**Note**

**Increase in S-Adenosylhomocysteine Content and Its Effect on the S-Adenosylhomocysteine Hydrolase Activity under Transient High Plasma Homocysteine Levels in Rats**

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Summary The objective of this study was to examine how transient high plasma homocysteine (Hcy) levels affect the metabolism of Hcy, the activity and expression of S-adenosylhomocysteine (SAH) hydrolase which catalyzes both SAH hydrolysis and SAH synthesis. Wistar ST rats (males) were cannulated in the right jugular vein for intravenous infusion of physiological saline or DL-Hcy solutions (15 and 30 mg/mL) for 1 h at 1.1 mL/h/rat. The content of S-adenosylmethionine (SAM), SAH-synthetic activity of SAH hydrolase and the expression of SAH hydrolase mRNA in liver extracts showed no significant difference in the Hcy infused groups as compared to the Control group. On the other hand, the contents of hepatic SAH in the Hcy infused groups were dose-dependent and significantly higher than that of the Control group. Thus, this study showed that hepatic SAH increased without any increase in the SAH-synthetic activity and the expression of SAH hydrolase mRNA under transient high plasma Hcy levels after intravenous infusion of Hcy.

Key Words plasma homocysteine, S-adenosylhomocysteine, S-adenosylhomocysteine hydrolase, methionine metabolism

An increase of plasma Hcy is caused by nutritional deficiencies or genetic mutations. Vitamin B₆ (B₆) deficiency (2, 3) and folic acid deficiency (4) were reported as the factors affecting Hcy accumulation. Furthermore, the relationship between elevated plasma Hcy and accumulated SAH was observed in several studies using rats (5, 6). Elevated plasma Hcy and accumulated SAH are likely to be caused by disturbed methionine metabolism. We reported in our previous study that the elevation of plasma Hcy and accumulation of hepatic SAH were observed in B₆-deficient rats (7). In B₆ deficiency, the transsulfuration pathway is suppressed due to the fact that B₆ is a coenzyme of CBS and γ-cystathionase. Therefore, abnormal methionine metabolism is caused by B₆ deficiency. Moreover, the increase in SAH-synthetic activity of SAH hydrolase was also observed in B₆ deficiency (7), the cause of which was not clarified. It was surmised that the elevation of Hcy, a substrate of SAH-synthetic reaction of SAH hydrolase, might induce the increase of SAH-synthetic activity of SAH hydrolase.

In this experiment, we examined the SAH content and the SAH-synthetic activity of SAH hydrolase in the liver of rats with transiently elevated plasma Hcy levels after intravenous infusion of Hcy.

Materials and Methods

Reagents. Somnopentyl® was purchased from...
heptanesulfonic acid sodium salt were purchased from Sigma Chemicals Co. (St. Louis, USA). dl-Hcy was purchased from Nacalai Tesque Inc. (Kyoto, Japan). SBD-F Kagaku Co. (Tokyo, Japan).

Methanol (HPLC grade) was purchased from Kanto Chemicals Co., Ltd. (Tokyo, Japan). The rats had free access to MF chow diet and water during the experimental period. The rats were housed in a temperature-controlled room (23 ± 1°C) with alternating 12 h cycle of light (light: 6:00 a.m. to 6:00 p.m.). The experiments were performed in accordance with the governmental legislated guidelines in Japan on the proper use of laboratory animals (1980), and our experiments were approved by the ethical committee of the Faculty of Applied Biological Sciences in Gifu University.

**Animal and diets.** Male Wistar ST rats, from 8 to 10 wk old, were obtained from Japan SLC, Inc. (Hamamatsu, Japan). The rats were used for the following infusion study.

**Homocysteine infusion.** dl-Hcy was dissolved in physiological saline at the concentration of 15 (15-Hcy group) or 30 (30-Hcy group) mg/mL, and infused at a rate of 4 mL/h/kg body weight using a syringe pump (Model IVAC P3000, IMI Co., Ltd., Saitama, Japan) for 1 h. Transient high plasma Hcy levels were achieved by the same amount of Hcy infusion (9). The rats in the Control group were given physiological saline at the concentration of 15-Hcy and 30-Hcy groups, respectively.

**Sample preparation.** Under Somnopenyl® anesthesia, blood samples were drawn from the abdominal aorta with a heparinized syringe, and then rat livers were immediately excised and weighed. Plasma was obtained by centrifugation at 2,000×g at 4°C for 20 min. Plasma and liver were stored at −20°C until analyses.

**Assay of plasma Hcy and cysteine.** Hcy in plasma was measured by the method of Yamaguchi et al. (10).

**Assay of SAM and SAH.** SAM and SAH were measured by the method of She et al. (11).

**Assay of hepatic SAH hydrolase.** The SAH-synthetic activity of SAH hydrolase was measured using a modified method of She et al. (11). Liver was homogenized with 5 volumes of 0.25 m sucrose/3.3 mm MgCl2/2 mm glutathione (reduced form)/50 mm Tris-HCl buffer (pH 7.4). The homogenate was centrifuged at 100,000×g (4°C) for 1 h. The supernatant was used for assay of SAH hydrolase.

For the assay of the SAH-synthetic activity of SAH hydrolase, the incubation mixture consisted of 100 mM potassium phosphate buffer (pH 7.3), 2 mm dithiotreitol, 5 mm dl-Hcy thiolactone, 100 μM adenosine and 50 μM liver extract (1 mg protein/mL) in a total volume of 0.3 mL. After incubation at 37°C for 10 min, the reaction was stopped by adding 50 μL of 3 N perchloric acid. The content was mixed and then centrifuged at 2,000×g for 10 min. The supernatant was filtered through a Millipore membrane (0.45 μm) and applied to HPLC for analysis of SAH. SAH-synthetic activity of SAH hydrolase was estimated by formed SAH.

**Extraction of total RNA.** Total RNA in each homogenate was isolated by the acid guanidium-phenol-chloroform method, using TRIzol (Invitrogen Corporation, Carlsbad, CA, USA). RNA concentration was determined by absorption at 260 nm using HITACHI U-2001 spectrophotometer (Tokyo, Japan).

**Assay for expression of SAH hydrolase mRNA.** Expression of SAH hydrolase mRNA was determined by RT-PCR using a TaKaRa One Step RNA PCR Kit (Takara Bio Inc., Otsu, Japan). The RT-PCR partly followed the method of Ohmori et al. (12). The primer of SAH hydrolase used was as follows: the upstream primer sequence was 5′-AAGCTGCCATGGAAGGCTACGA-3′ and the downstream primer sequence was 5′-GATGCCAGCTGGAAGTGAAAGG-3′. For the primer of β-actin (used as an invariant control), β-actin RT-PCR Primer set (Toyobo Co., Ltd., Osaka, Japan) was used.

The samples were amplified by 30 PCR cycles, where each consisted of denaturation at 94°C for 60 s, annealing at 58°C for 60 s and extension at 72°C for 75 s. Each PCR product was resolved by electrophoresis on 1% agarose gel stained with ethidium bromide, and photographed under UV light. Band intensity was evaluated by the NIH image program, which was developed by U.S. National Institutes of Health.

**Statistical analysis.** The statistical difference among mean was estimated at p<0.05 according to ANOVA and Scheffe’s test (Excel Statistics 2006 for Windows, Social Survey Research Information Co., Ltd., Tokyo, Japan).

**Results and Discussion**

Hcy is an important intermediate of methionine metabolism because it occupies a branch point in the metabolism. Therefore its metabolites and activity of relevant enzyme would change under disturbed Hcy metabolism. Elevated plasma Hcy and the accumulation of SAH were observed in rats with disturbed methionine metabolism (5, 6). This suggests that metabolic situations in which plasma Hcy is increased by intravenous infusion of Hcy is more likely to induce the accumulation of SAH.

In this study, intravenous infusion of Hcy was performed to make transient high plasma Hcy levels in rats. Plasma Hcy concentration after intravenous infusion of physiological saline or Hcy solution is shown in Fig. 1. The 15-Hcy and 30-Hcy groups showed significant increase in plasma Hcy concentrations. Plasma Hcy concentrations in the 15-Hcy and 30-Hcy groups were approximately 46 times and 73 times higher as...
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This result showed that the elevated plasma Hcy concentration was achieved by intravenous infusion of Hcy.

As shown in Fig. 2, the content of hepatic SAH in the 15-Hcy and 30-Hcy groups were significantly higher than that in the Control group. There was a positive correlation ($r=0.877$, $p<0.001$) between the amount of infused Hcy and SAH content in liver. On the other hand, the content of SAM in liver did not increase by Hcy infusion (Fig. 2). Fu et al. (9) and Kloor et al. (13) reported significantly increased SAH in examined tissues of the rats received Hcy infusion, while SAM was not significantly different as compared to the Control group. The results of our study were similar to their reports. The content of SAM in liver might not be affected by the elevated plasma Hcy unlike the case of SAH. Moreover, a decrease in the SAM/SAH ratio, which is used as an indicator of methylation capacity, was significant under transient high plasma Hcy levels (1.85±0.09, 1.33±0.08, and 0.96±0.02 in the Control, 15-Hcy, and 30-Hcy groups, respectively). The decrease of the ratio indicated a block in methylation reactions (14). Therefore, the accumulation of SAH might be caused by the SAH-synthetic reaction.

Under physiological conditions, SAH hydrolysis works predominantly, in which Hcy and adenosine are removed efficiently via the remethylation and transulfuration pathways. In contrast, when nutritional deficiencies (2–4) and genetic mutations (5, 6) induced plasma Hcy elevation, the SAH-synthetic reaction was surmised to be favored. However, under these conditions, the SAH-synthetic activity of SAH hydrolase was not examined. In this study, the SAH-synthetic activity of SAH hydrolase in liver extracts was assayed under transient high plasma Hcy levels (Fig. 3). SAH-synthetic activity of SAH hydrolase in liver extracts showed no significant difference among the three groups. In addition, the ratio of SAH hydrolase mRNA versus β-actin mRNA in liver extracts (0.81±0.06, 0.76±0.02, and 0.89±0.04 in the Control, 15-Hcy, and 30-Hcy groups, respectively) showed no significant difference among the three groups. Thus SAH hydrolase mRNA in liver extracts could not be upregulated by transiently elevated plasma Hcy concentration. These results indicate that the accumulation of hepatic SAH is likely to be caused by a SAH-synthetic reaction although its activity was unchanged in liver.

In our previous study when B$_6$-deficient rats were used, an increase in the SAH-synthetic activity of SAH hydrolase was observed under elevated plasma Hcy and accumulated hepatic SAH (7). However, in this study using normal rats, transient high plasma Hcy induced the accumulation of SAH without any change in SAH-synthetic activity of SAH hydrolase. In this study, Hcy rapidly decreased from plasma after discontinuance of intravenous Hcy infusion (data not shown), which showed that further metabolism of Hcy was working...
properly. SAH-synthetic activity of SAH hydrolase may change under conditions of abnormal methionine metabolism in which remethylation and transsulfuration pathways are disturbed. Our next concern is the effects of long-term infusion of Hcy on methionine metabolism including the SAH-synthetic activity of SAH hydrolase.

In conclusion, this study showed that there was an increase in hepatic SAH without any increase in SAH-synthetic activity of SAH hydrolase in liver extracts under transient high plasma Hcy levels.

REFERENCES


