Interorgan exchange of aminothiols in humans

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Aminothiols are linked in a complex series of intracellular metabolic pathways (15). Hcy is generated in the methyl donor transsulfuration pathway of methionine degradation. Hcy can be metabolized back to methionine by the remethylation pathway or converted by the transsulfuration pathway to cystathionine and cysteine (Cys). Cys is a major substrate for the synthesis of glutathione (GSH; glutamyl-cysteinyl-glycine), the most abundant intracellular thiol, which participates in reactions involving the synthesis of proteins and nucleic acids and in those that detoxify free radicals and peroxides (15, 19, 22). Cys-Gly is formed by the breakdown of GSH by γ-glutamyl transpeptidase (γ-GT), which is localized mostly on the external surface of the cell membrane but is also found in plasma (16, 19, 22). Besides catalyzing the hydrolysis of GSH to glutamate and Cys-Gly, this enzyme also catalyzes the transfer of amino acids or dipeptides to form γ-glutamyl peptides and Cys-Gly (22).

In vitro and in vivo studies in rats suggest that the kidney plays a major role in the maintenance of Hcy plasma levels (11, 17, 18). However, the concept of a role played by the kidney in Hcy metabolism may be challenged by studies performed in humans that have shown no significant uptake of this metabolite (39). In addition, the extrarenal exchange of Hcy and other aminothiols has never been evaluated. This issue may be particularly relevant if one takes into account the role of splanchnic organs and peripheral tissues in protein turnover and handling of several amino acids (37).

In the present study, to gain insight into the sites and mechanisms that regulate the homeostasis of aminothiols in blood, we measured the balance of total Hcy, Cys-Gly, and Cys across the kidney, splanchnic bed, and lower limb in humans in the postabsorptive state, a condition in which the steady state of arterial levels is maintained by the constancy of the uptake and tissue release rates.

MATERIALS AND METHODS

Subjects. Ten subjects (5 men and 5 women; age 23–58 yr) were studied in the postabsorptive state. Cardiac catheterization was considered necessary for hemodynamic evaluation in four subjects. Six subjects had arterial hypertension. In these subjects, renal vein catheterization for renin activity

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was considered helpful for diagnostic purposes. The final diagnosis was essential hypertension. Subjects were within 110% of their ideal body weight based on Metropolitan Life Insurance Tables (23) and had normal glucose tolerance tests. They were on a diet providing 30–35 kcal and 0.9–1.2 g of protein·kg⁻¹·day⁻¹, as assessed by dietary histories and urea excretion. Routine laboratory tests, acid-base, and electrolyte measurements were all normal. Plasma folate, B₁₂, and B₆ vitamin levels were within the normal range (10 ± 3 pg/ml, 408 ± 18 μmol/l, and 47 ± 8 μmol/l, respectively). None of the subjects had either a history or clinical evidence of gastrointestinal or liver diseases, congestive heart failure, diabetes mellitus, or other endocrinopathies.

The study was part of a larger protocol on interorgan metabolism approved by the Ethics Committee of the Department of Internal Medicine of the University of Genoa. All subjects were informed about the nature, purposes, procedures, and possible risks of the study before their informed consent was obtained. The procedures were in accordance with the Helsinki declaration.

**Protocol.** The subjects were studied in the basal, postabsorptive state. Between 9 and 10 AM, two introducer sets (8.5 F) were placed into the femoral vein. A Cobra 7F catheter (William Cook Europe, Bjaeverskov, Denmark) was then guided under fluoroscopic control through a femoral vein either to the right ventricular cavity or to a renal vein. A Teflon catheter was inserted percutaneously into a radial artery. After the diagnostic procedure was completed, another catheter was introduced to allow simultaneous sampling from a renal and a hepatic vein. The position of the catheter tip was visually ascertained through image intensification before blood withdrawal was started. The catheters were kept patent by intermittent flushing with saline. Three sets of blood samples were obtained at ~20-min intervals from the radial artery as well as from the renal, hepatic, and femoral veins. Ten subjects had leg catheterization (a femoral vein approach was common to all patients). Eight subjects had renal and leg and eight had hepatic and leg catheterizations. Thus leg, kidney, and splanchnic catheterizations were carried out in ten, eight, and eight subjects, respectively. Urine flow was collected via a bladder catheter. Urine, collected in sterile containers, was immediately frozen at −20°C.

The glomerular filtration rate (GFR) was measured by iothalamate infusion and clearance (27). True renal plasma flow (RPF) was determined in eight subjects by the paraminohippurate (PAH) method according to Smith (33). True renal plasma flow was calculated as (Xa − (Xv)) × plasma flow, where Xa and Xv are the concentrations of aminothiol(s) in arterial and venous plasma, respectively. Fractional extraction of aminothiol was calculated as [(Xa) − (Xv)]/Xa × 100, and urinary excretion was calculated as Xu × urinary flow. Renal clearance (ml·min⁻¹·1.73 m⁻²) was calculated as (Xa − Xv)/Xa plasma flow and urinary clearance as Xu/Xa × urine flow, where Xu is the renal urine level.

**Statistical analysis.** Statistical analysis was performed using ANOVA for repeated measures to compare arterial data with venous data. Linear regression and correlation were employed to evaluate the relation between two variables. A P value of <0.05 was considered statistically significant. All data are expressed as means ± SE. Statistical analysis was performed with the Statview Statistical Package (Abacus, Berkeley, CA).

**RESULTS**

Individual arterial and venous levels of Hcy, Cys, and Cys-Gly, as well as their exchange rates across organs, are reported in Table 1. Arterial values of individual aminothiols were within the range of those reported by other investigators in peripheral plasma in healthy subjects (1, 20). Arterial Cys and Hcy levels were directly related (r = 0.681, P < 0.03), whereas no relationship was found between Cys-Gly and Hcy or Cys plasma levels. Plasma flows across the kidney, splanchnic organs, and legs were 515 ± 36, 700 ± 20, and 620 ± 64 ml·min⁻¹·1.73 m⁻², respectively. GFR was 97 ± 5 ml·min⁻¹·1.73 m⁻².

Mean Hcy levels in the renal veins were only slightly lower (−2%, P = not significant [NS]), and those in the hepatic vein were slightly greater (+2%, P = NS) than

<table>
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<tr>
<th>Rate, ± SE</th>
<th>Artery μmol/l</th>
<th>Renal Vein μmol/l</th>
<th>Rate, ± SE</th>
<th>Artery μmol/l</th>
<th>Hepatic Vein μmol/l</th>
<th>Rate, ± SE</th>
<th>Artery μmol/l</th>
<th>Leg Vein μmol/l</th>
<th>Rate, ± SE</th>
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<tr>
<td>Homocysteine</td>
<td>10.3 ± 1.40</td>
<td>10.1 ± 1.60</td>
<td>0.11 ± 0.10</td>
<td>8.6 ± 1.29</td>
<td>8.7 ± 1.26</td>
<td>0.18 ± 0.94</td>
<td>9.2 ± 1.34</td>
<td>9.71 ± 1.34°</td>
<td>−0.31 ± 0.1</td>
</tr>
<tr>
<td>Cysteine</td>
<td>210 ± 12</td>
<td>219 ± 13†</td>
<td>4.6 ± 1.70</td>
<td>201 ± 13.8</td>
<td>196 ± 13.3°</td>
<td>3.56 ± 1.52</td>
<td>198 ± 12.5</td>
<td>196 ± 11.2</td>
<td>1.38 ± 1.5</td>
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<tr>
<td>Cysteinyl-glycine</td>
<td>19.0 ± 4.5</td>
<td>13.9 ± 3.6‡</td>
<td>2.5 ± 0.38</td>
<td>18.1 ± 1.80</td>
<td>19.5 ± 1.45†</td>
<td>−0.76 ± 0.04</td>
<td>18.8 ± 1.40</td>
<td>20.4 ± 1.5†</td>
<td>−1.07 ± 0.2</td>
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Values are means ± SE. *P < 0.05, †P < 0.02, ‡P < 0.01 or less vs. artery.
that aminothiol metabolism is a compartmentalized dipeptide Cys-Gly, to explore the possible sites of aminothiols, Cys and the GSH-derived organs. Cys levels in the femoral veins were not different from the arterial ones.

Cys-Gly was significantly released by splanchnic organs and peripheral tissues and taken up by the kidney. The concentrations of Cys-Gly in the hepatic and leg veins were ~7–8% greater than in the artery.

Table 2 reports the results regarding the handling of Cys-Gly by the kidney. Cys-Gly levels were markedly lower in the renal vein than in the artery, with a fractional extraction across the kidney of ~26%. The uptake of Cys-Gly by the kidney was directly proportional to its arterial level (r = 0.78, P < 0.02). Cys-Gly urinary excretion was, on average, only 0.13 μmol/min, with a urinary clearance of ~1 ml/min. The renal uptake of Cys-Gly was markedly greater than its urinary excretion, indicating that in the basal state the greatest portion of Cys-Gly is reabsorbed and degraded within the kidney. Renal Cys-Gly clearance tended to be greater than GFR, suggesting that a percentage of Cys-Gly is taken up by peritubular capillaries.

No significant relationship was observed among the exchange of Cys-Gly, Cys, and Hcy, individually considered, across leg and kidney tissues. Nevertheless, the exchange of Cys and that of Hcy were directly related across splanchnic organs (r = 0.792, P < 0.015). Moreover, the exchange of both Cys and Hcy was related (r = 0.68, P < 0.038 and r = 0.68, P < 0.038, respectively) to the release of Cys-Gly by splanchnic organs.

**DISCUSSION**

In the present study, we evaluated both the renal and the extrarenal interorgan exchange of Hcy and of two other aminothiols, Cys and the GSH-derived dipeptide Cys-Gly, to explore the possible sites of aminothiol metabolism in humans. Our data demonstrate that aminothiol metabolism is a compartmentalized interorgan process involving fluxes of Cys and Cys-Gly that are parallel and of opposite sign among peripheral tissues, splanchnic organs, and kidney. Cys-Gly is released from both peripheral tissues and splanchnic organs (both major reservoirs of GSH) and is taken up by the kidney in amounts that are equimolar to its release. On the contrary, Cys is produced by the kidney and taken up by the splanchnic organs. According to our data, interorgan fluxes of Cys appear to be regulated so as to preserve Cys from degradation and to ensure the availability of this amino acid for liver GSH synthesis and its related processes. On the other hand, only minor fluxes from peripheral tissues to the plasma pool are observed for Hcy, suggesting that Hcy is produced and metabolized mainly intracellularly.

Cys-Gly circulates in human plasma in 10–35 μmol/l concentrations, which is ~2–5 times greater than concentrations of circulating GSH (4–7 μmol/l) (2), thus suggesting a great propensity of tissues to release this peptide. In humans, Cys-Gly is thought to be formed by both reduced and oxidized GSH by the action of γ-GT, which is present in human plasma (2, 20) or to be released within the kidney (21). In the present study, we show that both peripheral tissues and splanchnic organs release significant amounts of Cys-Gly into the circulation, thus indicating the occurrence of an exquisite ability to degrade GSH by membrane-associated transpeptidases. The release of Cys-Gly from peripheral tissues (assuming that whole muscle tissue corresponds to 2× leg release) (37) accounts for ~3.1 mmol/day, whereas Cys-Gly release from splanchnic organs accounts for ~1 mmol/day. On the basis of our findings, the release of Cys-Gly by peripheral tissues over a day accounts for ~12–14% of the GSH contained in muscle (16), and the sum of peripheral plus splanchnic release accounts for ~12% of the daily GSH whole body turnover rate (7).

Previous studies evaluated the renal metabolism of GSH and Cys-Gly in rats (21, 22). Rat GSH metabolism appears to differ from what is observed in humans, since rat plasma contains much more GSH (~30 μmol/l) and less Cys-Gly (~12 μmol/l) (21, 22). In the rat kidney, Cys-Gly is formed by the filtered GSH in the proximal tubules and is further hydrolyzed by two extracellular brush-border membrane enzymes (21). As a new finding in humans, we observed that the kidney removes significant amounts of circulating Cys-Gly and that kidney Cys-Gly uptake is stoichiometric to its release by peripheral tissues and splanchnic organs (Fig. 1). Cys-Gly renal clearance is somewhat greater than GFR. Such behavior is similar to that of other peptides such as C-peptide, insulin, and leptin, which are taken up avidly from both tubule cells sides (13, 28, 42).

It has been observed that the endogenous appearance of Cys is greater than what can be calculated from whole body protein degradation, suggesting substantial de novo generation from GSH catabolism (12). In accord with our findings, the de novo Cys that is released by the human kidney can account for 19–23% of

**Table 2. Renal metabolism of cysteiny1-glycine**

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<th>FE, %</th>
<th>Renal Uptake, μmol·min⁻¹·1.73 m⁻²</th>
<th>Renal Qmet, μmol·min⁻¹·1.73 m⁻²</th>
<th>Renal Clearance, ml·min⁻¹·1.73 m⁻²</th>
<th>Urinary Clearance, ml·min⁻¹·1.73 m⁻²</th>
<th>Urinary Excretion, μmol·min⁻¹·1.73 m⁻²</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cysteinyl-glycine</td>
<td>26.4 ± 3.6</td>
<td>2.54 ± 0.38</td>
<td>2.41 ± 0.6</td>
<td>130 ± 16</td>
<td>0.93 ± 0.17</td>
<td>0.13 ± 0.02</td>
</tr>
</tbody>
</table>

Values are means ± SE. FE, fractional extraction; Qmet, net utilization by renal tissue.
the gap between the measured/calculated appearance of Cys in the body (12). As an additional finding, we observed that a parallel uptake of the same amino acid by splanchnic organs occurs. Therefore, a large part of the Cys delivered by splanchnic organs and peripheral tissues to the circulation as Cys-Gly dipeptide returns, through the kidney metabolism, to the splanchnic organs. The release of Cys by the kidney is somewhat greater than Cys-Gly uptake, indicating that part of Cys release may derive from other sources, such as intrarenal protein or peptide degradation.

Compared with the sizable interorgan flux of Cys-Gly and Cys, the amount of Hcy that is released or taken up by individual tissues is scanty. However, the finding of a net release of Hcy occurring from peripheral tissues is a new one. According to our data, Hcy is enriched by ~5% in the femoral vein after a single passage. This is an unexpected finding, considering that only a few tissues, including the liver, the kidney, and the lung, seem to possess the entire enzymatic machinery to metabolize methionine (9, 24, 25, 39). How can Hcy be generated in peripheral tissues? Most human tissue has some capacity to convert methionine to homocysteine (25). However, the partitioning between the transmethylation and the transsulfuration pathways varies markedly from tissue to tissue, with cystathionine synthase and cystathionase being more restricted in distribution and absent from rat skeletal muscle (24). Given these findings, it is possible that methionine, which is produced by the net protein breakdown occurring in muscle during fasting, generates some Hcy in the transmethylation process. Hcy output by the peripheral tissues could presumably occur as a result of incomplete remethylation, so that some Hcy leaves these tissues.

Splanchnic organs are in a positive amino acid balance and are therefore likely candidates for removing Hcy from the circulation. However, in the present study, we were not able to find evidence of uniform behavior in Hcy handling by splanchnic organs. It is of note that the exchange of Cys and Hcy across splanchnic organs was directly related (r = 0.792, P < 0.015), with a small uptake of Hcy taking place when Cys uptake was higher. Similarly, the fractional enrichment of Cys and Hcy across splanchnic organs (i.e., the percent variation in venous values vs. its respective arterial value) was closely and directly related (Fig. 2).
This suggests that there may be similar dependence of Cys and Hcy handling on ongoing intracellular events. It is tempting to suppose that Cys uptake by splanchnic organs is related to the need for GSH synthesis and that the high turnover rate of GSH raises the demand for Cys and Hcy, therefore depleting their intracellular pools. In agreement with this hypothesis, the exchange of Hcy and Cys across splanchnic organs was related, but inversely, to the release of Cys-Gly into the hepatic veins with greater Cys and Hcy uptake when Cys-Gly release was low (Fig. 3). It is of note that, even if the liver cells play a major role in Hcy production and uptake (34), the present study does not permit evaluation of the separate roles of liver and intestine in aminothiol metabolism.

The rat kidney is capable of clearing large quantities of Hcy (11). It is of note that Hcy circulates largely in rat plasma as a free, unbound amino acid (12), whereas only a small fraction of Hcy circulates in a free form in human plasma and is available for glomerular filtration. In agreement with a previous study (39), we observed no significant uptake of Hcy by the human kidney. These findings may represent some inaccuracies in the methods currently available to detect small a-v differences of Hcy. However, we found a great interindividual variability in Hcy metabolism across the kidney. The fractional extraction of Hcy across the kidney was positively related with RPF, with a net uptake taking place when RPF was >500 ml/min (Fig. 4). When the relationship between renal Hcy clearance and RPF is examined (Fig. 5), it appears that renal clearance declines rapidly from 70 ml/min to values close to zero when plasma flow declines from 650 to ~400–500 ml/min. This finding is in agreement with data indicating that whole body Hcy clearance is reduced from 100 to 30 ml/min when renal function is impaired (26). Taken together, these data suggest the dependence of Hcy uptake by the kidney on the rate of plasma flow.
the human kidney on a nonreduced blood supply. Besides glomerular filtration, which is restricted because of protein binding, Hcy may be taken up by the peritubular basolateral surface (10, 11, 31). In keeping with this renal transport modality, an increase in Hcy levels has been observed in association with diuretic therapy, aging, and heart failure (9, 32, 40), conditions that can preferentially decrease plasma flow across the kidney.

The peripheral release of Hcy observed in the present study was not completely matched by an uptake by the kidney, suggesting that other tissues might take part in the removal of Hcy from blood. The lung is a possible candidate, since it possesses methionine synthase and other enzymes involved in methionine degradation (14).

In this study, ethical reasons precluded the study of healthy subjects. Although GFR as well as renal and clinical chemistry parameters were in the normal range in the subjects herein reported, several of them were affected by hypertension, a condition that may influence oxidative processes and endothelial function. Therefore, some caution must be used to extrapolate findings obtained here to the normal, healthy condition.

In conclusion, the present study provides the first report of aminothiol exchange measured across the human kidney and compared with the splanchnic organs and peripheral tissues as well. Our data demonstrate that substantial fluxes of aminothiols take place from peripheral tissues to the kidney and that the kidney plays a unique role in aminothiol handling. The data reported in this study could be useful to understand the alterations in Hcy and aminothiol metabolism that are observed in many systemic and organ diseases.
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