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ORIGINAL ARTICLE

Alterations in the metabolomics of sulfur-containing substances in rat kidney by betaine

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Abstract Earlier studies have shown that betaine administration may modulate the metabolism of sulfur amino acids in the liver. In this study, we determined the changes in the metabolomics of sulfur-containing substances induced by betaine in the kidney, the other major organ actively involved in the transsulfuration reactions. Male rats received betaine (1 %) in drinking water for 2 weeks before killing. Betaine intake did not affect betaine–homocysteine methyltransferase activity or its protein expression in the renal tissue. Expression of methionine synthase was also unchanged. However, methionine levels were increased significantly both in plasma and kidney. Renal methionine adenosyltransferase activity and *S*-adenosylmethionine concentrations were increased, but there were no changes in *S*-adenosylhomocysteine, homocysteine, cysteine levels or cystathionine β-synthase expression. γ-Glutamylcysteine synthetase expression or glutathione levels were not altered, but cysteine dioxygenase and taurine levels were decreased significantly. In contrast, betaine administration induced cysteine sulfinate decarboxylase and its metabolic product, hypotaurine. These results indicate that the metabolomics of sulfur-containing substances in the kidney is altered extensively by betaine, although the renal capacity for methionine synthesis is unresponsive to this substance unlike that of the liver. It is suggested that the increased methionine availability due to an enhancement of its uptake from plasma may account for the alterations in the metabolomics of sulfur-containing substances in the kidney. Further studies need to be conducted to clarify the physiological/pharmacological significance of these findings.

Keywords Betaine · Methionine · Kidney · Sulfur amino acid metabolism · *S*-adenosylmethionine

Abbreviations

BHMT	Betaine–homocysteine methyltransferase
CβS	Cystathionine β-synthase
CDC	Cysteine sulfinate decarboxylase
CDO	Cysteine dioxygenase
CγL	Cystathionine γ-lyase
GCS	γ-Glutamylcysteine synthetase
GGT	γ-Glutamyltranspeptidase
GSH	Glutathione
MAT	Methionine adenosyltransferase
MS	Methionine synthase
SAH	*S*-Adenosylhomocysteine
SAM	*S*-Adenosylmethionine

Introduction

Betaine, trimethylglycine, is an important organic osmolyte that maintains the cell volume homeostasis in animal tissues (Garcia-Perez and Burg 1991; Wettstein et al. 1998). Also this substance is utilized as a methyl donor in the remethylation of homocysteine to methionine, which is catalyzed by betaine–homocysteine methyltransferase (BHMT). Methionine is metabolically converted to *S*-adenosylmethionine (SAM) by the activity of methionine

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adenosyltransferase (MAT). SAM has numerous important roles in cellular metabolism by acting as an essential substrate donor in biological transmethylation, transsulfuration, and aminopropylation reactions (Mato and Lu 2007). After donation of a methyl group from SAM in methylation reactions, the demethylated product, *S*-adenosylhomocysteine (SAH), is hydrolyzed to release homocysteine that is either remethylated to methionine by the action of BHMT or methionine synthase (MS), or condensed with serine into cystathionine in a reaction mediated by cystathionine β-synthase (CβS). Cystathionine is then cleaved by cystathionine γ-lyase (CγL), yielding cysteine. Cysteine is irreversibly metabolized to taurine, GSH, and inorganic sulfate. Production of GSH is catalyzed by γ-glutamylcysteine synthetase (GCS) and GSH synthetase, consecutively. Meanwhile, cysteine is oxidized to cysteine sulfinate by cysteine dioxygenase (CDO) in the cysteine sulfinate pathway. Cysteine sulfinate decarboxylase (CDC) mediates the conversion of cysteine sulfinate to hypotaurine that is transformed non-enzymatically to an end product, taurine (Pecci et al. 1999).

It has been acknowledged that an impairment in the sulfur amino acid metabolism is associated with various pathological states such as chronic liver injury, cancer, neurological disorders, and osteoarthritis (Bottiglieri 2002; Hardy et al. 2003; Mato et al. 2008), which suggests that the metabolomics of sulfur-containing substances must be strictly maintained for normal cellular functions. Previous studies have shown that betaine administration protects the liver from hepatotoxicants such as lipopolysaccharide, α-naphthylisothiocyanate, ethanol, and dimethylnitrosamine (Kim and Kim 2002; Kim et al. 2005, 2008, 2009), in which modulation of the sulfur amino acid metabolism by this substance appears to play a critical role. In fact, betaine supplementation was shown to have a significant impact on the sulfur amino acid metabolism in the liver (Kim et al. 2003; Kim and Kim 2005). Methylation of homocysteine was enhanced, resulting in a reduction of homocysteine and an elevation of methionine and SAM levels. Cystathionine was also reduced, but its metabolic product, cysteine, was not changed. Instead, the levels of taurine and hypotaurine were decreased, whereas GSH was unaffected, which would account for the maintenance of cysteine levels in the liver. It is well known that the liver is the major organ for the metabolism of sulfur amino acids; nevertheless, the kidney is also suggested to have a significant role in the regulation of the metabolomics of sulfur-containing substances as indicated by expression of the enzymes that may influence the transsulfuration reactions such as BHMT, MS, MAT, CβS, CγL, GCS, CDO and CDC in this organ (Stipanuk et al. 1990; House et al. 1997; Slow and Garrow 2006). In addition, the kidney plays a key role in the recycling of cysteine via breakdown of GSH by the renal γ-glutamyltranspeptidase (GGT) activity, while the liver has a high capacity to export this tripeptide into plasma (Griffith and Meister 1979; Kaplowitz et al. 1985). Therefore, it was of interest to examine a possibility that betaine may be capable of modulating the metabolomics of sulfur-containing substances in the kidney.

Methods

Animals and treatments

Male Sprague–Dawley rats were purchased from Orient-Bio (Sungnam, Korea). The use of animals was in compliance with the guidelines established by the Animal Care Committee in this institute. Animals were acclimated to temperature (22 ± 2 °C) and humidity (55 ± 5 %) controlled rooms with a 12-h light/dark cycle for 1 week before use. Regular rat chow and tap water were allowed ad libitum until killing. Betaine (Sigma-Aldrich, St. Louis, MO) was dissolved in drinking water (1 % w/v). Rats were 9-week-old and weighed 260–290 g when used in experiments. Rats were killed 2 weeks after the initiation of betaine intake for the collection of blood and kidney samples.

Determination of sulfur-containing substances

The left kidney was homogenized in 1 M $HClO_4$ for the determination of SAM, SAH, homocysteine, cysteine and GSH. GSH and GSSG were determined using a spectrophotometric method of Griffith (1980). Cysteine was determined by the acid-ninhydrin method (Gaitonde 1967). The method of She et al. (1994) was employed to quantify SAM and SAH. An HPLC system with a 3.5-μm Kromasil C18 column (4.6 × 250 mm, Eka Chemicals, Bohus, Sweden) and a UV detector was used. Homocysteine was determined using an HPLC method of Nolin et al. (2007) with slight modifications. Plasma was incubated with 5 mM dithiothreitol before the addition of 10 % sulfosalicylic acid; kidney homogenate was incubated with 1 M $HClO_4$. After centrifugation, the supernatant was mixed with 1.55 M NaOH. The reaction mixture, after addition of 7-fluorobenzofurazan-4-sulfonic acid (167 mg/L), was incubated at 60 °C for 1 h. An HPLC system with dual pumps, a 3.5-μm Symmetry C18 column (4.6 × 150 mm; Waters, Milford, MA), and a fluorescence detector was employed. Mobile phases used were 0.1 M sodium acetic acid (Solvent A) and methanol (Solvent B). The peak of homocysteine was detected at excitation and emission wavelengths of 385 and 515 nm, respectively. For the determination of methionine, taurine, hypotaurine, and cysteinesulfinic acid, plasma or kidney homogenate was mixed with cold methanol. These substances were derivatized with *O*-phthaldialdehyde/2-mercaptoethanol prior to quantification using the method of

Rajendra (1987). An HPLC system with a 3.5-μm Kromasil C18 column (4.6 × 250 mm, Eka Chemicals, Bohus, Sweden), dual pumps, and a fluorescence detector was used. Peaks were detected at excitation and emission wavelengths of 338 and 425 nm, respectively.

Enzyme assays

The right kidney was homogenized in 1.15 % KCl solution containing 1 mM EDTA (pH 7.4). The 104,000*g* supernatant fraction (cytosol) was used to determine the enzyme activity and protein expression. MAT activity was estimated by quantifying the SAM production. Reaction mixtures consisted of 80 mM Tris–HCl/50 mM KCl (pH 7.4), 5 mM ATP, 40 mM $MgCl_2$, 50 mM methionine, and 1.5 mg protein of cytosol in 1 mL. Incubation was carried out at 37 °C for 30 min. BHMT activity was determined by the method of Ericson and Harper (1956). Cytosol containing 0.167 % betaine and 0.167 % homocysteine was incubated at 37 °C for 60 min. The reaction was terminated by cold methanol. After centrifugation, the supernatant was analyzed for methionine using the method described above. CDO activity was measured by quantifying the production of cysteine sulfinate. Cytosol was incubated in 0.5 mM $Fe(NH_4)_2(SO_4)_2$, 5 mM $NH_2OH{\cdot}HCl$, 2 mM NAD^+, and 5 mM cysteine for 16 min at 37 °C. CDC activity was estimated by measuring the hypotaurine production. Cytosol was incubated with 0.1 M phosphate buffer (pH 7.4) containing 0.1 mM pyridoxal 5-phosphate, 2.5 mM dithiothreitol and 10 mM cysteinesulfinic acid at 37 °C for 30 min.

For Western blotting analysis, 20 μg of cytosolic proteins, separated by gel electrophoresis, was transferred to nitrocellulose membranes. The membranes were blocked with 5 % non-fat dry milk in Tris-buffered saline containing 0.1 % Tween 20. The blots were incubated overnight with primary antibodies in 5 % bovine serum albumin followed by incubation with secondary antibodies. Polyclonal antibodies against rat MATII, CβS (Santa Cruz Biotechnology, Santa Cruz, CA), BHMT, MS (Everest Biotech, Oxfordshire, UK), CDC (Abcam plc, Cambridge, UK), GCS (NeoMarkers, Fremont, CA), and CDO were used as probes. CDO antibody was a kind gift from Dr. Yu Hosokawa (Faculty of Human Sciences, Jissen Women's University, Tokyo, Japan). Proteins were detected by enhanced chemiluminescence.

Results

Changes in the concentrations of sulfur amino acids in the kidney

Betaine intake for 2 weeks induced significant changes in the concentrations of sulfur-containing substances in the kidney and plasma (Table 1). Renal methionine levels were increased markedly. SAM levels were also increased, but concentrations of its immediate metabolic products, SAH and homocysteine, were not altered by betaine intake. There were no changes in renal cysteine or GSH levels between the control and the betaine-fed rats. In contrast, taurine levels were decreased, while its precursor, hypotaurine, was elevated significantly. Plasma methionine levels were also elevated in the betaine-fed rats. But homocysteine levels in plasma were not affected by betaine intake.

Changes in the enzymes involved in sulfur amino acid metabolism

The changes in the enzyme protein expressions are summarized in Fig. 1. MATII, the extrahepatic form of MAT, was induced significantly in the kidney of the betaine-fed rats. Betaine intake did not affect MS or CβS expression. BHMT expression in renal tissue was negligible in the control rats, and not changed by betaine administration (data not shown). The expression of GCS was also unaffected. On the other hand, betaine administration reduced CDO expression significantly while inducing CDC in the kidney.

Renal MAT activity was increased, but BHMT activity was not altered in the betaine-fed rats (Fig. 2). CDO activity was depressed; however, CDC activity was induced markedly, which appeared to be directly related to the changes in their protein expressions.

Table 1 Effects of dietary betaine on sulfur-containing substances in kidney and plasma

	Control	Betaine
Kidney		
Methionine (nmol/g)	91.7 ± 4.1	130.2 ± 4.8***
SAM (nmol/g)	50.6 ± 1.5	56.7 ± 1.0**
SAH (nmol/g)	11.5 ± 0.9	11.7 ± 1.1
Homocysteine (nmol/g)	6.11 ± 0.52	6.88 ± 0.30
Cysteine (μmol/g)	3.41 ± 0.07	3.78 ± 0.21
GSH (μmol/g)	2.58 ± 0.05	2.78 ± 0.09
Hypotaurine (μmol/g)	0.35 ± 0.02	0.49 ± 0.03**
Taurine (μmol/g)	10.5 ± 0.5	8.8 ± 0.2*
Plasma		
Methionine (nmol/ml)	46.8 ± 3.0	86.3 ± 2.1***
Homocysteine (nmol/ml)	7.40 ± 0.38	6.56 ± 0.25

Rats were provided with drinking water containing 1 % betaine for 2 weeks. Each value represents the mean ± SE for six rats

*,**,*** Significantly different from the control (Student's *t* test, $P < 0.05$, 0.01, 0.001, respectively)

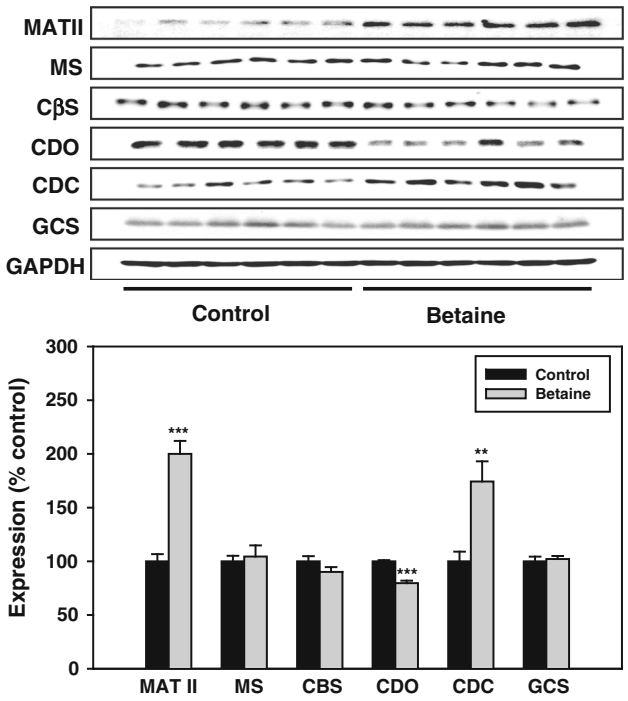

Fig. 1 Effects of dietary betaine on MATII, MS, CßS, CDO, CDC and GCS expressions in rat kidney. Rats were provided with drinking water containing 1 % betaine for 2 weeks. Each value represents the mean ± SE for six rats. **,***Significantly different from the control (Student's *t* test, $P < 0.01$, 0.001, respectively)

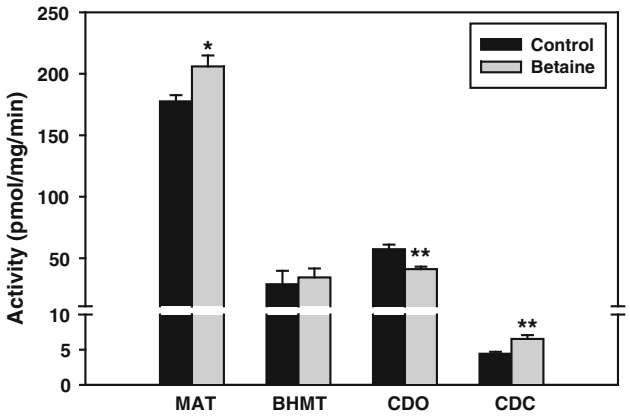

Fig. 2 Effects of dietary betaine on MAT, BHMT, CDO, CDC activities in rat kidney. Rats were provided with drinking water containing 1 % betaine for 2 weeks. Each value represents the mean ± SE for six rats. *,**Significantly different from the control (Student's *t* test, $P < 0.05$, 0.01, respectively)

Discussion

It is generally accepted that the liver has a central role in the metabolism of sulfur amino acids (Mudd and Poole 1975). However, an abnormality in the renal transsulfuration reactions results in serious conditions such as hyperhomocysteinemia, which suggests that the kidney is also active in maintaining the homeostasis of sulfur-containing substances in the body (Pin-Lan et al. 2007). In this study, we determined the changes in the transsulfuration reactions in the kidney induced by betaine that has been shown to be capable of modifying the sulfur amino acid metabolism in the liver.

The results of this study show that betaine administration alters the metabolomics of sulfur amino acids extensively in the kidney including changes in the levels of sulfur-containing substances as well as the related enzyme systems. Betaine intake resulted in an approximately 42 % increase in renal methionine levels. The protein expression or activity of BHMT was not changed by betaine intake. Neither was the MS enzyme, which mediates the regeneration of methionine from homocysteine using 5-methyltetrahydrofolate as a methyl donor, indicating that the increase in methionine may not be ascribed to an elevation of its synthesis in the kidney. Instead, betaine administration elevated plasma methionine levels markedly, which appeared to be associated with an enhancement of methionine synthesis from homocysteine in the liver (Kim and Kim 2005). In fact, the methionine concentration in plasma was shown to be regulated by its regeneration from homocysteine in hepatocytes (Shinohara et al. 2006; Korendyaseva et al. 2010). It is suggested that the elevation of renal methionine shown in this study may be due to an increase in its uptake from plasma.

Biosynthesis of SAM is mediated by the MAT enzyme. Three different forms of MAT (MATI, MATII, and MATIII) have been identified in mammalian tissues that are the products of two different genes (*MAT1A* and *MAT2A*). *MAT1A*, which is expressed only in the adult liver, encodes both MATI and MATIII (Mato et al. 1997). *MAT2A* and its gene product, MATII, are widely distributed in the body. *MAT2A* predominates in the fatal liver, but is progressively replaced by *MAT1A* during development (Gil et al. 1996). Therefore, MAT activity in the adult liver is attributed to the liver-specific MATI and MATIII, while its activity in the kidney is representative of extrahepatic MATII (Sullivan and Hoffman 1983). In the present study, betaine intake increased renal MAT activity and MATII expression, and this, in combination with the elevation of methionine availability, would be responsible for the enhancement of SAM synthesis in this organ. Despite the increase in renal SAM levels, the demethylated product, SAH, and homocysteine did not differ between the control rats and the betaine-fed rats. The reason for the failure of elevated SAM levels to affect the concentration of its immediate metabolic products is not clear. It has been acknowledged that the kidney is a major organ in the body for the elimination of homocysteine via the transsulfuration reactions (House et al. 1997, 1999; Yi and Li 2007). It is therefore suggested that a rapid removal of homocysteine

from the methionine cycle prevents an accumulation of this substance in the kidney in spite of the elevation of its metabolic precursors, methionine and SAM.

Homocysteine is condensed with serine into cystathionine that generates cysteine after cleavage by CγL. Cysteine is metabolically converted into either GSH or taurine. GCS is the rate-limiting enzyme in the synthesis of GSH. In this study, both GCS expression and GSH contents were unchanged by betaine intake. Meanwhile, cysteine is oxidized by the CDO activity to cysteine sulfinate that is the substrate for the CDC-mediated reaction to yield hypotaurine. The renal CDO protein and its activity were decreased in the betaine-fed rats. Taurine, a final product of the cysteine catabolism pathway, was also lowered in the kidney. This is in line with the results of our previous studies which showed a reduction of the CDO activity and taurine levels in the liver of the betaine-fed mice (Kim et al. 2003; Kim and Kim 2005). The reduction of taurine levels in the liver and kidney by betaine appeared to be associated with the essential function of both substances in the body. Aside from its function as a methyl donor, betaine (as well as taurine) is a major organic osmolyte to maintain the cell volumes in mammalian tissues (Rosas-Rodríguez and Valenzuela-Soto 2010). The physiological concentrations of betaine and taurine in major organs are similar (unpublished data), suggesting that intake of one substance at high levels could result in an increased efflux of the other from the kidney. Further studies need to be conducted to clarify the mechanism of taurine depletion by betaine. On the other hand, CDC expression and its metabolic product, hypotaurine, were elevated in the kidney. The failure of the increase in the kidney level of hypotaurine to alter that of its product, taurine, seems to be related to the much greater taurine level compared with that of hypotaurine. An earlier study showed an induction of CDC in animal tissues under taurine depletion (Rentschler et al. 1986). These results suggest a possibility that the reduction of taurine levels may be responsible for the enhancement of hypotaurine production.

In summary, the results of this study indicate that betaine intake results in extensive changes in the metabolomics of sulfur amino acids in rat kidney, although the renal capacity for methionine regeneration is unresponsive to betaine as shown by the lack of a change in MS and BHMT enzymes. Betaine intake increases renal methionine levels significantly, which is accompanied with an elevation of SAM and MAT activity. The increase in methionine is not associated with an enhancement of its synthesis in the kidney, but should be attributed to an elevation of its uptake from blood. There are no betaine-induced changes in the activity and expression of CβS or GCS in the kidney. However, the cysteine catabolism to taurine was inhibited significantly by betaine. Taken together, the results show that the metabolomics of sulfur-containing substances in the kidney is altered significantly by betaine, which is accounted for by the increase in regeneration of methionine in the liver, but not in the kidney. It is therefore suggested that the overall changes in the metabolomics of sulfur-containing substances in rat kidney induced by betaine are generally commensurate to the ones shown in the liver (Kim et al. 2003; Kim and Kim 2005), although the underlying mechanisms are entirely different. To our knowledge, this is the first study revealing that betaine may induce alterations in the metabolomics of sulfur-containing substances in the kidney. The physiological and pharmacological significance of the present findings is being studied in this laboratory.

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Conflict of interest The authors declare that there are no financial or other conflicts of interest.

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