Effects of nonsulfur and sulfur amino acids on the regulation of hepatic enzymes of cysteine metabolism

DEBORAH L. BELLA, CHRISTINE HAHN, AND MARTHA H. STIPANUK
Division of Nutritional Sciences, Cornell University, Ithaca, New York 14853

Bella, Deborah L., Christine Hahn, and Martha H. Stipanuk. Effects of nonsulfur and sulfur amino acids on the regulation of hepatic enzymes of cysteine metabolism. Am. J. Physiol. 277 (Endocrinol. Metab. 40): E144–E153, 1999.—To determine the role of nonsulfur vs. sulfur amino acids in regulation of cysteine metabolism, rats were fed a basal diet or diets supplemented with a mixture of nonsulfur amino acids (AA), sulfur amino acids (SAA), or both for 3 wk. Hepatic cysteine-sulfinate decarboxylase (CSDC), cysteine dioxygenase (CDO), and γ-glutamylcysteine synthetase (GCS) activity, concentration, and mRNA abundance were measured. Supplementation with AA alone had no effect on any of these measures. Supplementation of the basal diet with SAA, with or without AA, resulted in a higher CDO concentration (32–45 times basal), a lower CSDC mRNA level (49–64% of basal), and a lower GCS-heavy subunit mRNA level (70–76%). The presence of excess SAA and AA together resulted in an additional type of regulation: a lower specific activity of all three enzymes was observed in rats fed diets with an excess of AA and SAA. Both SAA and AA played a role in regulation of these three enzymes of cysteine metabolism, but SAA had the dominant effects, and effects of AA were not observed in the absence of SAA.
cysteine-sulfinate decarboxylase; cysteine dioxygenase; γ-glutamylcysteine synthetase; sulfate; taurine

Cysteine is utilized in the synthesis of protein and for the synthesis of several nonprotein compounds, including taurine, reduced inorganic sulfur, sulfate, and glutathione (GSH), which are essential for a wide variety of critical functions in the body. The activities of key regulatory enzymes of cysteine metabolism [cysteine-sulfinate decarboxylase (CSDC), EC 4.1.1.129; cysteine dioxygenase (CDO), EC 1.13.11.20; and γ-glutamylcysteine synthetase (GCS), EC 6.3.2.2] have been observed to change in liver of rats fed different levels of dietary protein (2–7). Although cysteine is metabolized to some extent by many tissues, the liver clearly plays the dominant role in cysteine metabolism response to sulfate, taurine, and GSH in intact rats (11, 12), and only the hepatic enzymes of cysteine metabolism are known to respond to changes in the levels of dietary protein (6). Thus the liver plays a major role in sulfur amino acid metabolism, and changes in CSDC, CDO, and GCS activities in response to diet can affect the utilization of cysteine for synthesis of essential metabolites.

It is generally assumed that the effects of dietary protein on CSDC, CDO, and GCS are due to the sulfur amino acid content of the protein. In support of this hypothesis, both CDO and GCS are strongly regulated in response to changes in dietary intake of either protein or sulfur amino acids alone (2–7). However, in studies done previously in our laboratory, a lower CSDC activity was observed in isolated hepatocytes in response to increased dietary protein levels (2, 4–7) but not to increased methionine levels (3, 4, 6, 7). When rats were fed diets with very high levels of sulfur amino acids (>2% of the diet), a lower CSDC activity was observed, but methionine was less effective in lowering CSDC activity than an equisulfur amount of protein (6, 7). Similarly, addition of methionine to primary cultures of rat hepatocytes resulted in upregulation of CDO and downregulation of GCS but had no effect on CSDC activity (24). The results from these studies collectively suggest that the hepatic signal for regulation of CSDC is related to the protein content of the diet and not solely to changes in the level of sulfur amino acids.

Jenkins et al. (16) saw a lower CSDC activity in response to an increased dietary protein level that corresponded to changes in CSDC concentration and CSDC mRNA level. In contrast to results from our laboratory, Jenkins and Steele (17, 18) reported a marked decrease in CSDC activity and CSDC protein levels in response to supplementation of a basal diet (10% casein) with methionine to yield a total dietary sulfur amino acid content of 11–16 g/kg of diet vs. 6 g/kg in the basal diet. Despite their observation of a greater response to sulfur amino acids alone compared with observations in our laboratory, Jenkins and co-workers (15, 17, 18) did observe a greater decrease in CSDC activity in response to protein than to an equisulfur amount of methionine, which is in agreement with our findings.

The purpose of this study was to determine the role of excess nonsulfur amino acids, excess sulfur amino acids, or a combination of the two in the regulation of CSDC activity. Although changes in CSDC activity were the major focus of this study, changes in CDO and GCS were also evaluated to more clearly define the dietary component(s) responsible for regulation of these enzymes. By using diets made with purified amino acids, we were able to more clearly elucidate the role of sulfur vs. nonsulfur amino acids in the regulation of CSDC, CDO, and GCS and of cysteine metabolism in vivo.

MATERIALS AND METHODS

Animals and Dietary Treatments

Semipurified diets were prepared as shown in Tables 1 and 2 to contain various levels of sulfur amino acids, nonsulfur

The costs of publication of this article were defrayed in part by the payment of page charges. The article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.
Table 1. Composition of semipurified diets supplemented with excess sulfur amino acids, nonsulfur amino acids, or both

<table>
<thead>
<tr>
<th>Ingredients</th>
<th>Diet, g/kg diet</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>B</td>
</tr>
<tr>
<td>Vitamin-free casein</td>
<td>100</td>
</tr>
<tr>
<td>Amino acid mixture*</td>
<td>300</td>
</tr>
<tr>
<td>L-Methionine</td>
<td>9.6</td>
</tr>
<tr>
<td>L-Cystine</td>
<td></td>
</tr>
<tr>
<td>Cornstarch</td>
<td>282.5</td>
</tr>
<tr>
<td>Dextrose</td>
<td>94</td>
</tr>
<tr>
<td>Sucrose</td>
<td>376.5</td>
</tr>
<tr>
<td>Cellulose</td>
<td>50</td>
</tr>
<tr>
<td>Corn oil</td>
<td>50</td>
</tr>
<tr>
<td>Vitamin mix (AIN 76A)</td>
<td>10</td>
</tr>
<tr>
<td>Mineral mix (AIN 76)</td>
<td>35</td>
</tr>
<tr>
<td>Choline chloride</td>
<td>2</td>
</tr>
</tbody>
</table>

Diets were prepared by Dyets (Bethlehem, PA) in pelleted form. B, basal; A, AA, basal + amino acids; B + M, basal + methionine; B + AA + C, basal + amino acids + cystine. *See Table 2 for composition of sulfur amino acid-free amino acid mixture.

amino acids, or a combination of the two. Diets were based on the AIN-76A formulation (1) and the amino acid mixtures of Rogers and Harper (25). Modifications were made to prepare diets that contained 100 g casein/kg diet and either no amino acid supplement (basal; B), 300 g of a sulfur amino acid-free amino acid mixture per kilogram diet (basal + amino acids; B + AA), 9.6 g of L-methionine/kg diet (basal + methionine; B + M), 300 g of the sulfur amino acid-free amino acid mixture plus 9.6 g L-methionine/kg diet (basal + amino acids + methionine; B + AA + M), or 300 g of the sulfur amino acid-free amino acid mixture plus 7.8 g L-cystine/kg diet (basal + amino acids + cystine; B + AA + C). Additions to the basal diet were made at the expense of sucrose. The molar amounts of sulfur amino acids (methionine plus half-cystine) were equivalent for the B + M, B + AA + M, and B + AA + C diets.

Male Sprague-Dawley rats that weighed ~140 g were purchased from Harlan Sprague Dawley (Indianapolis, IN). Rats were housed individually in stainless steel mesh cages in a room maintained at 20°C and 60–70% humidity with light from 2000 to 0800. Animals were fed a nonpurified diet (RHM 1000, Agway, Syracuse, NY) for 3 days before being assigned to a specific experimental diet. A total of 32 animals were grouped into seven blocks by body weight, and rats within each block were randomly assigned to receive one of the five diets. Thus six or seven animals were assigned to each of the five experimental diets, and the weight distribution of rats was similar for all treatment groups. Rats were fed the experimental diets for 21–23 days and had free access to diet and water for the duration of the experiment.

Beginning on day 18 of the dietary treatment, one-half of the rats from each group were placed in individual metabolic cages for collection of 24-h urine samples. Thymol was used as a preservative for the urine. At the end of the collection period, urine volume was measured and brought to 25 ml. Urine samples were then frozen and stored at −20°C. The same procedure was performed for the remaining rats on day 20 of dietary treatment.

Beginning on day 21 of dietary treatment, 10 or 12 rats per day were killed (CO2 anesthesia + decapitation) on each of 3 days. Rats were killed by assigned block, beginning with the block with the greatest initial body weights. Within assigned blocks, rats were killed in random order. Two to three rats per dietary group were killed on each of the 3 days. The care and use of animals were approved by the Cornell University Institutional Animal Care and Use Committee.

Liver was removed, rinsed with ice-cold saline, blotted, and weighed. Approximately 100–150 mg of liver from each animal were homogenized in denaturation solution (TOTALLY RNA kit, Ambion, Austin, TX) and then stored at −70°C for later measurement of CDO, GCS-heavy subunit (GCS-HS), and CSDC mRNA as we will describe. The liver was then minced and homogenized in appropriate ice-cold buffers; homogenate was immediately used for enzyme assays or to obtain the soluble fraction, which was stored at −70°C for subsequent determination of the concentrations of CSDC, CDO, and GCS-HS (Western blot analysis). Aliquots of homogenate or 20,000 g supernatant were frozen for later analysis of hepatic protein, taurine, and GSH.

Enzyme Assays

CSDC and CDO activities (4, 5) and GCS activity (4) were measured in liver homogenate with methods developed in our laboratory, as described previously.

Analysis of Protein, Taurine, GSH, Sulfate, and Creatinine

Urine taurine, sulfate, and creatinine levels were determined as previously described by Bella and Stipanuk (6). Hepatic protein, taurine, and total GSH levels were determined as described by Bella et al. (7).

Western, Northern, and Dot-Blot Methods

Sources of antibodies. Rabbit anti-CSDC serum was a gift from Dr. Owen Griffith (Medical College of Wisconsin, Milwaukee, WI). The purified IgG fraction from rabbit anti-CDO serum was a gift from Dr. Yu Hosokawa (National Institute of Health and Nutrition, Tokyo, Japan). Rabbit anti-GCS-HS serum was a gift from Dr. Henry Jay Forman (University of Southern California, Los Angeles, CA). The preparation of these antibodies against rat liver CSDC (31), rat liver CDO (32), and a peptide sequence of GCS-HS (27) has been reported.
Sources of cDNA. An EcoRI-cut cDNA for CDO (14) was a gift of Dr. Yu Hosokawa and Nobuyo Tsuboyama (National Institute of Health and Nutrition, Tokyo, Japan). Probes for CSDC and GCS-HS were prepared as described by Bella et al. (4). DECAProbe template-actin-mouse and DECAProbe template-18S-mouse (Ambion) were used as internal standards for Northern and dot-blot analyses, and were labeled with [32P]dCTP by use of the PrimeIt RmT random primer labeling kit (Stratagene, La Jolla, CA).

Western blot analysis. Western blot analyses (20, 30) were performed as previously described by Bella et al. (4). Briefly, total liver supernatant protein was separated by one-dimensional SDS-PAGE, and the proteins were electrophoretically transferred onto Immobilon-P membranes (Millipore, Medford, MA). Immunoreactive protein was detected by chemiluminescence with exposure to Kodak X-OMAT XRP film. The film image was scanned using a desktop scanner (Hewlett-Packard Scanjet 3c, Hewlett-Packard, Cambridge, CA). Two-dimensional quantitative densitometric analysis of the regions of interest was performed using Molecular Analyst software (Bio-Rad Laboratories, Hercules, CA). Single bands corresponding to 54 kDa for CSDC, 23.5 kDa for CDO, and 74 kDa for GCS-HS were detected as noted except in results for CDO; apparent molecular masses were consistent with previously published values (17, 26, 32). Graded amounts of rat liver supernatant were run on all gels and used to generate standard curves for conversion of relative absorbance units to relative protein concentrations.

Isolation of total RNA, Northern blot, and dot-blot hybridization analyses. Northern and dot-blot analyses were performed as previously described by Bella et al. (4). Briefly, total RNA was isolated from liver by use of the ToTALLY RNA kit (Ambion), based on the method of Chomczynski and Sacchi (10), and Northern blot analysis was conducted using 32P-labeled probes as described by Brown (8). Results were quantified using the Bio-Rad GS 363 Phosphorescence Imaging System (Bio-Rad Laboratories, Hercules, CA). Graded amounts of liver total RNA were loaded on each gel or membrane to generate standard curves for conversion of relative absorbance units to relative mRNA concentrations.

Statistics

Results were analyzed by ANOVA with factorial analysis for the main effects of nonsulfur amino acids and methionine (Minitab 81.1, State College, PA). Tukey’s or Tukey-Kramer’s test (28) was utilized to compare individual means.

RESULTS

Food Intake, Growth, and Hepatic Protein, Taurine, and GSH Concentrations

As shown in Table 3, daily weight gain and final body weights of rats fed both the B and B + AA diets were significantly lower (P < 0.05) than those of rats fed the sulfur amino acid-supplemented diets (B + M, B + AA + M, B + AA + C). These lower rates of weight gain were anticipated because the total sulfur amino acid content of the B and B + AA diets was 0.3% and the total protein content of the B diet was 10%, which are below the National Research Council’s recommendation of 0.6% for sulfur amino acids and 12% for protein (23). Despite the low levels of sulfur amino acids and protein, the B diet did support a reasonable rate of weight gain, and intake was similar to that of rats fed the sulfur amino acid-supplemented diets. The daily intake of rats fed the B + AA diet was significantly lower (P < 0.05) than the intake of rats fed the other four diets. The B + AA diet contained 100 g of casein plus 300 g of a sulfur amino acid-free amino acid mixture per kilogram diet; the imbalanced amino acid mixture may have contributed to the lower intake of the diet.

The relative liver weight (g liver/100 g body weight) was similar for rats fed the B, B + AA, B + AA + M, and B + AA + C diets but was slightly elevated in rats fed the B + M diet. The hepatic protein concentration (mg protein/g liver) was similar for rats fed all five experimental diets but was slightly elevated in rats fed the B + M diet. The hepatic protein concentration (mg protein/g liver) was similar for rats fed all five experimental diets, and enzyme concentrations and activities, as well as metabolite concentrations, have been reported per unit of liver protein.

Hepatic taurine and GSH concentrations were lower in rats fed sulfur amino acid-deficient diets (B and B + AA) than in those fed sulfur amino acid-supplemented diets (Table 3). The hepatic taurine concentration was lower and the hepatic GSH concentration was higher in rats fed the diet supplemented with methionine alone (B + M) than in rats fed the diets supplemented with both sulfur and nonsulfur amino acids (B + AA + M and B + AA + C). Analysis of hepatic taurine concentration for treatment effects indicated a significant effect of sulfur amino acid level (P < 0.001) but no effect of supplemental nonsulfur amino acids (Table 4). Analysis of hepatic GSH concentration for treatment effects indicated significant effects of both sulfur amino acids and methionine content, with the B + AA diet containing the lowest GSH concentration.

Table 3. Effects of diet on food intake, weight gain, and hepatic protein, taurine, and glutathione concentrations

<table>
<thead>
<tr>
<th></th>
<th>B</th>
<th>B + AA</th>
<th>B + M</th>
<th>B + AA + M</th>
<th>B + AA + C</th>
</tr>
</thead>
<tbody>
<tr>
<td>Daily diet consumption, g</td>
<td>16.9 ± 0.3b</td>
<td>16.5 ± 0.3a</td>
<td>20.1 ± 0.6b</td>
<td>19.5 ± 0.2b</td>
<td>19.4 ± 0.4b</td>
</tr>
<tr>
<td>Liver weight, g</td>
<td>3.7 ± 0.3a</td>
<td>2.8 ± 0.1a</td>
<td>5.3 ± 0.2b</td>
<td>5.9 ± 0.2b</td>
<td>5.8 ± 0.3b</td>
</tr>
<tr>
<td>Body weight at end of feeding period, g</td>
<td>234 ± 8.2a</td>
<td>211 ± 4.9a</td>
<td>275 ± 8.7b</td>
<td>293 ± 3.0b</td>
<td>279 ± 12b</td>
</tr>
<tr>
<td>Liver weight at end of feeding period, g</td>
<td>8.0 ± 0.7ab</td>
<td>6.6 ± 0.3a</td>
<td>11.9 ± 0.7a</td>
<td>9.8 ± 0.3ac</td>
<td>9.7 ± 0.8ac</td>
</tr>
<tr>
<td>Relative liver weight, g/100 g body wt</td>
<td>3.4 ± 0.3a</td>
<td>3.1 ± 0.1a</td>
<td>4.3 ± 0.1b</td>
<td>3.4 ± 0.1ab</td>
<td>3.5 ± 0.1a</td>
</tr>
<tr>
<td>Hepatic taurine concentration, nmol/mg protein</td>
<td>4.4 ± 0.5a</td>
<td>4.9 ± 0.2a</td>
<td>66.8 ± 5.5a</td>
<td>94.1 ± 6.4ac</td>
<td>102.7 ± 8.9b</td>
</tr>
<tr>
<td>Hepatic GSH concentration, nmol/mg protein</td>
<td>8.1 ± 0.6a</td>
<td>9.2 ± 0.6a</td>
<td>26.6 ± 2.0a</td>
<td>16.7 ± 1.2b</td>
<td>14.2 ± 1.0b</td>
</tr>
<tr>
<td>Liver protein, mg/g liver</td>
<td>208.0 ± 8.4</td>
<td>214.6 ± 5.19</td>
<td>221.8 ± 7.1</td>
<td>230.8 ± 4.82</td>
<td>221.9 ± 12.4</td>
</tr>
</tbody>
</table>

Values are means ± SE for 6–7 rats. Within a row, values with different superscripts are significantly different (P ≤ 0.05) by ANOVA and Tukey-Kramer’s test (28).
Table 4. Main effects of sulfur vs. nonsulfur amino acid supplementation on hepatic taurine and glutathione concentrations

<table>
<thead>
<tr>
<th></th>
<th>Hepatic Taurine Concentration</th>
<th>Hepatic Glutathione Concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nonsulfur amino acids</td>
<td>0.82</td>
<td>0.001</td>
</tr>
<tr>
<td>Methionine</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Interaction of amino acids and methionine</td>
<td>0.86</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

Significance values (P) are results of factorial analysis. Data for hepatic taurine concentration were transformed to log10 for statistical analysis.

Table 4 observations of methionine being less effective than protein at equisulfur levels (2–7).

CDO activity was significantly higher (P < 0.001) and GCS activity was significantly lower (P < 0.001) with addition of sulfur amino acids to the basal diet. The levels of CDO activity in liver of rats fed the B+M, B+AA+M, and B+AA+C diets were 178-, 138-, and 115-fold, respectively, the activity observed in rats fed the B diet. GCS activities in the liver of rats fed the B+M, B+AA+M, and B+AA+C diets were 52, 27, and 32%, respectively, of the activity in rats fed the B diet.

CSDC, CDO, and GCS-HS Protein Concentrations

As shown in Fig. 2, no difference in the relative protein level for any of the three hepatic enzymes was observed between rats fed the B and B+AA diets. Relative CSDC protein levels in rats fed the B+M, B+AA+M, and B+AA+C diets were 60, 50, and 47%, respectively, of the level observed in rats fed the B diet. Relative hepatic GCS-HS protein levels in the B+M, B+AA+M, and B+AA+C groups were 61, 62, and 54%, respectively, of the level observed in rats fed the B diet.

The relative CDO levels in the liver of rats fed the B+M, B+AA+M, and B+AA+C diets were 34, 45, and 32 times, respectively, the concentration of CDO observed in rats fed the B diet. In addition, the CDO band detected in samples from rats fed the B+M, B+AA+M, and B+AA+C diets resolved into two distinct bands, with the additional or lower band having an estimated molecular mass of 23.5 kDa compared with the upper or usual band with an estimated molecular mass of 25.5 kDa. The appearance of two bands in liver of rats fed excess protein or methionine has been observed previously (4). The quantitative results for relative CDO...
protein reported in Fig. 2 for rats fed the three sulfur amino acid-supplemented diets include both bands.

**CSDC, CDO, and GCS-HS mRNA Levels**

As shown in Fig. 3, no difference in the mRNA level for any of the three hepatic enzymes was observed between rats fed the B and B+AA diets. CSDC mRNA levels in rats fed the B+M, B+AA+M, and B+AA+C diets were 64, 49, and 63%, respectively, of the level observed in rats fed the B diet. As with CSDC activity, differences in CSDC mRNA levels were a result of sulfur amino acid (P = 0.001) but not of nonsulfur amino acid (P = 0.53) supplementation (Table 5). However, no significant interaction of sulfur amino acids and nonsulfur amino acids was observed for CSDC mRNA levels as was observed for CSDC activity.

No significant effect of dietary treatment on hepatic CDO mRNA levels was observed. CDO mRNA levels tended to be slightly higher in liver of rats fed the B+AA+M and B+AA+C diets than in rats fed the other three diets, but the magnitude of the apparent differences in CDO mRNA concentration was small compared with those observed in CDO activity and concentration.

The levels of GCS-HS mRNA in rats fed the B+M, B+AA+M, and B+AA+C diets were 70, 76, and 71%, respectively, of the level observed in rats fed the B diet. The GCS-HS mRNA levels were significantly affected by dietary methionine level (P = 0.003) but not by nonsulfur amino acid level, and they generally responded to dietary changes with a pattern similar to that observed for GCS-HS concentration.

**Urinary Taurine and Sulfate Excretion**

Consistent with the increased intake of sulfur amino acids, the absolute taurine excretion by rats fed the B+M, B+AA+M, and B+AA+C diets was 75, 13, and 32 times, respectively, the level excreted by rats fed the

---

**Table 5. Main effects of sulfur vs. nonsulfur amino acid supplementation on CSDC, CDO, and GCS enzyme activities and mRNA concentrations**

<table>
<thead>
<tr>
<th></th>
<th>CSDC</th>
<th>CDO</th>
<th>GCS</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Enzyme Activity</td>
<td>mRNA Conc</td>
<td>Enzyme Activity</td>
</tr>
<tr>
<td>Nonsulfur amino acids</td>
<td>0.068</td>
<td>0.53</td>
<td>0.063</td>
</tr>
<tr>
<td>Methionine</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Interaction of amino acids and methionine</td>
<td>0.003</td>
<td>0.34</td>
<td>0.78</td>
</tr>
</tbody>
</table>

Significance (P) values are results of factorial analysis. CSDC, cysteine-sulfinate decarboxylase; CDO, cysteine dioxygenase; GCS, γ-glutamylcysteine synthetase. *Data for CDO activity were transformed to log10 for statistical analysis.

---

![Fig. 2](image-url). Western blots of hepatic CSDC, CDO, and GCS-heavy subunit (GCS-HS) in rats fed diets supplemented with excess sulfur amino acids, nonsulfur amino acids, or both. Values are means for 3–5 separate determinations by quantitative Western analysis of pooled samples from each diet group; equal amounts of total soluble liver protein from each animal in a given group (n = 5–7 rats) were used to form the pooled samples. Between-gel variance for the Western blots (coefficient of variation) was 19% for CSDC, 3% for CDO, and 17% for GCS-HS. Sample Western blots are shown below bar graph; 5.25 µg of liver supernatant protein were loaded per lane for CSDC analysis, 210 µg per lane for CDO analysis, and 25 µg per lane for GCS-HS analysis. For quantitative analysis of CDO (data shown in bar graph), amounts of soluble protein loaded were 210 µg for the B and B+AA groups and 7.7 µg for the B+M, B+AA+M, and B+AA+C groups.
B diet (Table 6). Urinary taurine as a percentage of total sulfur excretion was also greater in rats fed the sulfur amino acid-supplemented diets than in rats fed the B or B+AA diets; this is probably the result of increased cysteine-sulfinate-dependent vs. cysteine-sulfinate-independent catabolism of cysteine in rats fed sulfur amino acid-supplemented diets (2). In addition to the effect of sulfur amino acids (P < 0.001), the addition of nonsulfur amino acids consistently and significantly (P < 0.001) lowered both the absolute amount of taurine excreted and the proportion of total sulfur excretion as taurine vs. sulfate (B vs. B+AA; B+M vs. B+AA+M) (Table 7). Cystine appeared to be slightly more effective than methionine in increasing taurine excretion by rats fed the amino acid-supplemented diets (B+AA+C vs. B+AA+M). In general, the patterns of urinary taurine and sulfate excretion in this study are similar to the patterns in previous studies in which rats were fed diets supplemented with protein or methionine (6).

**DISCUSSION**

Regulation of Cysteine Metabolic Enzymes in Response to Sulfur vs. Nonsulfur Amino Acids

By using purified amino acids instead of protein, we further elucidated the effects of dietary nonsulfur amino acids vs. methionine and their interaction in the regulation of CSDC, CDO, and GCS in vivo. We were also able to compare the effects of methionine vs. cystine in the presence of dietary nonsulfur amino acids on these same parameters. The degrees of change in enzyme activity, enzyme concentration, mRNA concentration, and specific activity (enzyme activity divided by the relative enzyme concentration) observed for CSDC,

**Table 6. Metabolites in 24-h urine collection from rats fed diets supplemented with excess sulfur amino acids, nonsulfur amino acids, or both**

<table>
<thead>
<tr>
<th>Metabolite</th>
<th>B</th>
<th>B + AA</th>
<th>B + M</th>
<th>B + AA + M</th>
<th>B + AA + C</th>
</tr>
</thead>
<tbody>
<tr>
<td>Creatinine, mg/day*</td>
<td>7.4 ± 0.8a</td>
<td>10.2 ± 0.3b</td>
<td>9.3 ± 0.5ab</td>
<td>13.4 ± 0.3c</td>
<td>13.4 ± 0.5c</td>
</tr>
<tr>
<td>Taurine, µmol/mg creatinine</td>
<td>0.4 ± 0.2a</td>
<td>0.05 ± 0.005ab</td>
<td>29.9 ± 0.9c</td>
<td>5.3 ± 0.8b</td>
<td>12.7 ± 1.1bc</td>
</tr>
<tr>
<td>Sulfate, µmol/mg creatinine</td>
<td>7.3 ± 1.9a</td>
<td>12.6 ± 1.2b</td>
<td>34.4 ± 3.1b</td>
<td>46.8 ± 5.0p</td>
<td>39.2 ± 1.1b</td>
</tr>
<tr>
<td>Taurine/(Taurine + Sulfate), %</td>
<td>4.6 ± 1.8a</td>
<td>0.46 ± 0.06a</td>
<td>46.8 ± 1.3d</td>
<td>10.9 ± 2.1c</td>
<td>24.4 ± 1.5ad</td>
</tr>
</tbody>
</table>

Values are means ± SE for 6–7 rats. Within a row, values with different superscripts are significantly different (P < 0.05) by ANOVA and Tukey’s ω-procedure. Data for urinary taurine and taurine as a percentage of urinary taurine plus sulfate excretion were transformed to \( \log_{10} \) for statistical analysis. *Urine was collected for 24 h but may not reflect total urine formation/24 h. Creatinine was used as a base for expression of taurine and sulfate excretion to correct for variation in completeness of urine collection as well as differences in lean body mass of rats.
Table 7. Main effects of sulfur vs. nonsulfur amino acid supplementation on urinary taurine and sulfate concentrations and urinary taurine as a percentage of total sulfur excretion

<table>
<thead>
<tr>
<th></th>
<th>Urinary Taurine Concentration</th>
<th>Urinary Sulfate Concentration</th>
<th>Urinary Taurine as a Percentage of Total Sulfur Excretion</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nonsulfur amino acids</td>
<td>&lt;0.001</td>
<td>0.012</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Methionine</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Interaction of amino acids and methionine</td>
<td>0.27</td>
<td>0.28</td>
<td>0.51</td>
</tr>
</tbody>
</table>

Significance (P) values are results of factorial analysis. Data for urinary taurine concentration and urinary taurine as a percentage of total sulfur excretion were transformed to log10 for statistical analysis.

CDO, and GCS-HS in this study are summarized in Table 8.

Regulation of CSDC. Consistent with previous reports (4, 16), we found that downregulation of CSDC in response to changes in dietary amino acid content occurred at the level of CSDC mRNA concentration. CSDC (protein) concentration and CSDC mRNA level were highly correlated with each other (r² = 0.94) and with enzyme activity (r² = 0.92 and r² = 0.92, respectively). By supplementing the basal diet with either a mixture of nonsulfur amino acids, a sulfur amino acid, or both nonsulfur and sulfur amino acids, we also were able to separate the effects of the sulfur and nonsulfur amino acid components of dietary protein. The CSDC mRNA levels were significantly affected by the dietary sulfur amino acid level (P < 0.001) and not at all affected by the level of nonsulfur amino acids alone (Table 5). It is not known whether the decrease in the CSDC mRNA concentration is a result of a decrease in the rate of transcription of the CSDC gene or of a decrease in CSDC mRNA transcript stability (an increased rate of degradation).

In contrast to changes in the CSDC mRNA level in response to sulfur amino acid intake, the response of CSDC activity to excess sulfur amino acid showed a strong interaction with the level of nonsulfur amino acids in the diet (P = 0.003), suggesting the possibility that additional regulation of CSDC occurs at the post-translational level. In agreement with previous studies in which a greater decrease in CSDC activity was observed in response to protein than to sulfur amino acids alone (2–4, 6, 7), we observed a greater decrease in CSDC activity in response to an excess of a complete mixture of amino acids (sulfur amino acid plus nonsulfur amino acids that simulated dietary protein) than to an excess of sulfur amino acid alone. A relative calculation of CSDC specific activity (CSDC activity divided by the relative CSDC protein concentration) revealed a slightly higher specific activity in liver of rats fed the B+M diet compared with rats fed the other two diets (B+AA+M and B+AA+C) in which the excess sulfur amino acids were “balanced” by excess nonsulfur amino acids. Changes in CSDC specific activity in response to methionine alone were not observed in a previous study, but changes may not have been apparent because of a relatively weak response of CSDC to either methionine or protein in that study (4). Although not large, this apparent posttranslational regulation of CSDC seems to account for the stronger effect of protein than of sulfur amino acids alone in bringing about downregulation of CSDC activity. The possibility of posttranslational regulation of CSDC activity deserves further study.

Regulation of CDO. As previously reported (4), markedly higher steady-state CDO activity and CDO protein concentration were seen in liver of rats fed sulfur amino acid-supplemented diets with or without additional nonsulfur amino acids, whereas no differences were observed in CDO mRNA levels in liver of rats fed these diets. These changes mainly occurred in response to sulfur amino acid supplementation, because supplementation with nonsulfur amino acids alone had no effect on either CDO activity or CDO protein level.

Although not always statistically significant, we have consistently found in several different studies (2–7) higher CDO activity in response to methionine supplementation vs. protein supplementation. We recently reported a lower CDO specific activity in liver of rats fed a protein-supplemented diet compared with rats fed a methionine-supplemented diet with an equivalent level of sulfur amino acid (4). In this study, a borderline significant (P = 0.063) overall effect of nonsulfur amino acids on CDO activity was observed: hepatic CDO activity, but not CDO protein concentration, was lower in rats fed the B+AA+M or B+AA+C diet than in rats fed the B+M diet. As a result, CDO specific activity (relative CDO activity/relative CDO protein concentra-
Acids in the Regulation of CSDC, CDO, and GCS

Neither CSDC, CDO, nor GCS activity changed in response to supplemental nonsulfur amino acids alone, which clearly underscores the important role of dietary sulfur amino acids in the regulation of these three enzymes. Changes in the abundance of all three enzymes occurred in response to sulfur amino acid supplementation. The changes in CDO protein and in CSDC and GCS-HS mRNA and protein levels all occurred in response to sulfur amino acid level with no apparent interaction with nonsulfur amino acid level.

Nevertheless, nonsulfur amino acids did seem to play a role in modulating the response of each of the three enzymes to sulfur amino acid supplementation. Although the main regulatory effects of sulfur amino acids did not require and were not affected by the presence of excess nonsulfur amino acids, the specific activity of all three hepatic enzymes was lower in rats fed the diet containing both excess methionine and nonsulfur amino acids than in those fed the diet containing only excess methionine.

Role of Methionine vs. Cyst(e)ine in Regulation of CSDC, CDO, and GCS-HS

Overall, the effect of methionine vs. cystine supplementation was similar for all three enzymes, regardless of their mechanism of regulation. Although methionine and cystine were compared in this study only in the presence of excess nonsulfur amino acids, similar effects of methionine and cystine on hepatic CDO and CSDC activities and on the capacity for GSH synthesis were observed in a previous study in which two levels of cystine and methionine were compared in the absence of excess nonsulfur amino acids (3). The similar response of all three enzymes to either methionine or cystine suggests that cysteine or a subsequent metabolite of cysteine probably plays a key role as a cellular signal for the adaptive regulation of hepatic CSDC, CDO, and GCS-HS.

Effect of Dietary Methionine or Cystine

The effect of changes in dietary methionine or cystine on the levels of hepatic CSDC and GCS-HS mRNAs is also particularly interesting. Expression of the asparagine synthetase gene (13) can be modulated by changes in amino acid concentrations. Marten et al. (21) demonstrated changes in expression of several genes in rat hepatoma cells in response to limitation of either a

Role of Methionine vs. Cyst(e)ine in Regulation of CSDC, CDO, and GCS-HS

Overall, the effect of methionine vs. cystine supplementation was similar for all three enzymes, regardless of their mechanism of regulation. Although methionine and cystine were compared in this study only in the presence of excess nonsulfur amino acids, similar effects of methionine and cystine on hepatic CDO and CSDC activities and on the capacity for GSH synthesis were observed in a previous study in which two levels of cystine and methionine were compared in the absence of excess nonsulfur amino acids (3). The similar response of all three enzymes to either methionine or cystine suggests that cysteine or a subsequent metabolite of cysteine probably plays a key role as a cellular signal for the adaptive regulation of hepatic CSDC, CDO, and GCS-HS.

Effect of Dietary Methionine or Cystine

The effect of changes in dietary methionine or cystine on the levels of hepatic CSDC and GCS-HS mRNAs is also particularly interesting. Expression of the asparagine synthetase gene (13) can be modulated by changes in amino acid concentrations. Marten et al. (21) demonstrated changes in expression of several genes in rat hepatoma cells in response to limitation of either a
single amino acid or of several amino acids for 24 h. Because dietary amino acids go directly to the liver, the liver is the major catabolic organ for most amino acids and plays a major role in the fate of amino acids in the body. It is possible that amino acid concentrations or secondary signals affected by changes in amino acid availability, such as the concentration of amino acid metabolites, could serve as cellular effectors for regulation of hepatic gene expression. This possibility seems worthy of further study, because expression of several genes in Escherichia coli is regulated by the cellular concentration of leucine via a leucine-responsive regulatory protein (9). Furthermore, the DNA-binding activities of several liver-specific transcription factors have been shown to be altered in liver of rats fed protein-restricted diets (22).

Dietary Sulfur and Nonsulfur Amino Acids Influence Cysteine Metabolism in Vivo

The factors that influence hepatic taurine and GSH levels have been reviewed extensively elsewhere (5). As seen in other studies, differences in hepatic GSH concentrations in this study reflect the level of cysteine availability, with some apparent modulation by GCS activity. The increases in cysteine availability and in hepatic CDO activity in rats fed the three diets supplemented with sulfur amino acids presumably were associated with greater conversion of cysteine to cysteine-sulfinate and, hence, to taurine. Increased taurine synthesis was reflected by increases in both hepatic taurine level and urinary taurine excretion.

Among the three sulfur amino acid-supplemented groups, higher activities of both CDO and CS defense were observed in rats fed the diet supplemented with methionine alone (B+M) than in rats fed the diet supplemented with both methionine and nonsulfur amino acids (B+AA+M). These higher enzyme activities may have resulted in a greater partitioning of cysteine to taurine vs. sulfate (taurine:taurine+sulfate ratio of 47 in the B+M group vs. 11 in the B+AA+M group) and in the much greater rate of taurine excretion (29.9 µmol/mg creatinine in the B+M group vs. 5.3 µmol/mg creatinine in the B+AA+M group) in rats fed the B+M diet than in those fed the B+AA+M diet. These findings are in agreement with those reported previously (6).

Hepatic taurine concentration is less likely to reflect the rate of taurine synthesis in vivo than is urinary taurine excretion. The hepatic taurine concentration in rats fed the B+M diet tended to be lower than that in rats fed the B+AA+M or B+AA+C diet, which is in contrast to observations for urinary taurine. Besides substrate availability and CDO and CS defense activities, the level of hepatic taurine efflux, the plasma taurine concentration, and the regulation of taurine reabsorption in the epithelium of the renal proximal tubules can also affect hepatic and urinary taurine levels and may explain observed differences. Little is known about regulation of these processes except that renal taurine concentration seems to be the signal for changes in renal taurine transporter activity (19, 29).

An independent effect of excess nonsulfur amino acids on urinary taurine excretion is indicated by the significantly lower taurine excretion in rats fed the B+AA diet than in rats fed the B diet. This difference occurred despite similar hepatic taurine concentrations, cysteine availability, and hepatic CDO and CS defense activities in rats fed these two diets. This, along with the lower rate of taurine excretion of rats fed the B+AA+M vs. the B+M diet, suggests the possibility that the partitioning of cysteine-sulfinate between sulfate and taurine may be affected by the presence of excess nonsulfur amino acids. There may be an increase in flux of cysteine-sulfinate to pyruvate and sulfate via transamination to β-sulfinylpyruvate owing to a high rate of amino acid catabolism/gluconeogenesis or an elevated keto acid cosubstrate (α-ketoglutarate) concentration for transamination. In addition, the rate of renal reabsorption of taurine may be different in rats fed diets with excess levels of nonsulfur amino acids than in rats fed diets supplemented with both sulfur and nonsulfur amino acids.

The regulation of both CDO and CS defense enzyme activities by changes in diet clearly affects the rates of taurine and sulfate synthesis in vivo, but regulation of CDO activity seems to play the dominant role. In a previous study (4), CDO activity was increased ~10-fold when dietary protein was increased from 10 to 20% casein, whereas no change was observed for CS defense activity over this range of dietary protein. Even greater degrees of change in CDO activity (>100-fold increases) were observed with sulfur amino acid supplementation in this study, whereas only a 23–59% decrease in CS defense activity was observed. Little regulation of CS defense activity, which could alter the partitioning of hepatic cysteine-sulfinate between taurine vs. sulfate, is observed unless the levels of dietary protein (or complete amino acid mixture) are clearly in excess. Even at the lowest observed levels of CS defense activity in this study, increases in CDO and cysteine availability were accompanied by large increases in rates of taurine synthesis and excretion (i.e., the B+AA+M group). Therefore, changes in CDO activity most likely play a greater role in regulation of taurine synthesis in animals consuming levels of protein that are near the requirement, and even in animals consuming very high levels of protein, than do changes in CS defense activity.

We gratefully acknowledge the guidance and advice of Dr. Patrick Stover and the technical assistance of Larry Hirschberger. We also thank Dr. Jay Ferman for the anti-GCS-HS serum, Dr. Owen Griffith for the anti-CS defense serum, Dr. Yu Hosokawa for the anti-CDO IgG, and Dr. Yu Hosokawa and Nobuyo Tsujoysama for the EcoRI-cut cDNA for CDO.

This research was supported in part by National Research Initiative Competitive Grants Program/US Department of Agriculture (USDA) Grant 92–37200–7583, USDA/Cooperative State Research, Education, and Extension Service Grant 94–34324–0987, and by the President’s Council of Cornell Women. D. L. Bella was supported by a National Institute of Diabetes and Digestive and Kidney Diseases training grant (T32-DK-07158).

Address for reprint requests and other correspondence: M. H. Stipanuk, 225 Savage Hall, Cornell Univ., Ithaca, NY 14853–6301 (E-mail: mhs6@cornell.edu).

Received 10 December 1998; accepted in final form 25 March 1999.
REFERENCES


