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ORIGINAL ARTICLE

Altered D-methionine kinetics in rats with renal impairment

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Abstract D-Amino acids are now recognized to be widely present in mammals. In rats, exogenously administered D-methionine is almost converted into the L-enantiomer via 2-oxo-4-methylthiobutylic acid as an intermediate. D-Amino acid oxidase is associated with conversion of D-methionine into the 2-oxo acid. Since D-amino acid oxidase is present at the highest activity in the kidney compared to other organ, kidney injury is suggested to cause accumulation of D-methionine. The purpose of the present study is to assess the role of kidney in the elimination of D-methionine and metabolic conversion into L-methionine in rats using a stable isotope methodology. After a bolus i.v. administration of D-[$^2H_3$]methionine to 5/6-nephrectomized rats, plasma concentrations of D-[$^2H_3$]methionine, L-[$^2H_3$]methionine, and endogenous L-methionine were determined by a stereoselective GC–MS method. Renal mass reduction slowed down the elimination of D-[$^2H_3$]methionine. The clearance values of conversion of D-[$^2H_3$]methionine into the L-enantiomer in 5/6-nephrectomized rats were one-sixth of those in sham-operated rats. The elimination behavior of D-[$^2H_3$]methionine observed in rats demonstrated that kidney was the principal organ responsible for chiral inversion of D-methionine.

Keywords D-Methionine · Chiral inversion · GC–MS · Stable isotope · Renal impairment

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Abbreviations

$AUC_{L(\leftarrow D)}$	AUC of L-amino acid formed from D-amino acid
CL	Clearance
$CL_{D\rightarrow L}$	The metabolic clearance associated with conversion of D-amino acid into the L-enantiomer
$F_{D\rightarrow L}$	Fraction of conversion of D-amino acid into the L-enantiomer
MTPA	α-Methoxy-α-trifluoromethyl-phenylacetyl
OMe	Methyl ester
Nx	Nephrectomy
SIM	Selected ion monitoring

Introduction

Several D-amino acids are used for growth in some species of animals (Berg 1959). D-Methionine is known as a D-amino acid used most effectively by animals in place of the L-enantiomer (Man and Bada 1987; Funk et al. 1990). Because the utilization of exogenous D-amino acid depends on whether it can be effectively transformed to the L-enantiomer, it has been postulated that most of the D-methionine is converted into the L-enantiomer (Wretlind and Rose 1950; Cho and Stegink 1979). However, little information was available on the extent that D-methionine is converted into the L-enantiomer in vivo because the L-methionine formed is indistinguishable from endogenous L-methionine.

Stable isotope methodology has provided a useful tool for metabolic and pharmacokinetic investigations for endogenous compounds. Our stable isotope tracer method coupled with chiral separation of DL-methionine offered

unique advantages because it allowed determining plasma concentrations of exogenously administered D-[$^2H_3$] methionine and the L-[$^2H_3$]methionine formed by chiral inversion simultaneously with endogenous unlabeled L-methionine (Hasegawa et al. 2005a). In the previous study, we have first shown that over 90% of D-methionine administered intravenously to rat is converted into the L-enantiomer (Hasegawa et al. 2005b).

D-Methionine is supposed to be converted into the L-enantiomer by two steps (Kaji et al. 1980; Sugiyama and Muramatsu 1987; London and Gabel 1988). The initial step is an oxidative deamination by D-amino acid oxidase to form 2-oxo-4-methylthiobutylic acid (Fig. 1). Subsequently, the 2-oxo acid is stereospecifically reaminated by some transaminases to form L-methionine. L-Methionine formed is then metabolized by transmethylation and transsulfuration pathways. Since the fraction of conversion of D-methionine into the L-enantiomer is thought to be the product of the fraction of deamination and that of

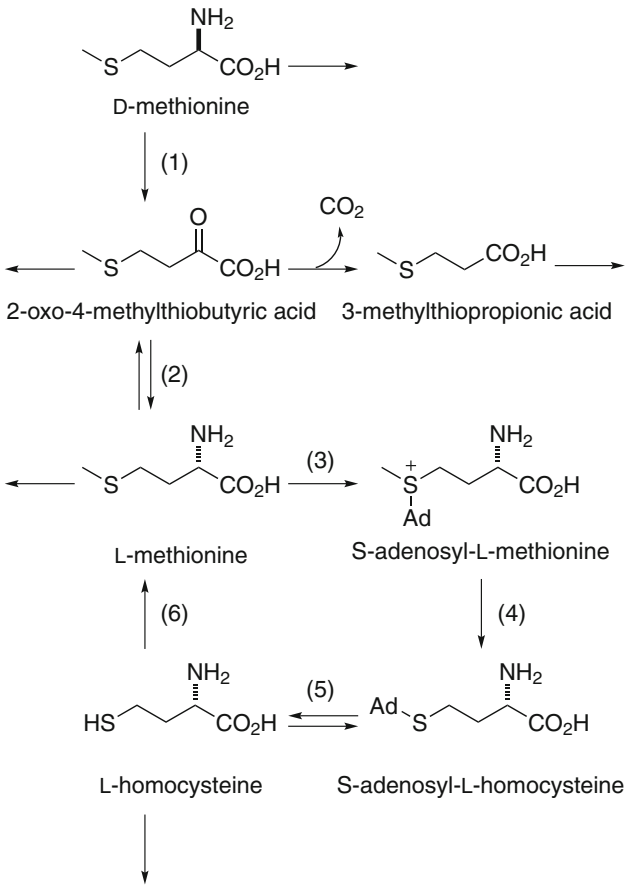

Fig. 1 Proposed metabolic pathways and excretion of D-methionine and L-methionine. The numbers in parenthesis represent the following enzymes or sequences: (*1*) D-amino acid oxidase, (*2*) transaminases, (*3*) methionine adenosyltransferase, (*4*) S-adenosylmethionine-dependent transmethylation, (*5*) adenosylhomocysteine hydrolase, (*6*) betaine-homocysteine methyltransferase or methionine synthase

reamination, over 90% conversion of D-methionine into the L-enantiomer suggests that both steps (deamination and reamination) proceed over 90% yield, respectively. We showed that mutant mice lacking D-amino acid oxidase could not convert D-leucine into the corresponding 2-oxo acid, 2-oxo-4-methylpentanoic acid, and L-leucine (Hasegawa et al. 2004). Because the mutant mice have the ability to convert 2-oxo-4-methylpentanoic acid into L-leucine, their conversion failure is due to a defect in D-amino acid oxidase activity. This result revealed that D-amino acid oxidase was indispensable for the process of chiral inversion of D-amino acids.

D-Amino acid oxidase is expressed in the kidney, liver, and brain of most vertebrates. Since the expression of D-amino acid oxidase is highest in the kidney, renal injury is suggested to cause accumulation of D-amino acids. Plasma concentration of D-amino acids was significantly higher in patients with kidney diseases than that in healthy subjects (Brückner and Hausch 1993; Nagata et al. 1987). This suggests that D-amino acid oxidase in kidney plays a prominent role in elimination of D-amino acids. However, it is not known how renal injury affects the conversion of D-methionine into the L-enantiomer.

The purpose of this study is to assess the role of kidney in the kinetic behavior of D-[$^2H_3$]methionine in rats. To achieve the purpose, we have investigated the effects of renal mass reduction by 5/6-nephrectomy on elimination of D-methionine and formation of the L-enantiomer in rats.

Materials and methods

Chemicals

Optically pure D- and L-[*S*-methyl-$^2H_3$]methionine (D-[$^2H_3$] methionine and L-[$^2H_3$]methionine, respectively) were prepared from DL-[$^2H_3$]methionine (99.2 atom% ^2H; CDN isotopes) as described previously (Hasegawa et al. 2005b). DL-[3,3,4,4,*S*-methyl-$^2H_7$]methionine (DL-[$^2H_7$]methionine) was prepared from DL-[3,3,4,4-$^2H_4$]methionine (>99 atom% ^2H; CDN isotopes) as described previously (Hasegawa et al. 2001). (*S*)-(+)-α-Methoxy-α-trifluoromethyl-phenylacetyl chloride [(+)-MTPA-Cl, >99% e.e.] and 10% HCl in methanol were purchased from Tokyo Kasei (Tokyo, Japan). A strong cation-exchange solid-phase extraction column BondElut SCX (H^+ form, size 1 ml/100 mg) was purchased from Varian (Harbor city, OH).

Animals

The experimental protocols were approved by the Institutional Animal Care Committee of Tokyo University of Pharmacy and Life Sciences. Male Sprague–Dawley rats

were purchased from SLC Animal Research Laboratories (Hamamatsu, Japan). The rats were housed in stainless-steel cages in an air-conditioned room maintained at 23 ± 1°C and 55 ± 5% humidity with a 12-h dark/light cycle. All rats were acclimated for at least 7 days, and they were allowed free access to water and food (CE-2, Clea Japan, Tokyo, Japan).

Rats weighing 180–200 g were anesthetized with sodium pentobarbital (50 mg/kg body weight, i.p.) and the anterior and posterior apical segmental branches of the left kidney were removed. At 1 week after the first surgery, the right kidney was removed under anesthesia. Rats underwent sham-operations with decapsulation of both kidneys at the same intervals as nephrectomy. After surgery, rats were allowed free access to water and food for 2 weeks.

D-Amino acid oxidase activity

D-Amino acid oxidase activity in the kidney homogenates was determined using D-alanine as the substrate by the method of Watanabe et al. (1978).

Plasma creatinine

Plasma creatinine was determined by HPLC with cation exchange Supelcosil LC-SCX column (250 × 4.6 mm i.d., particle size 5 μm; Supelco, Bellefonte, PA) using the following conditions: mobile phase, 50 mM sodium phosphate buffer (pH 5.1)–acetonitrile (3:2, v/v); flow rate, 1.3 ml/min; column temperature, ambient; detection, UV 234 nm. Creatinine was eluted at t_R 10.4 min as a single peak.

Dose experiments

After overnight food deprivation, rats were anesthetized with sodium pentobarbital (50 mg/kg body weight, i.p.). D-[$^2H_3$]Methionine (35 μmol/kg body weight) or L-[$^2H_3$]methionine (35 μmol/kg body weight) dissolved in saline (0.5 ml dosing solution/kg body weight) were administered into the femoral vein. Heparinized blood samples (150 μl) were obtained from the jugular vein 10 min before and 0.5, 1, 3, 5, 10, 15, 20, 30, 60, 90, 120, 180, 240, 300 and 360 min after dosing. The blood was centrifuged at 1,000×*g* for 10 min and the plasma was stored at −20°C until analysis. In administration of D-[$^2H_3$]methionine, rats were placed in individual metabolic cages and urine was collected at 0–24 h postdose. The urine was stored at −20°C until analysis.

Sample preparation

Plasma concentrations of D-[$^2H_3$]methionine, L-[$^2H_3$]methionine, and L-methionine were determined by the isotope dilution method as described previously (Hasegawa et al. 2005a).

To urine samples (200 μl each) was added DL-[$^2H_7$]methionine (20 nmol) as an internal standard. The samples were deproteinized with 10% trichloroacetic acid (200 μl) on a vortex mixing for ca. 0.5 min. After centrifugation at 1,000×*g* for 10 min, the supernatant was applied to a BondElut SCX cartridge. The cartridge was pre-washed and activated with 3 ml of methanol, 3 ml of a mixture of methanol and 0.1 M HCl (1:1, v/v) and 3 ml of 0.1 M HCl. The cartridge was washed with 1 ml of water and 1 ml of methanol, and then eluted with 1 ml of 2 M NH_3 in methanol. After removal of the solvent under a stream of nitrogen, the residue was dissolved in 10% HCl in methanol and heated at 60°C for 1 h to form methyl ester. After removal of the solvent under a stream of nitrogen, 100 μl of 2% triethylamine in chloroform and 100 μl of 2% (+)-MTPA-Cl in chloroform were added, shaken for 30 s on a vortex mixer and left at room temperature for 1 h to yield *N*-MTPA methyl ester (MTPA-OMe) derivative. After washing the reaction mixture with water (1 ml × 2), the solvent was evaporated under a stream of nitrogen. The residue was dissolved in 50 μl of chloroform and subjected to thin-layer chromatography on Kieselgel 60F_{254} plates (0.25 mm thickness, Merck, Darmstadt, Germany). The TLC plate was developed with chloroform–ethyl acetate (20:1, v/v) and the UV positive zone corresponding to standard MTPA-OMe derivative of methionine with R_f value of 0.48 was scraped off. The derivative was eluted with 2 ml of ethyl acetate and the solvents were evaporated under a stream of nitrogen. The residue was dissolved in ethyl acetate and a 1–2 μl of the solution was subject to GC–MS.

Gas chromatography–mass spectrometry-selected ion monitoring

Gas chromatography–mass spectrometry-selected ion monitoring (GC–MS-SIM) was made with a Shimadzu (Kyoto, Japan) QP1000EX gas chromatograph–mass spectrometer equipped with a data processing system. The operating conditions were the same as those described in our previous paper (Hasegawa et al. 2005a). Briefly, the GC column was a fused-silica capillary column SPB-1 (15 m × 0.25 mm i.d., 0.25 μm thin film; Supelco), and the mass spectrometer was operated in chemical ionization mode with isobutane as the reagent gas. SIM was performed on the quasimolecular-ions of the MTPA-OMe derivatives of methionine (*m/z* 380), [$^2H_3$]methionine (*m/z* 383), and [$^2H_7$]methionine (*m/z* 387). The MTPA-OMe derivatives of D- and L-methionine underwent baseline separation within 10 min and eluted in that order on GC. The lower limit of quantitation (LOQ) for the method was

around 500 pmol/ml plasma for [$^2H_3$]methionine. The intra- and inter-day precision of the assay for D-[$^2H_3$]methionine spiked to rat plasma in the range of 1–100 nmol/ml were less than 5 and 1%, respectively. Similarly, the intra- and inter-day precision for L-[$^2H_3$]methionine were less than 7 and 2%, respectively.

Data analysis

The AUC_{0-t} of D- and L-[$^2H_3$]methionine were calculated by the trapezoidal rule. The half-lives of D- and L-[$^2H_3$]methionine were determined from slope obtained in the last four or five time points. The extrapolation of AUC was based on the last plasma concentration and the terminal slope. Systemic plasma clearance (CL_{total}) was determined as Dose/$AUC_{0-\infty}$. Renal excretion clearance was calculated by dividing the total amounts of urinary excretion by $AUC_{0-\infty}$. The pharmacokinetic parameters were calculated by model-independent analysis using a macro-program MOMENT(EXCEL) (Tabata et al. 1999) running on Microsoft Excel.

The fraction of conversion of D-[$^2H_3$]methionine into L-[$^2H_3$]methionine ($F_{D\to L}$) after administration of D-[$^2H_3$]methionine is calculated as follows:

$$F_{D\to L} = \frac{\text{amount of L-[}^2H_3\text{]methionine formed}}{\text{amount of D-[}^2H_3\text{]methionine administered}} = \frac{CL_{D\to L}\cdot AUC_D}{Dose_D} \quad (1)$$

where $CL_{D\to L}$ is the metabolic clearance associated with conversion of D-[$^2H_3$]methionine into L-[$^2H_3$]methionine and AUC_D is the AUC of D-[$^2H_3$]methionine. According to mass balance, the rate of change of L-[$^2H_3$]methionine in the body is represented as follows:

$$\text{Rate of change of L-[}^2H_3\text{]methionine} = CL_{D\to L}\cdot C_D - CL_{L-total}\cdot C_{L(\leftarrow D)} \quad (2)$$

where $CL_{L\text{-}total}$ is the total clearance of L-[$^2H_3$]methionine and C_D and $C_{L(\leftarrow D)}$ are the respective plasma concentrations of D-[$^2H_3$]methionine and L-[$^2H_3$]methionine. Because no L-[$^2H_3$]methionine is present in the body at zero or at infinite time, integrating Eq. (2) between these time limits yields the following:

$$CL_{D\to L} = CL_{L-total}\cdot\frac{AUC_{L(\leftarrow D)}}{AUC_D} \quad (3)$$

where $AUC_{L(\leftarrow D)}$ is the AUC of L-[$^2H_3$]methionine after administration of D-[$^2H_3$]methionine. Substitution for $CL_{D\to L}$, according to Eq. 3, into Eq. 1 yields the following:

$$F_{D\to L} = \frac{AUC_{L(\leftarrow D)}}{Dose_D}\cdot CL_{L-total} \quad (4)$$

Statistical analysis

Values are mean ± SD. The statistical analyses were performed using StatView version 5 (SAS Institute). Differences between means were evaluated with Student's t test and Welch's t test. $p < 0.05$ was considered statistically significant.

Results

5/6-Nephrectomy had adverse effects on body growth, as the 5/6 nephrectomized rats weighed less than the sham-operated rats (Table 1). The levels of plasma creatinine were significantly increased in 5/6 nephrectomized rats in comparison with sham-operated rats. No significant difference of plasma L-methionine between 5/6 nephrectomized rats and sham-operated rats were observed. In both rats, endogenous D-methionine was detected but the levels were around the limit of quantitation (500 pmol/ml plasma). D-Amino acid oxidase activities in remnant kidney of 5/6 nephrectomized rats were as same as those in untreated kidney.

Figure 2 shows the plasma concentration–time profiles of D-[$^2H_3$]methionine, L-[$^2H_3$]methionine, and L-methionine after i.v. administration of D-[$^2H_3$]methionine (35 μmol/kg body weight) to 5/6-nephrectomized rats and sham-operated rats. D-[$^2H_3$]methionine disappeared biexponentially in both rats (Fig. 2a), but the systemic plasma clearance ($CL_{D\text{-}total}$) was significantly lower in 5/6-nephrectomized rats than in sham-operated rats (4.7 ± 0.6 vs. 13.8 ± 1.8 ml/min/kg body weight, $p < 0.001$; Table 2).

L-[$^2H_3$]Methionine appeared quickly in the plasma (Fig. 2b). In sham-operated rats, the plasma concentration of L-[$^2H_3$]methionine reached maximal concentration (13.5 ± 2.0 nmol/ml) at 1 min after administration. In 5/6-nephrectomized rats, the plasma concentration of L-[$^2H_3$]methionine reached maximal concentration (4.8 ± 1.3 nmol/ml)

Table 1 Characteristics of the 5/6-nephrectomized and sham-operated rats

	5/6Nx ($n = 15$)	Sham ($n = 13$)
Body weight (g)	248.9 ± 13.0***	331.2 ± 39.4
Plasma creatinine (mg/dl)	0.8 ± 0.3***	0.3 ± 0.1
L-Methionine (nmol/ml)	53.2 ± 4.9	51.1 ± 4.1
Renal D-amino acid oxidase activity[a] (nmol/min/mg protein)	31.0 ± 14.4	31.9 ± 5.2

*** $p < 0.001$ versus sham-operated rats

[a] $n = 4$

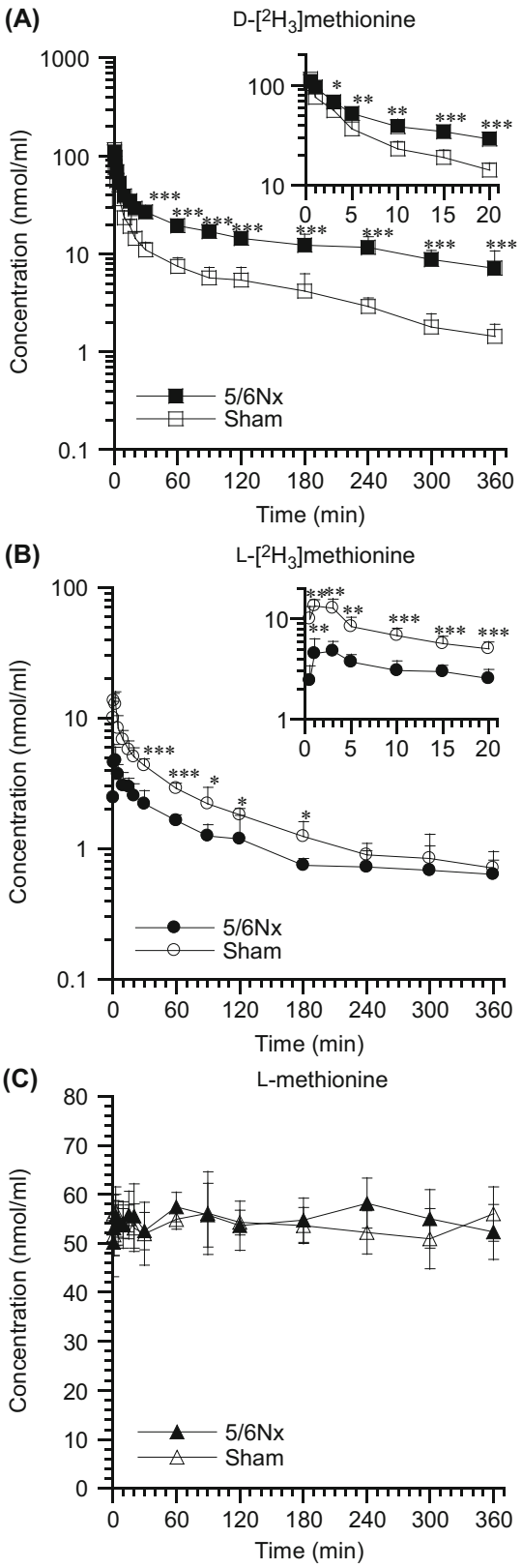

Fig. 2 Plasma concentration–time profiles for D-[$^2H_3$]methionine (**a**), L-[$^2H_3$]methionine (**b**), and endogenous L-methionine (**c**) in 5/6-nephrectomized (5/6Nx) and sham-operated rats after intravenous bolus administration of D-[$^2H_3$]methionine (35 μmol/kg body weight). Values represent mean ± SD (5/6Nx, $n = 7$; sham, n = 6). $^*p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$

at 3 min after administration. The AUC values of L-[$^2H_3$]methionine ($AUC_{L(\leftarrow D)}$) were markedly decreased to 62% compared with those in sham-operated rats (516 ± 78 vs. 824 ± 86 min nmol/ml, $p < 0.001$; Table 2).

In both the groups, the plasma concentration of endogenous L-methionine was almost constant for 6 h after administration (Fig. 2c).

No significant but slight increase of renal excretion clearance of D-[$^2H_3$]methionine in 5/6-nephrectomized rats was observed compared with those in sham-operated rats [0.088 ± 0.024 ($n = 6$) vs. 0.066 ± 0.020 ($n = 4$) ml/min/kg body weight, $p = 0.122$].

Figure 3 shows the plasma concentration–time profiles of L-[$^2H_3$]methionine and L-methionine after i.v. administration of L-[$^2H_3$]methionine (35 μmol/kg body weight) to 5/6-nephrectomized rats and sham-operated rats. In both rats, plasma concentration of L-[$^2H_3$]methionine declined rapidly and reached beyond the limit of quantitation (500 pmol/ml plasma) at 180 min after dosing (Fig. 3a). There was no significant difference in the $CL_{L\text{-total}}$ value between 5/6-nephrectomized rats and sham-operated rats (31.2 ± 5.6 vs. 37.4 ± 4.6 ml/min/kg body weight, $p = 0.051$; Table 3). Administration of L-[$^2H_3$]methionine produced no detectable amount of D-[$^2H_3$]methionine in the plasma. Plasma concentration of endogenous L-methionine was almost constant during 6 h periods after administration (Fig. 3b).

The metabolic clearance of D-[$^2H_3$]methionine into L-[$^2H_3$]methionine ($CL_{D\rightarrow L}$) was calculated by the product of total clearance for L-[$^2H_3$]methionine after administration of L-[$^2H_3$]methionine and the ratio between the AUC for D-[$^2H_3$]methionine and that for L-[$^2H_3$]methionine after administration of D-[$^2H_3$]methionine using Eq. 3. The $CL_{D\rightarrow L}$ value in 5/6-nephrectomized rats were one-sixth of that in sham-operated rats (2.2 ± 0.6 vs. 12.8 ± 2.3 ml/min/kg body weight, $p < 0.001$). The $F_{D\rightarrow L}$ value in 5/6-nephrectomized rats was decreased to 50% compared with that in sham-operated rats (46 ± 11 vs. 92 ± 20%, $p < 0.001$).

Discussion

We have found that renal mass reduction by 5/6-nephrectomy slowed down the elimination of D-[$^2H_3$]methionine, as $CL_{D\text{-total}}$ value was one-third of that in sham-operated rats (Table 2). It should be noted that the decrease in $CL_{D\text{-total}}$ of D-[$^2H_3$]methionine in 5/6-nephrectomized rats might be due to decreased excretion and/or metabolism of D-[$^2H_3$]methionine. Since the values of urinary excretion

Table 2 Pharmacokinetic parameters of D-[$^2H_3$]methionine and the formed L-[$^2H_3$]methionine after i.v. administration of D-[$^2H_3$]methionine (35 μmol/kg body weight) to 5/6-nephrectomized and sham-operated rats

	5/6Nx ($n = 7$)	Sham ($n = 6$)
D-[$^2H_3$]Methionine		
$t_{1/2}$ (min)	191.5 ± 24.5**	132.1 ± 28.7
AUC (min nmol/ml)	7251.1 ± 1,064.6***	2,451.1 ± 393.1
$CL_{D\text{-total}}$ (ml/min/kg weight)	4.7 ± 0.6***	13.8 ± 1.8
L-[$^2H_3$]Methionine		
$t_{1/2}$ (min)	165.2 ± 54.7	151.4 ± 34.7
$AUC_{L(\leftarrow D)}$ (min nmol/ml)	516.5 ± 77.8***	824.4 ± 85.7

** $p < 0.01$, *** $p < 0.001$

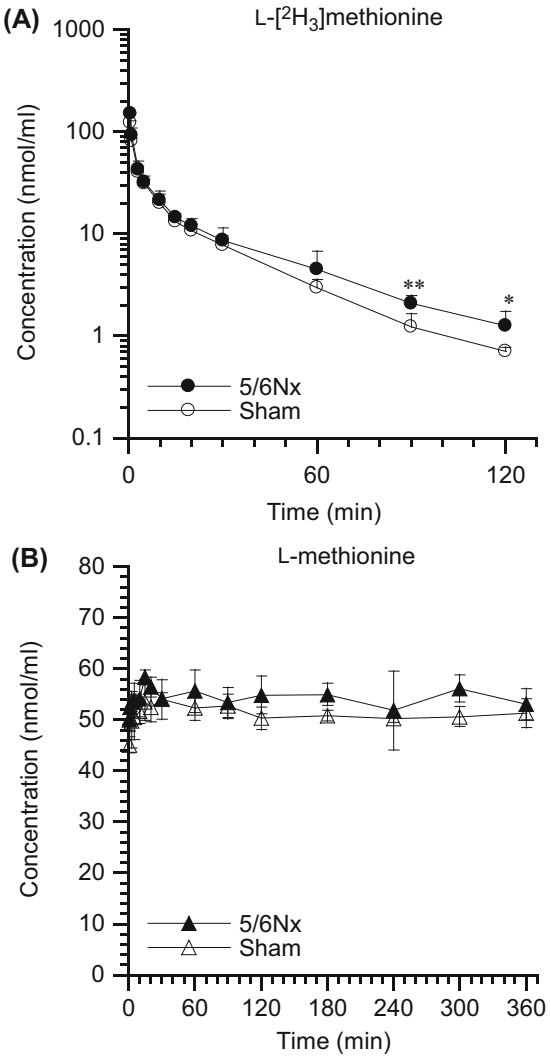

Fig. 3 Plasma concentration–time profiles for L-[$^2H_3$]methionine (**a**) and endogenous L-methionine (**b**) in 5/6-nephrectomized (5/6Nx) and sham-operated rats after intravenous bolus administration of L-[$^2H_3$]methionine (35 μmol/kg body weight). Values represent mean ± SD (5/6Nx, $n = 6$; sham, $n = 7$). *$p < 0.05$, **$p < 0.01$

Table 3 Pharmacokinetic parameters of L-[$^2H_3$]methionine after i.v. administration of L-[$^2H_3$]methionine (35 μmol/kg body weight) to 5/6-nephrectomized and sham-operated rats

	5/6Nx ($n = 6$)	Sham ($n = 7$)
$t_{1/2}$ (min)	30.6 ± 3.0***	24.4 ± 1.6
AUC_L (min nmol/ml)	1,133.7 ± 198.3	941.3 ± 117.2
$CL_{L\text{-total}}$ (ml/min/kg weight)	31.2 ± 5.6	37.4 ± 4.6

*** $p < 0.001$

clearance were two figures smaller than $CL_{D\text{-total}}$, the decrease in $CL_{D\text{-total}}$ in 5/6-nephrectomized rats might be due to decreased metabolism of D-[$^2H_3$]methionine.

Our previous study showed that almost all D-[$^2H_3$]methionine was converted into the L-enantiomer in normal rats (Hasegawa et al. 2005b). Assuming that the chiral inversion of D-methionine to the L-enantiomer is the only pathway for the metabolism of D-methionine, the metabolic clearance of D-[$^2H_3$]methionine into L-[$^2H_3$]methionine ($CL_{D\rightarrow L}$) was estimated from the values of the $AUC_{L(\leftarrow D)}$, $AUC_{D\text{-total}}$, and $CL_{L\text{-total}}$ using Eq. 3. This approach does not depend on where L-[$^2H_3$]methionine is formed or whether D-[$^2H_3$]methionine is eliminated by other routes, although i.v. administration of L-[$^2H_3$]methionine is necessary. In administration of L-[$^2H_3$]methionine, no significant decrease of the $CL_{L\text{-total}}$ value of L-[$^2H_3$]methionine in 5/6-nephrectomized rats was observed compared to that in sham-operated rats (Table 3). The $F_{D\rightarrow L}$ value in sham-operated rats indicated that almost all D-[$^2H_3$]methionine was eliminated by metabolic conversion into the L-enantiomer, which was consistent with the previous study in normal rats (Hasegawa et al. 2005b). The marked decrease of $CL_{D\rightarrow L}$ and $F_{D\rightarrow L}$ values in 5/6-nephrectomized rats implied that the renal mass reduction mainly caused decrease of the conversion.

As shown in Fig. 1, D-methionine is supposed to be converted into 2-oxo-4-methylthiobutylic acid by D-amino acid oxidase and the subsequent reamination by transaminases to form L-methionine. The transamination between 2-oxo-4-methylthiobutylic acid and L-methionine is a reversible reaction, catalyzed by enzymes such as glutamine transaminase L (Cooper and Meister 1972), glutamine transaminase K (Cooper and Meister 1974), asparagine transaminase (Cooper 1977) and leucine transaminase (Ikeda et al. 1976). The in vivo reaction by glutamine transaminases favors the synthesis of L-methionine from the 2-oxo acid. In human subjects, only minute amounts of 2-oxo-4-methylthiobutylic acid are excreted in urine (Kaji et al. 1980; Martensson 1986), whereas an increased excretion has been found after a peroral loading of D-methionine (Kaji et al. 1987) and in hypermethioninemia (Favier and Caillat 1977). 2-Oxo-4-methylthiobutylic acid also may be decarboxylated to form

3-methylthiopropionic acid (Steele and Benevenga 1978). In normal rats (Hasegawa et al. 2005b) and chicks (Dilger et al. 2007), the reamination of 2-oxo-4-methylthiobutylic acid to give L-methionine is favorable reaction rather than the urinary excretion or further degradation to 3-methylthiopropionic acid. Dixon and Benevenga (1980) suggested that the oxidative decarboxylation of 2-oxo-4-methylthiobutylic acid may be important in condition where the metabolism of L-methionine is altered. In the present study, the decrease of $F_{D\rightarrow L}$ value in 5/6-nephrectomized rats might depend on not only the decrease of renal D-amino acid oxidase and transaminase contents by 5/6-nephrectomy but also the urinary excretion and decarboxylation of [$^2H_3$]2-oxo-4-methylthiobutylic acid.

In conclusion, the present study shows that kidney plays the principal role in the conversion of D-methionine into the L-enantiomer.

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