Characterization of homocysteine metabolism in the rat kidney

James D. HOUSE, Margaret E. BROSNAN and John T. BROSNAN[1]
Department of Biochemistry, Memorial University of Newfoundland, St John's, Newfoundland, A1B 3X9, Canada

Epidemiological studies have provided strong evidence that an elevated plasma homocysteine concentration is an important independent risk factor for cardiovascular disease. We have shown, in the rat, that the kidney is a major site for the removal and subsequent metabolism of plasma homocysteine [Bostom, Brosnan, Hall, Nadeau and Selhub (1995) Atherosclerosis **116**, 59–62]. To characterize the role of the kidney in homocysteine metabolism further, we measured the disappearance of homocysteine in isolated renal cortical tubules of the rat. Renal tubules metabolized homocysteine primarily through the transulphuration pathway, producing cystathionine and cysteine (78 % of homocysteine disappearance). Methionine production accounted for less than 2 % of the disappearance of homocysteine. Cystathionine, and subsequently cysteine, production rates, as well as the rate of disappearance of homocysteine, were sensitive to the level of serine in the incubation medium, as increased serine concentrations permitted higher rates of cystathionine and cysteine production. On the basis of enrichment profiles of cystathionine β-synthase and cystathionine γ-lyase, in comparison with marker enzymes of known location, we concluded that cystathionine β-synthase was enriched in the outer cortex, specifically in cells of the proximal convoluted tubule. Cystathionine γ-lyase exhibited higher enrichment patterns in the inner cortex and outer medulla, with strong evidence of an enrichment in cells of the proximal straight tubule. These studies indicate that factors that influence the transulphuration of homocysteine may influence the renal clearance of this amino acid.

INTRODUCTION

Recent epidemiological studies indicate a strong link between increased total plasma homocysteine concentrations and the risk for the development of atherosclerosis [1]. Although several factors may lead to hyperhomocysteinaemia [2], the ultimate cause of elevated plasma homocysteine concentrations must lie in the balance between its rate of appearance in and disappearance from the plasma compartment. We have recently investigated one side of this equation and have determined that, in the rat, the kidney is a major site for the removal of plasma homocysteine [3]. Approx. 20 % of the arterial plasma homocysteine was removed on passage through the kidney. Furthermore this extracted homocysteine must be further metabolized, as the urinary excretion of homocysteine is negligible in healthy rats [3].

Previous research has shown that the rat kidney possesses the enzymes for both the remethylation of homocysteine, methyltetrahydrofolate–homocysteine methyltransferase (EC 2.1.1.13), and its removal via the transulphuration pathway, cystathionine β-synthase (EC 4.2.1.22) and cystathionine γ-lyase (EC 4.4.1.1) [4,5]. However, the relative contributions of the remethylation and transulphuration pathways to the disposal of homocysteine by the kidney remain ill-defined. Therefore the present study was designed to investigate the major routes of disposal of homocysteine in kidney tissue. In addition, the regional distribution of the major enzymes of homocysteine metabolism was determined in order to characterize the disposal of homocysteine more fully. We report here that renal homocysteine metabolism proceeds predominantly through the enzymes of the transulphuration pathway in proximal tubules and is sensitive to serine supply.

MATERIALS AND METHODS

Animals

In all experiments, male Sprague–Dawley rats (Charles River, Montreal, Canada), weighing 350–450 g, were used. They were allowed water and commercial rat chow (Agway PROLAB, Agway Inc., C.G., Syracuse, NY, U.S.A.) *ad libitum*. Animals were exposed to a 12 h dark/12 h light cycle, with the dark cycle commencing at 20:00 h. At 08:00 h on each day of study, animals were anaesthetized with sodium pentobarbital (Somnitol; MTC Pharmaceuticals, Cambridge, ON, Canada; 65 mg/kg intraperitoneally). After a midline abdominal incision, both kidneys were rapidly excised, decapsulated and placed in either ice-cold Krebs–Henseleit [6] saline (KHB; for tubule incubation studies) or ice-cold 0.05 M potassium phosphate buffer, pH 6.9 (for enzyme studies). The animals were then killed by exsanguination. The care and use of the animals were in accordance with our institution's animal care policy, and adhered to the guidelines established by the Canadian Council on Animal Care.

L-Homocysteine metabolism in kidney cortical tubules

Kidney cortical tubules were prepared by the collagenase digestion method of Guder et al. [7], using the kidney cortical tissue of three rats per digestion. Final cortical tubule fractions were resuspended in 10 vol. of KHB. Tubule viability was assessed by measuring the leakage of lactate dehydrogenase [8], and was more than 93 %. Tubule incubations were performed in stoppered 25 ml Erlenmeyer flasks, previously treated with a silanizing agent (Sigmacote; Sigma Chemical Co., St. Louis, MO, U.S.A.). Tubules (approx. 10 mg dry weight) were incubated in a total volume of 2.5 ml of KHB with substrates, for 30 min at 37 °C, in a Dubnoff shaking water bath (90 cycles/min). Incubations contained 0.5 mM L-homocysteine, prepared from L-homocysteine thiolactone [9], 1 mM L-serine, 1 mM glycine, 5 mM lactate and 0.5 mM pyruvate, unless otherwise indicated. Incubations were terminated with 0.3 ml of 30 % $HClO_4$, and the protein was removed by centrifugation. Supernatants were frozen until analysed for methionine, cystathionine, cysteine, homo-

Abbreviations used: KHB, Krebs–Henseleit saline.
[1] To whom correspondence should be addressed.

cysteine and *S*-adenosylhomocysteine. Incubations were performed without homocysteine to correct for the contribution of amino acids from endogenous sources. Homocysteine metabolism was shown to be linear with both time and tubule amount.

Methionine and cystathionine were determined by HPLC, utilizing pre-column derivatization with *o*-phthalaldehyde and fluorescence detection [10]. Total cysteine, homocysteine and *S*-adenosylhomocysteine were determined by HPLC [11,12].

Preparation of homogenates from kidney regions

Kidneys, in ice-cold 0.05 M potassium phosphate, pH 6.9, were bisected, cut into cones and further dissected into five regions: outer cortex, inner cortex, outer medulla, inner medulla and papilla, as previously described [13]. Tissue regions from three animals were pooled, and 10 % (w/v) homogenates were prepared in 0.05 M potassium phosphate, pH 6.9. The tissue was homogenized with a Polytron (Brinkman Instruments, Toronto, ON, Canada) for 20 s at 50 % output. Aliquots of the 10 % homogenates were further centrifuged at 18000 ***g*** at 4 °C for 30 min and the supernatants retained. Samples of the 10 % homogenates and the supernatants were assayed for enzyme activity and total protein.

Fractionation of kidney cortical tubules

Kidney cortical tubules were prepared as described above, and fractionated on a Percoll (Pharmacia, Montreal, Que, Canada) gradient, by the method of Vinay et al. [14]. The fractionation of the total cortical-tubule suspension (F_T) yielded four distinct fractions (F_1–F_4). Fractions F_2 and F_3 were pooled, because of their low yield, and the combined F_{2+3} fraction, as well as the F_T, F_1 and F_4 fractions were washed three times in ice-cold KHB and resuspended in 10 vol. of 0.05 M potassium phosphate, pH 6.9. Homogenates were prepared as described above, and assayed for enzyme activity and total protein.

Enzyme assays

All enzyme assays were carried out on the same day on freshly isolated tissue and tubule fractions. All were demonstrated to be linear with respect to time and protein concentration. As marker enzymes for specific kidney regions, γ-glutamyltranspeptidase [15], phosphoenolpyruvate carboxykinase [16] and hexokinase [17] were measured. The activities of the marker enzymes were determined in the 10 % homogenates. Cystathionine β-synthase activity was measured by the method of Miller et al. [18], with cystathionine concentrations measured by HPLC, as described above. Cystathionine γ-lyase activity was measured by the method of Stipanuk [19]. The activities of the enzymes of the transulphuration pathway were determined in the supernatants of the 10 % homogenates. Total and soluble protein was measured using the biuret method [20] after solubilization with deoxycholate [21]. BSA was used as a standard.

Chemicals

All chemicals, except where noted above, were obtained from Sigma Chemical Co., Oakville, ON, Canada.

Statistical analyses

Data are presented as means ± S.D. Data from the tubule-incubation studies were subjected to one-way analysis of variance (SAS V 6.10; SAS Institute Inc., Cary, NC, U.S.A.), and the statistical significance of the difference between control and test incubation results was assessed by Dunnett's procedure [22]. A value of $P < 0.05$ was chosen to represent a statistically significant difference.

RESULTS AND DISCUSSION

L-Homocysteine metabolism in isolated renal cortical tubules

When incubated in the presence of 1 mM serine, glycine and oxidizable substrates, tubules metabolized L-homocysteine predominantly through the transulphuration pathway (Table 1). Approx. 78 % of the disappearance of L-homocysteine could be accounted for by the production of both cystathionine and cysteine, with less than 2 % of the disappearance attributed to the production of methionine. Furthermore both the production of cystathionine and cysteine and the disappearance of L-homocysteine were sensitive to the level of serine in the incubation medium. Cystathionine formation, through the cystathionine β-synthase reaction, involves the condensation of one homocysteine molecule with one serine molecule. When no additional serine (0 serine) was included in the medium, tubules metabolized homocysteine mainly to cystathionine and cysteine; however, the rate of homocysteine disappearance was 20 % that of the controls (1 mM serine). In previous studies [13,23], serine-production rates in isolated renal cortical tubules, incubated in the absence of direct serine precursors, were approx. 0.5 nmol/min per mg dry weight, virtually identical with the cystathionine-production rates in tubules incubated without serine (Table 1). These data suggest that endogenous serine production was adequate for the rates of synthesis of cystathionine observed when tubules were incubated with 0.5 mM L-homocysteine.

When tubules were incubated at lower serine concentrations (0 and 0.1 mM), the recovery of homocysteine metabolites, relative to the disappearance of homocysteine, was quantitative and showed excellent agreement (Table 1). At higher serine concentrations (0.5 and 1.0 mM), recovery approximated 80 % (79 and 83 % respectively). Although these values are less than 100 %, it must be kept in mind that only the initial metabolites of homocysteine were measured. Further catabolism of cysteine in isolated renal cortical tubules has been demonstrated [23]. This further catabolism of cysteine undoubtedly accounts for the lower recovery of metabolites, relative to the measured rates of homocysteine disappearance, in tubules incubated at higher concentrations of serine.

Table 1 L-Homocysteine metabolism in isolated rat kidney cortical tubules

Data represent the means ± S.D. for four experiments and are expressed as nmol/min per mg dry weight. Statistically significant differences ($P < 0.05$) from the control group were assessed by Dunnett's procedure, and are represented by *. Control incubation conditions included 0.5 mM L-homocysteine, 1 mM serine, 1 mM glycine, 5 mM lactate and 0.5 mM pyruvate. Specific changes to the control conditions are noted in the Table, otherwise the conditions were identical.

Incubation conditions	Methionine production	Cystathionine production	Cysteine production	Homocysteine disappearance
Control	0.034 ± 0.016	1.429 ± 0.156	0.622 ± 0.066	2.625 ± 0.162
No serine	0.031 ± 0.019	0.417 ± 0.121*	0.183 ± 0.061*	0.544 ± 0.297*
0.1 mM Serine	0.048 ± 0.010	0.716 ± 0.121*	0.234 ± 0.011*	0.978 ± 0.128*
0.5 mM Serine	0.053 ± 0.019	1.269 ± 0.160	0.467 ± 0.032	2.146 ± 0.295
No serine or glycine	0.056 ± 0.010	0.432 ± 0.088*	0.145 ± 0.010*	0.492 ± 0.458*
No glycine	0.062 ± 0.025	1.642 ± 0.211	0.499 ± 0.164	2.901 ± 0.173

The inclusion of glycine had no effect on the rates of production of any of the amino acids studied. Glycine, as well as serine, can supply one-carbon units for folate metabolism. However, its presence or absence in the incubation medium did not influence the level of homocysteine remethylation. Furthermore glycine can also serve as a substrate for renal serine synthesis, both *in vivo* and *in vitro* [13,24], but the inclusion of 1 mM glycine in the incubation medium, in the absence of serine, did not further enhance cystathionine formation over the production rates observed in the absence of both serine and glycine. Previous studies investigating serine production from glycine have utilized higher concentrations (5 mM) of glycine in the medium, and the lower levels used in the present study may have been too low to enhance serine synthesis.

Methionine production remained low and was not influenced by the level of serine or glycine in the incubation medium. The low rates of methionine production observed in the tubule incubations strongly indicate that the remethylation of the homocysteine moiety is not a major route of disposal in the kidney. Our results are consistent with those of Foreman et al. [25]. These researchers measured the uptake and metabolism of ^{35}S-labelled homocystine in isolated renal cortical tubules. Their studies focused on measuring the percentage of label found in intracellular metabolites of homocystine after 5 and 30 min incubations. They found no detectable levels of ^{35}S associated with methionine when tubules were incubated with either 0.025 or 0.5 mM L-homocystine. Taken together, these studies provide good evidence that the remethylation pathway is not a major route of disposal for homocysteine in the kidney. A recent study conducted *in vivo* supports the data obtained *in vitro*. Guttormsen et al. [26], studying folate- and cobalamin-deficient subjects, suggested that the methionine synthase reaction was not an important determinant for the elimination of homocysteine from the plasma, based on the similarities of the clearance patterns in control and deficient subjects. If methionine synthase were a major route for the disposal of plasma homocysteine, one would expect decreased rates of disposal in folate or cobalamin deficiency. If the human kidney plays as important a role in clearing plasma homocysteine as does the rat kidney, these observations are strongly supportive of the transulphuration pathway being the predominant route of disposal of plasma homocysteine cleared by the kidney.

Foreman et al. [25] recovered a significant amount ($\approx 22\%$) of the ^{35}S label from homocysteine in *S*-adenosylhomocysteine. *In vivo*, *S*-adenosylhomocysteine is an intermediate in the metabolism of methionine to homocysteine. *S*-Adenosylhomocysteine produced from *S*-adenosylmethionine after transmethylation reactions is hydrolysed to produce adenosine and homocysteine through the action of the reversible enzyme *S*-adenosylhomocysteine hydrolase (EC 3.3.1.1.). Surprisingly, the equilibrium constant of this enzyme heavily favours *S*-adenosylhomocysteine synthesis [27]. However, hydrolysis predominates *in vivo*, because of the rapid metabolism of adenosine and homocysteine. In our study, we did observe *S*-adenosylhomocysteine production rates equivalent to about 1% of homocysteine disappearance. This accumulation was not influenced by the serine or glycine levels in the incubation medium. The apparent discrepancies between our results and those of previous investigators [25] may be due to methodological differences, as the previous studies focused on the measurement of intracellular metabolites. *S*-Adenosylhomocysteine, an intracellular metabolite, would be expected to be represented in a higher proportion in studies investigating intracellular metabolites solely, as opposed to our studies, which considered total production rates. However, we question whether *S*-adenosylhomocysteine production would be a major route of homocysteine disappearance, because of low levels of adenosine in the kidney [28].

Localization of enzymes of the transulphuration pathway in the rat kidney

On the basis of our data showing that the transulphuration pathway is the predominant pathway for homocysteine metabolism in kidney tubules, we examined the localization of cystathionine β-synthase and cystathione γ-lyase in the rat kidney. The strategy we employed for determining the regional and cellular location of these enzymes relied on the use of marker enzymes whose location has been defined. Phosphoenolpyruvate carboxykinase is found throughout the proximal tubule, but the enrichment of this enzyme is higher in the early convoluted portion [29]. γ-Glutamyltranspeptidase is highly enriched in proximal straight tubules [30]. Hexokinase is predominantly enriched in the distal tubules, with low activities in the proximal tubule segments [31]. We therefore compared the regional distribution of the enzymes of the transulphuration pathway with that of the markers. We have used this technique for the localization of enzymes of renal serine [13] and arginine [32] metabolism, and this procedure has yielded similar enrichment patterns to those determined by other methods (e.g. arginase [32,33]).

The specific and total activities of the enzymes of the transulphuration pathway and the marker enzymes are reported in Table 2. The majority of the total protein, 82%, was recovered in the cortical region, with 16.2% and 1.7% recovered in the medulla and papilla respectively. A similar recovery pattern for the soluble protein was also observed. These recoveries are similar to those reported previously [32]. More than 90% of the total activity of phosphoenolpyruvate carboxykinase was found in the cortex. The specific activity of this enzyme was highest in the outer cortex, and decreased towards the inner regions of the kidney, with low activity in the papilla. The total activity of the brush-border enzyme γ-glutamyltranspeptidase was highest in the cortex, with the inner cortex having the highest total activity (53.2%). Similarly, the specific activity of this enzyme was highest in the inner cortex and was also high in the outer medulla. Although the total activity of hexokinase was highest in the outer cortex (41.5%), the specific activity of this enzyme was highest in the outer medulla region of the kidney. Vinay et al. [14] demonstrated that the activity of hexokinase in the cortical region was associated with distal tubules. The enzymes of the transulphuration pathway showed different patterns of enrichment, with cystathionine β-synthase activity showing a similar localization pattern to phosphoenolpyruvate carboxykinase, and cystathionine γ-lyase showing a similar localization pattern to γ-glutamyltranspeptidase.

For both enzymes of the transulphuration pathway, the bulk of the total activity was confined to the cortical region. However, as the kidney cortex is a heterogeneous tissue, we fractionated renal cortical tubules on a Percoll gradient to permit the collection of tubules from specific sections along the nephron.

Distribution of enzymes of the transulphuration pathway in kidney cortical-tubule fractions

The method of Vinay et al. [14] allows the fractionation of a kidney cortical-tubule suspension (F_T) into a fraction that is predominantly proximal convoluted tubules (F_4) and one that is a mixture of proximal straight tubules and distal tubules (F_1). A total of four fractions are recovered after separation on a Percoll gradient (F_{1-4}), with fractions F_2 and F_3 containing a mixture of tubules. For our purposes, we pooled the F_2 and F_3, because of the

Table 2 Distribution of the major enzymes of homocysteine metabolism in different regions of the rat kidney

Enzyme activities were assayed as described in the Materials and methods section. All specific activities are expressed as μmol/min per g of protein. The specific activities of the marker enzymes are expressed per mg of total protein, and the specific activity of the cystathionine β-synthase and cystathionine γ-lyase, which are assayed in supernatants, are expressed per mg of soluble protein. The total activity of each enzyme refers to that activity in each region of rat kidney tissue (μmol/min per g wet weight of kidney). Values represent the means ± S.D. for three experiments. Values in parentheses represent percentage of protein or enzyme activity in each fraction. ND, Not detected.

		Outer cortex	Inner cortex	Outer medulla	Inner medulla	Papilla
Total protein	mg/g wet weight of kidney	81.6 ± 5.3 (51)	50.2 ± 10.3 (31)	11.3 ± 2.2 (7)	14.5 ± 2.2 (9)	2.8 ± 2.1 (2)
Soluble protein	mg/g wet weight of kidney	31.6 ± 1.8 (54)	18.5 ± 2.4 (31)	4.0 ± 0.8 (7)	4.4 ± 0.4 (7)	0.5 ± 0.3 (1)
γ-Glutamyltranspeptidase	Special activity	920 ± 205	2752 ± 651	1962 ± 318	1545 ± 223	127 ± 27
	Total activity	75.115 ± 17.194 (29.5)	132.777 ± 21.888 (53.2)	22.318 ± 5.464 (8.9)	22.157 ± 1.832 (8.9)	0.379 ± 0.360 (0.2)
Hexokinase	Specific activity	19 ± 3	22 ± 4	53 ± 7	31 ± 5	30 ± 13
	Total activity	1.555 ± 0.309 (41.5)	1.066 ± 0.229 (28.7)	0.602 ± 0.151 (16.0)	0.439 ± 0.049 (11.9)	0.065 ± 0.018 (1.8)
Phosphoenolpyruvate carboxykinase	Specific activity	37 ± 8	29 ± 6	11 ± 3	11 ± 2	5 ± 3
	Total activity	3.037 ± 0.714 (62.6)	1.524 ± 0.475 (31.5)	0.126 ± 0.068 (2.5)	0.154 ± 0.037 (3.2)	0.011 ± 0.004 (0.2)
Cystathionine β-synthase	Specific activity	5.7 ± 0.6	6.2 ± 1.3	4.7 ± 1.0	4.5 ± 1.9	
	Total activity	0.180 ± 0.007 (54.4)	0.113 ± 0.009 (34.2)	0.018 ± 0.002 (5.6)	0.019 ± 0.007 (5.8)	ND
Cystathionine γ-lyase	Specific activity	1.9 ± 0.5	4.8 ± 0.9	4.6 ± 1.9	3.8 ± 0.8	
	Total activity	0.059 ± 0.012 (32.2)	0.088 ± 0.014 (48.9)	0.018 ± 0.004 (9.6)	0.017 ± 0.002 (9.2)	ND

Table 3 Distribution of the major enzymes of homocysteine metabolism in rat kidney cortical-tubule fractions

Enzyme activities were assayed as described in the Materials and methods section. All specific activities are expressed as μmol/min per g of protein. The specific activities of the marker enzymes are expressed per mg of total protein whereas the specific activity of the cystathionine β-synthase and cystathionine γ-lyase, which are assayed in supernatants, are expressed per mg of soluble protein. The total activity of each enzyme refers to that activity in each fraction (μmol/min). Values represent the means ± S.D. for five experiments. Values in parentheses represent percentage recovery.

		F_T	F_1	F_{2+3}	F_4	Recovery (%)	F_4/F_1
Total protein	mg/fraction	183 ± 65 (100)	25 ± 8 (14)	10 ± 9 (5)	82 ± 22 (45)	64	
Soluble protein	mg/fraction	65 ± 24 (100)	10 ± 3 (15)	4 ± 2 (6)	29 ± 8 (44)	65	
γ-Glutamyltranspeptidase	Specific activity	2066 ± 206	4515 ± 1076	1694 ± 951	1052 ± 62	55	0.23
	Total activity	387 ± 171 (100)	118 ± 61 (30)	13 ± 9 (3)	86 ± 21 (22)		
Hexokinase	Specific activity	9.6 ± 1.9	17.9 ± 5.5	11.5 ± 6.7	6.1 ± 1.3	58	0.34
	Total activity	1.85 ± 0.93 (100)	0.45 ± 0.16 (24)	0.11 ± 0.13 (6)	0.52 ± 0.26 (28)		
Phosphoenolpyruvate carboxykinase	Specific activity	20.5 ± 8.7	11.6 ± 5.7	21.1 ± 19.7	29.3 ± 16.3	79	2.53
	Total activity	3.57 ± 1.78 (100)	0.28 ± 0.13 (8)	0.17 ± 0.15 (5)	2.36 ± 1.52 (66)		
Cystathionine β-synthase	Specific activity	7.2 ± 1.7	5.8 ± 1.2	7.0 ± 0.8	10.0 ± 2.9	83	1.70
	Total activity	0.515 ± 0.135 (100)	0.065 ± 0.008 (13)	0.034 ± 0.018 (7)	0.328 ± 0.113 (64)		
Cystathionine γ-lyase	Specific activity	8.3 ± 2.0	15.8 ± 8.8	3.1 ± 2.1	3.5 ± 2.1	51	0.22
	Total activity	0.531 ± 0.229 (100)	0.165 ± 0.130 (31)	0.011 ± 0.006 (2)	0.097 ± 0.067 (18)		

small size of these fractions. The data are shown in Table 3. The total recovery of protein from the original F_T fraction was 64 %, slightly lower than the 70 % reported previously [14], as cell debris and isolated cells sediment between the bands and further losses occur during the washing procedure after Percoll fractionation. The highest protein recovery was found in the F_4 fraction. Similar to the results from the regional distribution study, similar recovery patterns for the total and soluble protein were found.

The specific activity of phosphenolpyruvate carboxykinase was enriched in the F_4 band, as judged by an F_4/F_1 ratio of 2.53. The specific activity of hexokinase was enriched in the F_1 band, as judged by an F_4/F_1 ratio of 0.34. The specific activity of γ-glutamyltranspeptidase was enriched in F_1 band, as judged by an

F_4/F_1 ratio of 0.23. These data are consistent with previous studies [13,14,32]. From these data, we can conclude that F_1 is enriched in distal tubules, based on the high activity of hexokinase and low activity of phosphoenolpyruvate carboxykinase, and F_4 is enriched in proximal convoluted tubules, based on the low activity of hexokinase and high activity of phosphoenolpyruvate carboxykinase. As previously observed [13,32], the enrichment of hexokinase and γ-glutamyltranspeptidase activity in F_1 indicates that this fraction contains both proximal straight tubules and distal tubules.

Cystathionine β-synthase activity was enriched in F_4, as judged by an F_4/F_1 ratio of 1.70. The identical enrichment patterns of cystathionine β-synthase and phosphoenolpyruvate carboxykinase in both the defined kidney cortical regions (Table 2) and the fractionated cortical tubules (Table 3) strongly support the enrichment of cystathionine β-synthase in proximal convoluted tubules. As demonstrated by Lowry et al. [13], the enzymes for serine synthesis are also located in this segment of the nephron. The co-localization of these enzymes could allow serine produced endogenously in the kidney to be available for renal cystathionine synthesis.

Cystathionine γ-lyase activity was enriched in the F_1 fraction. As this fraction contains both proximal straight and distal tubules, this enrichment profile does not permit definite localization of this enzyme. However, the similar enrichment profile for cystathionine γ-lyase and γ-glutamyltranspeptidase in both the defined kidney regions (Table 2) and the fractionated cortical tubules (Table 3) support the enrichment of cystathionine γ-lyase in the proximal straight-tubule segment of the nephron. Nishi et al. [34], when immunostaining for the location of the nuclear protein probasin, showed that a cytosolic 40 kDa protein, related to probasin on the basis of antigenic properties, was expressed in a specific region of that rat kidney corresponding to proximal straight-tubule cells. Further studies by this group [35], using molecular-cloning techniques, identified this protein as cystathionine γ-lyase. However, unlike the present results, these investigators were unable to detect cystathionine γ-lyase activity in their samples, presumably because of the loss of activity on isolation of the protein. Although their data support our results, allowing us to tentatively conclude that cystathionine γ-lyase is highly enriched in proximal straight tubules, definitive experiments employing microdissected nephron segments or *in situ* hybridization are needed to define the localization of this enzyme specifically.

Our data suggest that cystathionine β-synthase and cystathionine γ-lyase are not enriched in the same segments of the nephron. However, sufficient cystathionine γ-lyase activity is present within the cells of the proximal convoluted tubule to account for the production of cysteine during incubations of cortical tubules with L-homocysteine. We measured cysteine production in both whole cortical-tubule suspensions and proximal convoluted tubules, separated on a Percoll gradient. The rates of cysteine production were similar in the two suspensions (results not shown) and compared well with the control incubations presented in Table 1. The presence of higher activities of cystathionine γ-lyase in cells 'downstream' of the proximal convoluted tubule is suggestive of an alternative function for this enzyme *in vivo*. Cystathionine γ-lyase, apart from its role in cystathionine metabolism, is involved in the β-cleavage of cyst(e)ine to form thiocysteine, pyruvate and ammonia, as part of the cysteine sulphate-independent pathway of cyst(e)ine catabolism in rat renal cortical tubules [23]. This pathway accounts for approx. 50 % of the cyst(e)ine catabolism in renal cortical tubules [23]. Cystathionine γ-lyase activity in the proximal straight-tubule segment of the nephron may serve in the catabolism of cyst(e)ine arising from the metabolism of glutathionine, initiated by γ-glutamyltranspeptidase, which is also located in this tubule segment [30]. Indeed, Abbott et al. [36], in measuring the metabolism of [^{35}S]glutathione in the perfused rat kidney, discovered approx. 10 % of the recovered label in the venous effluent as 'acidic' metabolites of glutathionine, indicating that cysteine catabolism could occur at the site of glutathionine metabolism (i.e. proximal straight tubules). Localization of the enzymes of cyst(e)ine catabolism in renal cortical tubules would provide some insight into this potential mechanism.

Concluding remarks

In summary, L-homocysteine is metabolized in isolated renal cortical tubules mainly via the transulphuration pathway. The catabolism of homocysteine was sensitive to the level of serine in the incubation medium, as serine is required for the synthesis of cystathionine. Since the transulphuration pathway was found to be the main route of homocysteine metabolism in the kidney, we investigated the regional and cellular locations of both cystathionine β-synthase and cystathionine γ-lyase. Cystathionine β-synthase was localized primarily in the outer cortex, and showed significant enrichment in the proximal convoluted tubule segment of the rat kidney nephron. Cystathionine γ-lyase, on the other hand, was localized primarily toward the inner cortex and outer medulla, with a pattern of enrichment that strongly suggested its enrichment in the proximal straight tubules of the nephron. This difference in enrichment patterns deserves further attention, but may be related to the alternative role of cystathionine γ-lyase in the catabolism of cyst(e)ine in the rat kidney.

Our previous *in vivo* study identified the kidney as a major site for the disappearance and metabolism of plasma homocysteine [3]. The current *in vitro* data provide strong evidence that homocysteine removed by the kidney is metabolized primarily through the transulphuration pathway. These data suggest that factors that may influence the activity of the enzymes of the transulphuration pathway (e.g. vitamin B_6 availability) could affect the renal clearance of homocysteine. However, *in vivo* studies designed to test this hypothesis remain to be conducted.

We thank Mrs. Beatrice Hall for expert technical assistance in the preparation of the renal cortical tubules. Funding for this study was provided by the Kidney Foundation of Canada and the Medical Research Council of Canada.

REFERENCES

1 Kang, S. S., Wong, P. W. K. and Malinow, M. R. (1992) Annu. Rev. Nutr. **12**, 279–298
2 Refsum, H. and Ueland, P. M. (1990) Trends Pharmacol. Sci. **11**, 411–416
3 Bostom, A., Brosnan, J. T., Hall, B., Nadeau, M. R. and Selhub, J. (1995) Atherosclerosis **116**, 59–62
4 Finkelstein, J. D., Kyle, W. E. and Harris, B. J. (1971) Arch. Biochem. Biophys. **146**, 84–92
5 Mudd, S. H., Finkelstein, J. D., Irreverre, F. and Laster, L. (1966) J. Biol. Chem. **240**, 4382–4392
6 Krebs, H. A. and Henseleit, K. (1932) Hoppe-Seyler's Z. Physiol. Chem. **210**, 33–66
7 Guder, W. G., Wiesner, W., Stukowski, B. and Wieland, O. (1971) Hoppe-Seyler's Z. Physiol. Chem. **352**, 1319–1328
8 Dhanakoti, S. N., Brosnan, J. T., Herzberg, G. R. and Brosnan, M. E. (1990) Am. J. Physiol. **259**, E437–E442
9 Duerre, J. A. and Miller, C. H. (1966) Anal. Biochem. **17**, 310–315
10 Jones, B. N. and Gillingham, T. P. (1983) J. Chromatogr. **266**, 471–482
11 Vester, B. and Rasmussen, K. (1991) Eur. J. Clin. Chem. Clin. Biochem. **29**, 549–554
12 Wilson, M. J., Shivapurkar, N. and Poirier, L. A. (1984) Biochem. J. **218**, 987–990
13 Lowry, M., Hall, D. E. and Brosnan, J. T. (1986) Am. J. Physiol. **250**, F649–F658
14 Vinay, P., Gougoux, A. and Lemieux, G. (1981) Am. J. Physiol. **241**, F403–F411
15 Tate, S. S. and Meister, A. (1974) J. Biol. Chem. **249**, 7593–7602
16 Seubert, W. and Huth, W. (1965) Biochem. Z. **343**, 176–191

17 Vinuela, E., Salas, M. and Sols, A. (1963) J. Biol. Chem. **238**, 1175–1176
18 Miller, J. W., Nadeau, M. R., Smith, J., Smith, D. E. and Selhub, J. (1994) Biochem. J. **298**, 415–419
19 Stipanuk, M. H. (1979) J. Nutr. **109**, 2126–2139
20 Gornall, A. G., Bardawill, C. J. and David, M. M. (1949) J. Biol. Chem. **177**, 751–766
21 Jacobs, E. E., Jacob, J., Sanadi, D. R. and Bradley, L. B. (1956) J. Biol. Chem. **223**, 147–156
22 Zar, J. H. (1984) Biostatistical Analysis, 2nd edn., Prentice-Hall, Englewood Cliffs, NJ
23 Stipanuk, M. H., De La Rosa, J. and Hirschberger, L. L. (1990) J. Nutr. **120**, 450–458
24 Lowry, M., Hall, D. E., Hall, M. S. and Brosnan, J. T. (1987) Am. J. Physiol. **252**, F304–F309
25 Foreman, J. W., Wald, H., Blumberg, G., Pepe, L. M. and Segal, S. (1981) Metabolism **31**, 613–619
26 Guttormsen, A. B., Schneede, J., Ueland, P. M. and Refsum, H. (1996) Am. J. Clin. Nutr. **63**, 194–202
27 De La Haba, G. and Cantoni, G. (1959) J. Biol. Chem. **234**, 603–608
28 Miller, W. L., Thomas, R. A., Berne, R. M. and Rubio, R. (1978) Circ. Res. **43**, 390–397
29 Guder, W. G. and Schmidt, U. (1974) Hoppe-Seyler's Z. Physiol. Chem. **355**, 273–278
30 Heinle, H., Wendel, A. and Schmidt, U. (1977) FEBS Lett. **73**, 220–224
31 Schmidt, U., Marosvari, J. and Dubach, U. C. (1975) FEBS Lett. **53**, 26–28
32 Dhanakoti, S. N., Brosnan, M. E., Herzberg, G. R. and Brosnan, J. T. (1992) Biochem. J. **282**, 369–375
33 Levillain, O., Hus-Citharel, A., Morel, F. and Bankir, L. (1989) Renal Physiol. Biochem. **12**, 302–312
34 Nishi, N., Kagawa, Y., Miyanaka, H., Oya, H. and Wada, F. (1992) Biochim. Biophys. Acta **117**, 47–54
35 Nishi, N., Tanabe, H., Oya, H., Urushihara, M., Miyanaka, H. and Wada, F. (1994) J. Biol. Chem. **269**, 1015–1019
36 Abbott, W. A., Bridges, R. J. and Meister, A. (1984) J. Biol. Chem. **259**, 15393–15400

Received 10 April 1997/30 June 1997; accepted 30 July 1997