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Interrelations Between Plasma Homocysteine and Intracellular *S*-Adenosylhomocysteine

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***S*-Adenosylhomocysteine, a potent intracellular methylation inhibitor, is suggested as a potential mediator for hyperhomocysteinemia-related vascular changes. We investigated the effect of acute and chronic hyperhomocysteinemia on intracellular *S*-adenosylhomocysteine and *S*-adenosylmethionine in rats and humans. Elevated plasma homocysteine in rats infused with homocysteine produced an increase in *S*-adenosylhomocysteine ($P < 0.001$) but not *S*-adenosylmethionine levels ($P > 0.05$) in various rat tissues. However intraerythrocyte *S*-adenosylhomocysteine and *S*-adenosylmethionine levels were not changed in homocysteine-infused rats and human subjects with experimentally acute hyperhomocysteinemia by methionine loading test. In contrast, erythrocyte *S*-adenosylhomocysteine levels were significantly higher in chronic renal failure patients, who had chronically elevated plasma homocysteine levels, than in either vascular disease patients or healthy controls ($P < 0.05$). In conclusion, acute hyperhomocysteinemia can increase intracellular *S*-adenosylhomocysteine levels in tissues actively involved in homocysteine metabolism. The findings are relevant to homocysteine-related endothelial dysfunction since *S*-adenosylhomocysteine modulates endothelial cell apoptosis.** © 2000 Academic Press

While it is a subject of debate (1), considerable clinical and experimental data suggest that homocysteine may contribute to atherogenic and thrombotic events in homocysteinuric and hyperhomocystinemic patients. Elevated plasma homocysteine levels have been found in patients with coronary artery (2, 3), cerebrovascular (2, 4) and occlusive peripheral vascular disease (5, 6), and chronic renal disease (7, 8). However, these cross-sectional associations are yet to be supported by longitudinal studies. Several mechanisms have been proposed for the homocysteine related vascular changes. However, none of the hypotheses is experimentally substantiated.

Homocysteine is a metabolic intermediate formed by demethylation of methionine. The first step is the formation of *S*-adenosylmethionine (SAM) which is then demethylated to *S*-adenosylhomocysteine (SAH). SAH is further hydrolyzed to adenosine and homocysteine. SAM is an important methyl donor in many transmethylation reactions (9). SAH, the metabolite of SAM, on the other hand is a powerful inhibitor of methyltransferase enzymes, competing for the SAM binding site. It is a noncytotoxic hypomethylating agent (10). A concurrent decrease in SAM and an increase in SAH levels would inhibit methylation of many tissue components including proteins, DNA, RNA, phospholipids and other small molecules (9). Given the special metabolic relationship between the SAH and homocysteine, SAH has recently been explored for its role in homocysteine related vascular changes (11). While a point of view that high plasma homocysteine could be resulted from high intracellular SAH levels in cells with active repair process has been discussed (1), it remains to see if this high circulating homocysteine can equally increase intracellular SAH levels. Establishment of the relationship is important since high plasma homocysteine derived either from dietary intake or tissues active in methionine metabolism, e.g., hepatocytes, could increase the SAH burden to endothelial cells and potentially modulate apoptosis process (12–14). Indeed, homocysteine *in vitro* significantly increased levels of SAH, and then inhibited methyltransferase activity and growth of vascular smooth muscle cells (15). However, the production of SAH was also

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reportedly not to be influenced by extracellular homocysteine in smooth muscle cells, and the decrease of carboxyl methylation was absent (12).

As pathologically elevated SAH levels could cause inappropriate inhibition of important metabolic steps, in the present study we explored whether acute or chronic elevation of plasma homocysteine can affect intracellular SAH levels. For the acute effect, we investigated the hypothesis both in rat model with homocysteine infusion and in human after methionine loading. For the chronic effect, we measured erythrocyte SAH levels in chronic renal failure (CRF) patients who were known to have chronically elevated plasma homocysteine levels.

EXPERIMENTAL PROCEDURES

Chemicals

SAM *p*-toluenesulfonate salt, SAH, $HClO_4$ solution (70%), methanol (HPLC grade), L-cysteine, 7-fluorobenzo-2-oxa-1,3-diazole-4-sulfonate (SBD-F), *N*-acetylcysteine, and tri-*n*-butylphosphine (TBP) were purchased from Sigma Chemical Co. (St. Louis, MO). 1-Heptanesulfonic acid sodium salt and sodium acetate were obtained from Ajax Chemical Co. (Sydney, Australia). D,L-Homocysteine was obtained from Fluka Chemicals, Buchs, Switzerland. *N*,*N*-Dimethylformamide (Spectrosol; Ajax Chemical Co.) was passed through a short Al_2O_3 column immediately before use. Amino acid calibration mixtures standard and AN^+ were purchased from Beckman Instruments, Sydney, Australia. Solution standard contained cysteine (1.25 mmol/L) plus other amino acids in an acid buffer; AN^+ contained homocystine (2.5 mmol/L) plus different amino acids, also in an acid buffer. Human serum, halothane, and inactin were obtained from ICN Pharmaceuticals Inc., Sydney, Australia. Methanol and other reagents were of analytical grade.

Animal Experiments

Male Sprague–Dawley rats, body weight 230–258 g, were kept at room temperature (23°C) and had free access to food and water. The animals were sedated with halothane prior to intraperitoneal injection of anaesthetic inactin (120 mg/kg body wt). Tracheotomy was performed to facilitate breathing during experiments, and a Model 7025 rodent ventilator (UGO, Basile, Italy) was used when necessary. The right femoral artery was cannulated and attached to a Spectramed TXX-R pressure transducer connected to a Model 79D chart recorder (Grass, MA) to measure mean arterial blood pressure and heart rate. The left femoral vein was cannulated for the infusion of D,L-homocysteine solution ($n = 8$) or physiological saline ($n = 8$). Homocysteine was dissolved in physiological saline 10 mg/ml, and infused at a rate 4 ml/h/kg body weight. Rats were killed by decapitation after 1.5 h infusion with homocysteine or saline. Liver, kidney, heart, intestine, skeletal muscle, lung and brain were then rapidly removed, frozen in liquid nitrogen and stored at −70°C. Plasma and red blood cells were also collected at the same time. The project was approved by the Ethics Committee of University of New South Wales for Animal Research.

Patients and Healthy Controls

CRF patients. We recruited 10 CRF patients (7 males and 3 females, mean ± SD age = 55.4 ± 12.3 years) who were on standard hemodialysis therapy thrice weekly at the Renal Department of Prince Henry Hospital, Sydney. They were clinically stable at the time of investigation. Venous blood was collected into an EDTA tube before and after hemodialysis.

Vascular disease patients. We enrolled 13 patients with vascular disease (11 males and 2 females, mean ± SD age = 46.1 ± 7.8 years) for our study. They were diagnosed as having occlusive cerebrovascular ($n = 6$) and peripheral vascular ($n = 1$) diseases, and angiographically confirmed coronary artery disease ($n = 6$).

Healthy controls. There were 34 healthy adults (age = 40–75 years; 17 males and 17 females) volunteered for the study. They had no known clinical history of cardiovascular or renal diseases. There was no overt infection on the day of blood sample collection.

Signed consent was obtained from every participant and the study was approved by the Ethics Committee of University of New South Wales for Human Subjects.

Oral Methionine Loading Test (MLT)

MLT was carried out in all vascular disease patients and healthy controls. No MLT was conducted in CRF patients due to their medial conditions. After a 10-h overnight fast a 4-ml venous EDTA blood sample was drawn and then each individual was given an oral methionine loading of 4 g/m^2 of body surface area (approximately 0.1 g/kg body wt) mixed in orange juice. Further venous blood samples were drawn 4 and 8 h later. After the 4-h sample each subject ate a standard chicken sandwich lunch as an additional methionine load. Plasma was separated immediately, and stored at −70°C until analysis of homocysteine and methionine with HPLC.

Homocysteine Release from Red Blood Cells

To explore the hypothesis of the sources of plasma homocysteine we conducted the experiments of homocysteine released from red blood cells. *In vitro* export of homocysteine from erythrocytes into plasma was measured in freshly obtained blood samples. Venous blood was collected in 10-ml EDTA tubes from six CRF patients before and after hemodialysis, and from 6 healthy controls with similar ages to CRF patients. The whole blood was separated into 4 EDTA tubes (2.5 ml/tube). One tube was used for determination of routine hematology analysis including full blood count, hemoglobin, hematocrit and white blood cell differentiation. The other three tubes with caps open to the air were incubated in a water bath at 30°C with gentle shaking. Blood was then centrifuged at 3000*g* after 0, 3, and 6 h incubation. Plasma was removed and stored at −70°C for analysis of homocysteine. Since anemia is a common feature in CRF patients, and their hematocrits are generally lower than in healthy controls. The homocysteine concentration released into the plasma from blood cells was corrected by the formula corrected plasma homocysteine = measured plasma homocysteine/hematocrit.

Measurement of Volatile Materials Evaporating from Blood

If the increased release of homocysteine by blood cells reflects greater intraerythrocyte transmethylation activity, this can be tested by measuring the rate of accumulation of methylated product. It is known that erythrocyte membrane proteins undergo regular repair, involving a SAM-dependent methylation step (16, 17), and a by-product of this is methanol (18). Therefore we also measured this volatile product in venous blood from pre- and posthemodialysis CRF patients ($n = 4$) and health controls ($n = 4$). Blood samples of 200 μl aliquots were placed in Eppendorf tubes. Each tube was added 5 μl [L-methyl-^{3}H]methionine (71.4 Ci/mmol, 14 nmol/ml, Dupont NEN Products, Boston, MA), and then incubated at 37°C for periods of 0, 15, 30, 60, and 90 min. Immediately after this incubation, the samples were placed in ice for 2 min and centrifuged for 1 min at 10,000*g* for plasma collection. Plasma (60 μl) was applied to filter paper strips, and each strip was suspended over 5 ml scintillant

(Aqueous Counting Scintillant, Amersham, Arlington Heights, IL) in a capped counting vial, and incubated at 23°C for 2 h. The paper strip was then removed from the vial and discarded. The vial was counted using a beta counter.

Determination of Homocysteine Levels in Plasma and Tissues

We assayed homocysteine levels in plasma and rat tissue extracts using the method described previously (19). Blood was collected in an EDTA tube, and plasma was separated immediately. Plasma samples were stored at −70°C. The derivatization of plasma was performed by the Ubbink method (20). We supplemented plasma samples (200 μl) with an internal recovery standard N-acetylcysteine (1.1 mmol/L, 20 μl), and then treated with TBP solution (20 μl, 0.55 mmol/L in dimethylformamide). After one hour incubation at 0°C, the preparations were precipitated with 10% cold trichloroacetic acid (200 μl). Precipitated proteins were removed by centrifugation, and 100 μl of supernatant was incubated with borate/EDTA buffer (250 μl, 0.125 mol/L, pH 9.2, containing 4 mmol/L EDTA), NaOH solution (20 μl, 1.5 mol/L) and SBD-F solution (100 μl of a 1 g/L solution in borate/EDTA buffer) for 60 min at 60°C. This was following by cooling to room temperature before chromatographed. Human serum standards were prepared by adding either Beckman standard solution (2 ml) plus Beckman AN^+ solution (1 ml), or phosphate-buffered saline (PBS; 3 ml), to pooled human serum (50 ml). After thorough mixing, these two serum preparations were stored at −70°C in 10-ml aliquots. These standards were run alongside plasma samples in every batch of samples assayed.

The frozen tissues were weighed and cut into small pieces, then homogenized [1:8 (w/v), unless otherwise stated] in-cold PBS at 0°C using an Omni 2000 homogenizer (Omni International, Waterbury, CT). The homogenized tissue suspension (0.5 ml) was treated with 0.05 ml TBP solution (0.55 mmol/L in dimethylformamide), mixed thoroughly, and incubated for 60 min at 0°C. After adding 0.5 ml TCA and mixing, the precipitated proteins were immediately removed by centrifugation (1200*g* for 10 min). The supernatant was derivatized with SBD-F as described above.

All derivatized samples were filtered (0.22 μm) before HPLC. Samples (20 μl) were injected through a Nova-Pak C18 guard column onto a Microbondapak C18 column, 3.9 × 150 mm (from Waters Australia Pty, Sydney, Australia). Isocratic elution occurred at 2.2 ml/min with potassium phosphate buffer (0.1 mol/L) containing 35 ml/L CH_3CN, adjusted to pH 3.50, and peaks were detected using a RF 535 fluorometer (Shimadzu Oceania, Sydney, Australia). Data were collected and analyzed with a Shimadzu CR4A Chromatopac integrator or Millennium Software 1994 (Waters Corp., Milford, MA).

Determination of SAM and SAH in Tissues

All sample preparation prior to chromatography was performed at 0–2°C. The frozen rat tissues were weighed, homogenized with an Omni 2000 homogenizer using various volumes of 0.5 M $HClO_4$ (w/v 1:2 brain, 1:4 lung, skeletal muscle and thyroid, and 1:8 heart, liver, kidney, and intestine). After centrifugation at 12,500*g* for 5 min, each supernatant was filtered through a Millipore membrane (0.22 μm). Aliquots of acid extracts were directly applied to HPLC for analysis. The frozen packed erythrocytes were treated with an equal volume of 0.5 mol/L $HClO_4$. The precipitated proteins were removed by centrifugation and supernatant was filtered as described above.

The column was a 250 × 4.6 mm ODS-Hypersil, 5-μm particle size (Shandon, Runcorne, Cheshire, UK). Other HPLC instruments were the same as used for the homocysteine assay. The mobile phase consisted of 0.1 mol/L sodium acetate, 2.4 mmol/L heptanesulfonic acid, 4.2% acetonitrile, and 50 mmol/L sodium perchlorate. The pH value was adjusted to 3.5 with 70% perchloric acid. The isocratic elution was carried out at 1.3 ml/min. Injection volume was 25 μl. The UV detector (Model 441, Waters Corp., Milford, MA) was set at 254 nm. During HPLC analysis, SAM and SAH in biological samples were co-chromatographed with authentic standards and identified according to their retention time. Standards of SAM and SAH (1 mmol/L) were dissolved in 0.5 mol/L $HClO_4$ solution, and then diluted with 0.05 mol/L $HClO_4$ to final concentrations.

Determination of Plasma Methionine Levels

These were analyzed by routine amino acid analysis of HPLC methods at Biochemistry Department, Royal Prince Alfred Hospital, Sydney, and Department of Pharmacology, University of Sydney, Sydney, Australia.

Statistics

Results were expressed as means ± SD. The unpaired Student's *t* test was used to compare the differences between homocysteine-treated rats and saline-treated rats. The paired *t* test was used to evaluate plasma homocysteine and methionine levels, erythrocyte and plasma SAM and SAH amounts before and after MLT among patients of vascular disease and CRF and healthy controls. Homocysteine exportation from blood cells was assessed by one-way ANOVA. The Kruskal–Wallis Nonparametric ANOVA test was used to evaluate volatile evaporation from blood cells before and after hemodialysis. Two tailed $P < 0.05$ is reported as statistical significance.

RESULTS

Effects of Acute Hyperhomocysteinemia on SAH and SAM Contents in Rat Tissues

Homocysteine levels in plasma and tissues. One and a half hour after the homocysteine infusion, plasma homocysteine levels (318 ± 22.2 μmol/L) were about 30-fold higher than those in control rats infused with saline only (9.7 ± 2.2 μmol/L). The increased homocysteine levels were also detected in all tissues in a range of 15.5–684 nmol/g wet tissues (Fig. 1). The highest homocysteine levels were found in intestine which was followed by liver, kidney and heart in both control and homocysteine infused groups. However, while lung had low homocysteine level in control rats, homocysteine infusion increased the level comparable to those of liver and kidney. Both skeletal muscle and brain were found to have low contents of homocysteine.

SAM and SAH concentrations in rat tissues. We determined the amount of SAM and SAH in the rat tissues. As shown in Figs. 2A and 2B, SAM and SAH in saline infused rats varied from 38 to 112 and from 2 to 51 nmol/g wet tissues, respectively. The highest concentrations of SAM and SAH were detected in liver. For SAM, levels in kidney, heart and intestines are comparable and slightly higher than those in skeletal muscle, lung and brain (Fig. 2B). SAH levels are minimal in tissues of brain, lung and skeletal muscle, and the levels in heart and intestine were intermediate (Fig. 2A). While levels of SAH in homocysteine infused rats increased in all tissues, there was relatively little change in tissue SAM levels (Figs. 2A and 2B). The SAH levels in liver remained to be the highest among

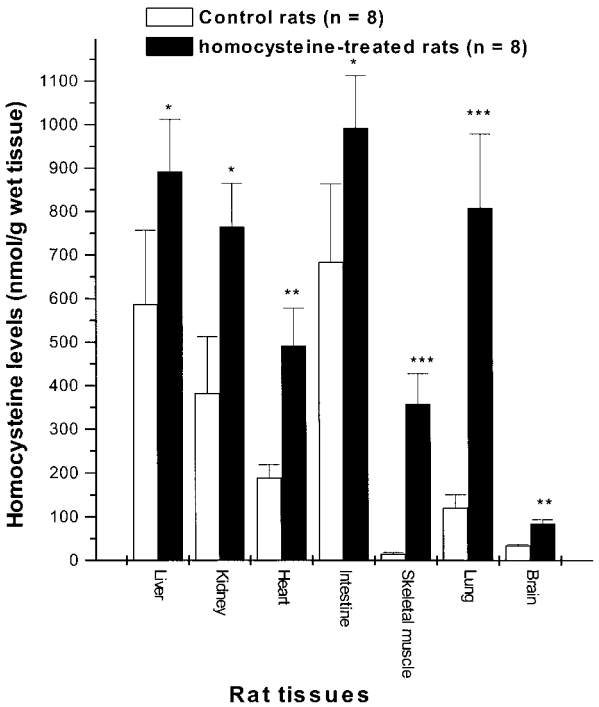

FIG. 1. Effect of homocysteine infusion on tissue homocysteine concentrations in rats. D,L-Homocysteine solution (10 mg/ml) was infused at 4 ml/h/kg body wt for 1.5 h. Rats were killed by decapitation, and tissues were removed. Values are presented as means ± SEM. $*P < 0.05$, $**P < 0.01$ and $***P < 0.001$, compared with control rats.

all tissues. But the degrees of the increase were more dramatic in skeletal muscle, heart, lung and intestine. In contrast, there was only a slight but nonsignificant increase of SAH in erythrocytes from 1.84 ± 0.9 in control rats to 2.24 ± 0.5 nmol/ml packed cells in homocysteine rats.

Effects of Acute Hyperhomocysteinemia on SAH and SAM Contents in Human Erythrocytes

As shown in Table 1 the pre-MLT plasma homocysteine levels were more than 20% higher in vascular disease patients than those in healthy controls. MLT increased both plasma homocysteine levels and methionine levels dramatically. As observed in rat model, there was no change in erythrocyte SAM and SAH levels in both vascular disease patients and healthy controls.

Effects of Chronic Hyperhomocysteinemia on SAH and SAM Contents in Human Erythrocytes

To explore the effect of chronic hyperhomocysteinemia on intracellular SAH, we studied CRF patients who were known to have elevated plasma homocysteine. As expected, the pre-dialysis plasma homocysteine levels (21.2 ± 4.5 μmol/L) were significantly higher than the baseline levels of vascular disease patients (15.2 ± 2.9 μmol/L, $P < 0.05$) and of healthy controls (12.0 ± 3.8 μmol/L, $P < 0.001$). Hemodialysis decreased the homocysteine levels to 16.5 ± 5.5 μmol/L.

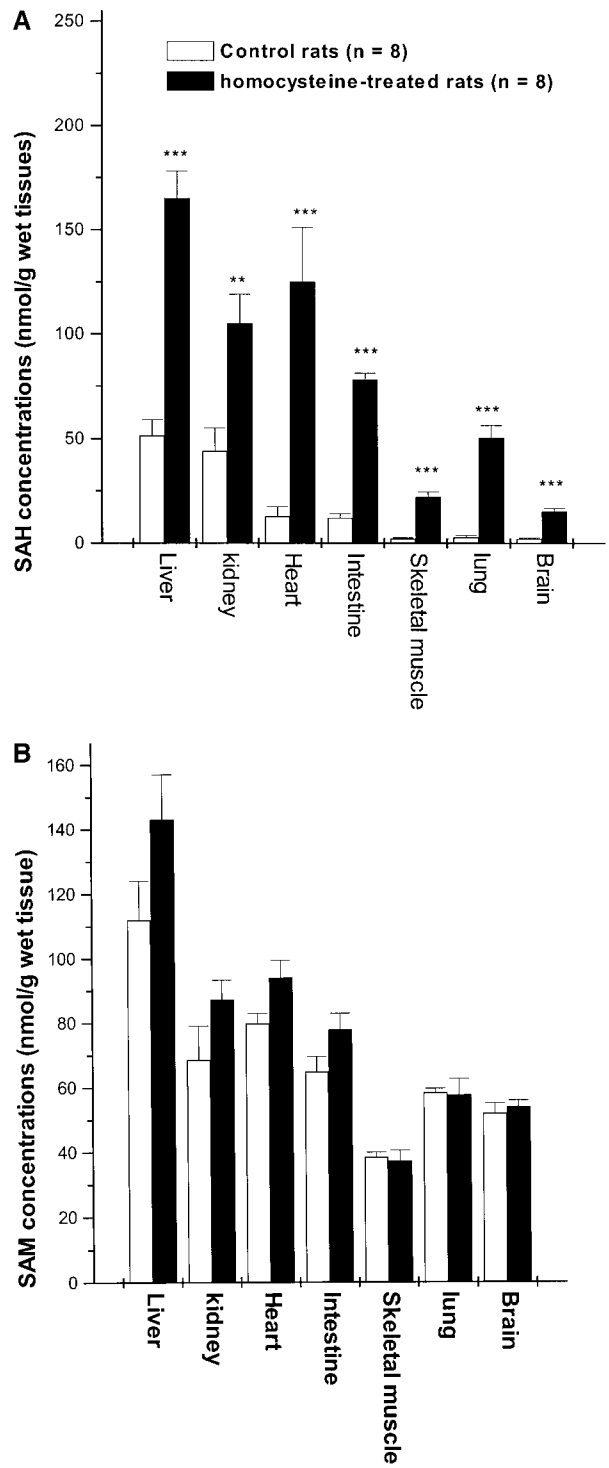

FIG. 2. Effect of homocysteine infusion on SAH (A) and SAM (B) levels of rat tissues. D,L-Homocysteine solution (10 mg/ml) was infused at 4 ml/h per kg body wt for 1.5 h. Rats were killed by decapitation, and tissues were removed. Values are presented as means ± SEM. $**P < 0.01$ and $***P < 0.001$, compared with control rats.

TABLE 1

Levels of Plasma Methionine and Homocysteine, SAM, and SAH in Erythrocytes and Plasma in Controls (n = 34) and in Vascular Disease Patients (n = 13)

	Vascular disease patients		Control subjects	
	Pre-MLT	4 h post-MLT	Pre-MLT	4 h post-MLT
Plasma homocysteine (μmol/L)	15.2 ± 2.9	38.3 ± 11.1*	12.0 ± 3.8	35.0 ± 9.3*
Plasma methionine (μmol/L)	34.2 ± 6.5	628 ± 180*	37.0 ± 9.4	762 ± 15*
Erythrocyte SAM (μmol/L of packed cells)	1.04 ± 0.4	1.08 ± 0.5	1.02 ± 0.8	1.0 ± 0.7
Erythrocyte SAH (μmol/L of packed cells)	0.34 ± 0.12	0.37 ± 0.2	0.45 ± 0.4	0.44 ± 0.3

Note. Values are presented as means ± SD. MLT, methionine loading test.
* $P < 0.001$, compared with pre-MLT samples.

In contrast to methionine loaded patients, the pre-dialysis erythrocyte SAH levels (0.95 ± 0.3 μmol/L) were significantly higher than those of vascular disease patients (0.34 ± 0.12 μmol/L, $P < 0.05$) and healthy controls (0.45 ± 0.40 μmol/L, $P < 0.05$). The intraerythrocyte SAM levels (1.17 ± 0.5 μmol/L) were only slightly higher than those in vascular disease patients and healthy controls. The ratio of SAH:SAM, which regulates SAM-dependent methyltransferases, was increased by 2.5-fold compared with vascular disease patients and healthy controls (Fig. 3). As SAH is produced by SAM-dependent methylation reactions, it may serve as a parameter to indicate intracellular methylation.

Contribution of Blood Cells to Plasma Homocysteine Levels

To explore whether the elevated SAH in erythrocytes of CRF patients was produced by erythrocytes intracellularly or altered by plasma homocysteine levels, we conducted experiments to compare accumulations of plasma homocysteine in blood incubated *in vitro* over a period of 6 hours. Increased homocysteine in plasma was observed in whole blood of both CRF patients and healthy controls after 6 h incubation (Fig. 4). However, blood samples of CRF patients showed a much greater increase in homocysteine pre-dialysis blood compared to the post-dialysis samples or healthy controls (Fig. 4). This is consistent with the elevated SAH in erythrocytes of CRF being the results of excess intracellular production. To further support this, we also measured volatile substances evaporating from the whole blood. There was a release of volatile (^{3}H)material produced *in vitro* in the whole blood of CRF patients (Fig. 5). The

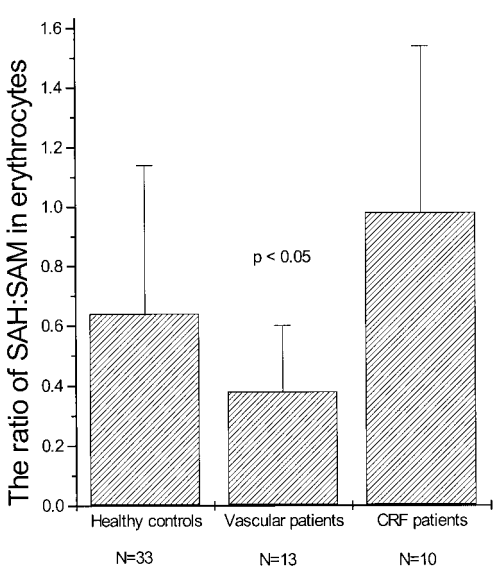

FIG. 3. Ratios of SAH:SAM in erythrocytes of CRF, vascular disease patients and healthy controls. Values are expressed as means ± SD and compared by ANOVA.

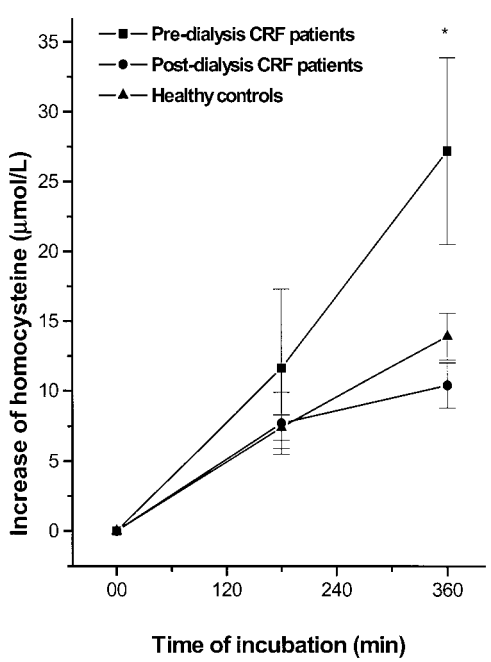

FIG. 4. Changes of total plasma homocysteine after the whole blood (n = 6 for each group) was incubated for different time period at 30°C. Values were expressed as means ± SD. *$P < 0.05$, compared with healthy controls.

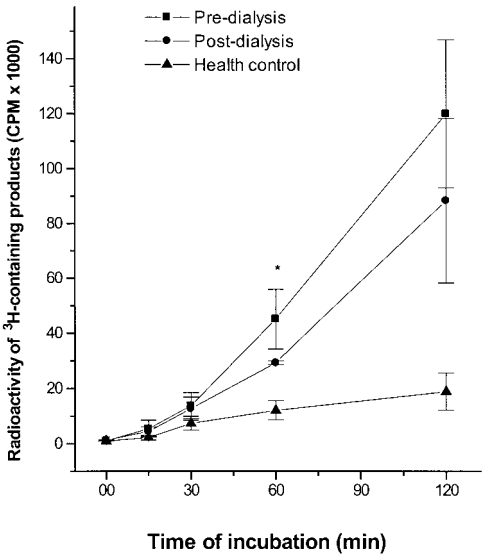

FIG. 5. Volatile radioactive product metabolized from [L-methyl-^{3}H]methionine by whole blood of CRF patients pre- and posthemodialysis. At each designed time point, the plasma was separated, and the volatile product was transferred from plasma by gaseous diffusion into scintillation fluid in capped counting vials. Values are presented as means ± SD ($n = 4$). *$P < 0.05$ compared with postdialysis samples.

predialysis blood cells produced more volatile material compared with post-dialysis blood cells. This reached statistical significance after 60 min ($P < 0.05$) incubation.

DISCUSSION

Our study demonstrated SAH and SAM contents varied from tissue to tissue which may reflect the metabolic differences between different tissues. While acute homocysteine infusion to rats increased the tissue contents of SAH, SAM levels changed very little 1.5 h after infusion. There was no increase in erythrocyte SAH levels after the infusion. This finding was further confirmed in human subjects following MTL with an increased plasma homocysteine levels but no change in erythrocyte SAH levels. We also report an elevated SAH levels in erythrocytes of CRF patients who have had chronic hyperhomocysteinemia. The *in vitro* incubation experiments of more homocysteine released from blood cells, demonstration of volatile substances from the whole blood of CRF suggest that the accumulated SAH in erythrocytes may reflect increased intracellular methylation reactions, rather than the effect of elevated plasma homocysteine as suggested by Perna *et al.* (21).

The animal model we used was healthy rats without any deficiency of vitamin B-6, and folate. Elevated plasma homocysteine levels were obtained by exogenous homocysteine infusion. The homocysteine-infused rat model is metabolically simplified, avoiding unnecessary complexities introduced by prolonged diets deficient in folate and/or vitamin B6 (22). The simplified rat model may be more clinically relevant to human hyperhomocysteinemia, which is often not complicated by concurrent deficiency of folate or vitamin B6. In this rat model, we have confirmed that liver and kidney play important roles in homocysteine metabolism (23). The homocysteine levels in both infused and non-infused rat livers and kidneys are exceedingly high, much higher than SAM and SAH levels, which have not been reported before. To our surprise, however, high amounts of homocysteine, SAM and SAH were observed in intestinal tissue. It suggests that intestine may also play an important role in homocysteine metabolism, which has never been proposed. Because homocysteine is a metabolic intermediate, formed by the demethylation of dietary methionine absorbed in the intestine, the present finding may be valuable in exploring the origin of homocysteine in plasma.

The results of a pronounced increase in SAH but only a slight change in SAM levels in rat tissues are consistent with that the increase in SAH were partly by a direct combination of homocysteine with intracellular adenosine, catalyzed by SAH hydrolase. An alternative pathway is the intracellular remethylation of homocysteine to methionine, followed by formation and demethylation of SAM.

There was no difference of erythrocyte SAM concentration among CRF, vascular disease patients and healthy controls. Although plasma homocysteine levels were mildly elevated in vascular disease patients, their erythrocyte SAH levels in fact tended to be lower than in control subjects (Table 1). For CRF patients however, SAH accumulated in erythrocytes consistent with alterations of erythrocyte membrane in CRF patients, as expressed by hemolysis, reduced deformability and increased osmotic fragility, and of various membrane proteins (21). It should be noted that these alterations in erythrocytes are not characteristic of patients with homocysteinemia and homocystinuria who do not have renal failure, although both groups have elevated plasma homocysteine.

As SAH is hydrolyzed to adenosine and homocysteine, we predicted that in fresh blood homocysteine would be released into plasma more quickly in predialysis blood from CRF patients since their erythrocytes had higher SAH. This was indeed the case. After incubating blood for 6 h, homocysteine accumulation in plasma was 2-fold faster in CRF blood than in blood from healthy controls, and 2.7-fold faster than in post-dialysis blood. The hypothesis of accelerated protein methylation is supported further by our measurement of (^{3}H)methanol, an index of the rate of protein methylation (18), from blood of CRF patients which was significantly faster in pre-dialysis than in post-dialysis. However, a higher SAH:SAM ratio, which inhibits

SAM-dependent methylations, in erythrocytes of CRF patients appears contradictory to an accelerated protein methylation. Further experiments are clearly needed for this paradox.

In conclusion, liver, kidney and intestines are the preferred metabolic sites for plasma homocysteine, which has little effect on erythrocyte SAH and/or homocysteine levels. While hyperhomocysteinemia in post myocardial infarction patients could be a result of increased release from ischemic tissues (1), the findings that high plasma homocysteine can increased intra-cellular SAH levels in certain tissues are significant. This increased intracellular SAH, when occurs in endothelial cells, would lead to excessive endothelial apoptosis, which is both pro-atherogenic and pro-thrombotic.

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