Cth−/− mice, the amino acid levels were generally comparable between wild-type and Cth−/− mice or between virgin and L1 mice (Table 1). Meanwhile, the CSF samples displayed completely altered amino acid profiles, and their amino acid levels were generally much lower than their serum levels (except taurine). In particular, the CSF levels of total Hcy in virgin and L1 Cth−/− mice were only 3.69 and 3.62 µmol/L, respectively, and both those levels in wild-type mice were below the limit of quantification (LOQ: <0.21 µmol/L). The CSF/serum level ratio in Cth−/− L1 mice was the lowest (1/51.9) among all the measured amino acids (Table 1), and the ratios in wild-type mice were also estimated to be less than 1/35.8 (virgin) or 1/45.0 (L1) (Table 1). There results suggest that the blood–brain barrier protects the CNS from the circulatory homocysteine, especially in Cth−/− dam mice.

In our previous study, we observed an impaired peripheral oxytocin response (milk ejection) in the epithelial ducts of the mammary gland of Cth−/− mice. Furthermore, preadministration of Hcy was found to interfere with oxytocin-induced uterine contraction in ex vivo experiments. We hypothesized that the high level of circulatory Hcy somehow counteracts...
with oxytocin, for example, by forming Hcy-oxytocin dimers via disulfide bonds between their Cys. Milk containing high levels of total Hcy (49.6 µmol/L) produced from homocysteinemia (111 µmol/L) Cth−/− L14 dam mice had no influence on the growth of their Cth−/− pups, perhaps because of its lack of impact on their blood Hcy levels.12) Similarly, the relatively very low levels of total Hcy in the CSF of virgin or L1 Cth−/− mice may underlie their normal oxytocin-regulated maternal and social behaviors.12) Serum oxytocin levels were found to be comparable between wild-type and Cth−/− mice,12) and their CSF levels could not be measured because of its low yields.

Guiraud et al. reported that plasma levels of total Hcy in patients with Alzheimer’s disease and their control subjects were 8.1 ± 6.9 and 5.3 ± 1.6 µmol/L, respectively, whereas both of their CSF levels were < 0.25 µmol/L (LOQ).17) Okino-mid et al. reported that plasma and CSF levels of total Hcy in 35 adult women were 11.9 µmol/L and 64.4 ± 16.4 nmol/L, respectively, whereas their serum levels were 13.0, 263, and 184 µmol/L and 64.4 nmol/L, respectively.19) Therefore, dysfunction of the barrier could cause amino acid leakages to the CSF as observed in more homocysteinemic Cbs−/− mice20) that have the CNS disorders.9)

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Conflict of Interest The authors declare no conflict of interest.

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