Excretion of Sulfate and Taurine in Rats Fed with a High Protein Diet

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Abstract

Sulfate and taurine are the main metabolites of L-cysteine in mammals and are excreted in the urine. The effect of a high protein diet on the ratio of sulfate to taurine excretion was studied in rats using synthetic 25% (standard protein diet group, group A) and 40% (high protein diet group, group B) casein diets. Average taurine and sulfate excretions (mmol/kg of body weight per day) were 280.4 +/- 93.8 and 943.2 +/- 144.8 in group A and 553.4 +/- 124.5 and 2675.0 +/- 390.9 in group B, respectively. Thus, the average taurine/sulfate ratio in group A was 0.30 +/- 0.08. By a single administration of 5 mmol of L-cysteine/kg of body weight in group A, the average taurine and sulfate excretions increased to 1127.5 +/- 120.2 and 4043.0 +/- 305.6, respectively, but the taurine/sulfate ratio changed only slightly (0.28). The average taurine/sulfate ratio in group B was 0.22 +/- 0.07, a significantly lower ratio than that in group A, which means that daily intake of a high protein diet resulted in more sulfate excretion. The taurine/sulfate ratio in group B was affected only slightly (0.19) by the cysteine administration as well. These results suggest that the ratio of taurine and sulfate production was determined by dietary protein content and that the increase in sulfate production is larger than that of taurine production when the intake of dietary protein is increased.

KEYWORDS: high protein diet, sulfate, taurine, cysteine metabolism

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Sulfate and taurine are the main metabolites of L-cysteine in mammals and are excreted in the urine. The effect of a high protein diet on the ratio of sulfate to taurine excretion was studied in rats using synthetic 25% (standard protein diet group, group A) and 40% (high protein diet group, group B) casein diets. Average taurine and sulfate excretions (μmol/kg of body weight per day) were 280.4 ± 93.8 and 943.2 ± 144.8 in group A and 553.4 ± 124.5 and 2675.0 ± 390.9 in group B, respectively. Thus, the average taurine/sulfate ratio in group A was 0.30 ± 0.08. By a single administration of 5 mmol of L-cysteine/kg of body weight in group A, the average taurine and sulfate excretions increased to 1127.5 ± 120.2 and 4043.0 ± 305.6, respectively, but the taurine/sulfate ratio changed only slightly (0.28). The average taurine/sulfate ratio in group B was 0.22 ± 0.07, a significantly lower ratio than that in group A, which means that daily intake of a high protein diet resulted in more sulfate excretion. The taurine/sulfate ratio in group B was affected only slightly (0.19) by the cysteine administration as well. These results suggest that the ratio of taurine and sulfate production was determined by dietary protein content and that the increase in sulfate production is larger than that of taurine production when the intake of dietary protein is increased.

Key words: high protein diet, sulfate, taurine, cysteine metabolism

Sulfate and taurine are both important end products of cysteine metabolism in mammals (1, 2). Sulfate plays various important roles in the mammalian body. Anatomically, sulfate is a constituent of the animal body as a component of sulfated polysaccharides (3) and consequently a component of proteoglycans. Physiologically, sulfate is involved in the detoxification of xenobiotics and of endogenous metabolites (4). Taurine is also anatomically important as it is contained in high concentrations in the heart and skeletal muscles (5). It plays physiologically important roles as a component of taurine acids (5) and in membrane protection (6) and antioxidant action (6).

Cysteine is supplied to the mammalian body as a component of dietary proteins and as a metabolite of methionine which is also contained in dietary proteins. In mammalian tissues, cysteine sulfur is ultimately oxidized to sulfate and taurine, which are excreted in the urine as its major constituents (1). Medes reported that almost 100% of sulfur from ingested L-cysteine and L-methionine was excreted as sulfate in the urine of human subjects (7). In rats, 95% of sulfur from intraperitoneally injected L-cysteine was excreted as free sulfate and taurine (8). Thus, it has been suggested that the intake and excretion of sulfur in rats is in a state of sulfur equilibrium (8, 9). We have studied the effect of high and low (10) protein diets on the urinary excretion of sulfate and taurine. In this paper, we report the nutritional effects of high protein content in the diet and cysteine administration on the urinary excretion of sulfate and taurine, and evaluate in vivo production of these metabolites.

Materials and Methods

Materials. Male Wistar rats, 7 weeks of age, weighing 180–200 g were used. These rats had been maintained on a laboratory diet MF of Oriental Yeast Co., Ltd., Tokyo, Japan until the diet was changed to the synthetic diet, the composition of which is shown below.

Casein, corn starch (α), potato starch (α), cellulose powder and a mixture of vitamins (formula of Oriental Yeast Co.) (11) were obtained from Oriental Yeast Co. A sulfate-free mineral mixture was prepared according to the

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formula of Oriental Yeast Co. (11), in which magnesium sulfate, manganese sulfate and copper sulfate were replaced with carbonate salts. These minerals were obtained from Wako Pure Chemical Ind., Ltd., Osaka, Japan. The composition, based on the composition of metal ions of the Oriental Yeast formula (11), was as follows: CaHPO₄·2H₂O, 14.56 g; KH₂PO₄, 25.72 g; NaH₂PO₄, 9.35 g; NaCl, 4.66 g; calcium lactate, 35.09 g; MgCO₃ (basic), 5.96 g; ZnCO₃, 0.11 g; MnCO₃, 0.067 g; CuCO₃ (basic), 0.014 g; KI, 0.01 g; ferric citrate, 3.18 g. Constituents (% in weight) of the synthetic 25% casein diet were corn starch, 38; milk casein, 25; potato starch, 10; cellulose powder, 8; sucrose, 5; corn oil, 6; mineral mixture, 6; and vitamin mixture, 2. In the synthetic 40% casein diet milk casein was increased to 40% and corn starch was reduced to 23%. L-Cysteine was a product of Sigma Chemical Co., St. Louis, MO, USA.

Feeding of rats, administration of l-cysteine and collection of urine. Ten rats were divided into two groups (A and B, 5 rats per group) and each rat was housed in a metabolic cage. After the initial feeding with the MF diet for one week, rats of groups A and B were fed with the 25% and 40% casein diets, respectively, and water ad libitum. At two and three weeks after the start of feeding with the synthetic diets, 5 mmol of l-cysteine per kg of body weight was injected intraperitoneally. Body weight and food intake were weighed daily. The 24 hr urine was collected in a 100-ml Erlenmeyer flask containing 5 ml of 50% acetic acid and one ml of toluene. The urine was centrifuged at 1,200 × g for 10 min, filtered and used for the determination of sulfate and taurine.

Determination of urinary sulfate. Sulfate is excreted in the urine as free sulfate (inorganic sulfate) and bound sulfate (ester sulfate). In the present study, total sulfate (free + ester) was determined by ion chromatography after hydrolysis of ester sulfate as follows: Urine (one ml) was heated at 80°C with one ml of 0.4M hydrochloric acid for 2 h. The hydrolyzed urine was diluted 1:50 with water and 100 µl of the diluted urine was subjected to ion chromatography (12). The ion chromatography system used in this study consisted of a Tosoh ion chromatograph IC-8010 (Tosoh Co., Tokyo, Japan), a TSK-gel IC-Anion-PW column (4.5 × 50 mm) with a TSK guardcolumn IC-A (4.5 × 50 mm) and a Chromatocorder 12 integrator. Chromatography was performed with a pH 8.5 buffer solution consisting of 1.3 mM sodium tetraborate, 5.8 mM boric acid, 1.3 mM potassium gluconate, 12% acetonitrile, 3% n-butanol and 0.5% glycerol at a flow rate of 1.0 ml per min at 40°C. Sulfate was eluted at 22.5 min and its contents were calculated from the standard curve prepared with standard potassium sulfate solutions.

Determination of urinary taurine and hypotaurine. Taurine and hypotaurine were determined by reversed-phase high-performance liquid chromatography (RP-HPLC) as previously reported (13). Briefly, 10 µl of a urine sample and 50 µl of 5.0 mM S-carboxymethylcysteine (CMC) solution, an internal standard, were mixed and amino acids were derivatized with 4-dimethylaminoazobenzene-4'-sulfonyl (dabsyl) chloride. An aliquot of a sample containing dabsyl-amino acids was analyzed with a RP-HPLC system (Toosho Co.) consisting of a CCPM pump, a TSKgel ODS-80Ts column (4.6 × 150 mm) with a guard column of TSKguard ODS-80Ts (3.2 × 15 mm), a UV-8010 detector and a Chromatocorder 12 integrator. Chromatography was performed at 16°C with a linear gradient of a solvent system prepared with 50 mM sodium acetate (pH 4.0) and acetonitrile. Taurine and hypotaurine contents were calculated from the ratios of peak areas of dabsyl-taurine and dabsyl-hypotaurine to dabsyl-CMC. The term “total taurine” was used in this paper to describe the sum of taurine and hypotaurine.

Data obtained in the present study were analyzed with the Student’s t-test. Atypical data obtained from rats in which cysteine injection appeared to have been unsuccessful were omitted from statistical analyses.

Results and Discussion

Fig. 1 shows the growth curves and food intake of rats fed with the 25% and 40% casein diets. There was no significant difference in the growth curve and food intake (Table I) between these two groups although the body weight gain of rats fed with the 40% casein diet was slightly higher than that of rats fed with the 25% casein diet. This result seems to indicate that the 25% casein diet provides sufficient protein and that a 1.6-fold increase in protein content in the diet had little effect on the growth of these rats. The food intake after the first cysteine administration decreased in both groups of rats, but the decrease was not observed after the second cysteine administration. The mechanism of the first drop in food intake is unknown at present and is still under investiga-
Fig. 1  Growth curve and diet intake of rats. Two groups of rats (5 animals per group) were fed with 25% (●) or 40% (○) casein diet. Mean ± SD of body weight and mean of diet intake are plotted. Day 0 is the start of feeding with synthetic diets. Arrows indicate injection of L-cysteine.

Fig. 2  Urinary excretion of total (free + ester) sulfate in rats. Rats (the same as those in Fig. 1) were fed with 25% (●) or 40% (○) casein diet. See legend to Fig. 1. Mean ± SD is shown.

Fig. 3  Urinary excretion of total taurine (taurine + hypotaurine) in rats. Rats (the same as those in Fig. 1) were fed with 25% (●) or 40% (○) casein diet. See legend to Fig. 1. Mean ± SD is shown.

Fig. 4  Ratio of urinary excretion of total taurine (taurine + hypotaurine) and total (free + ester) sulfate in rats fed with 25% (●) or 40% (○) casein diet. Rats are the same as those in Fig. 1. See legend to Fig. 1. Mean ± SD is shown.

Fig. 2 shows the excretion of total sulfate by rats fed with the 25% and 40% casein diets. The sulfate excretion (μmol/kg of body weight per day) in group A after adaptation to the synthetic diet (mean ± SD of days 6–14) was 943.2 ± 144.8, and that in group B was 2675.0 ± 390.9. A sharp increase in sulfate excretion occurred in both groups after the administration of cysteine as shown in Fig. 2 and the total excretions in groups A and B were 4043.0 ± 305.6 (n = 8) and 5833.2 ± 321.7 (n = 10), respectively. Sulfate excretion returned to the basal level on the following day. The average increase in sulfate excretion after the cysteine administration compared to the previous day was 2910.4 ± 107.1 in group A (n = 8) and 3149.2 ± 54.6 in group B (n = 10). Thus, there was no
statistically significant difference between these groups in average increase in the sulfate excretion.

Fig. 3 shows the excretion of total taurine by rats fed with the 25% and 40% casein diets. The average taurine excretion (μmol/kg of body weight per day) after adaptation to the synthetic diet (mean ± SD of days 6-14) was 280.4 ± 93.8 and 553.4 ± 124.5 in groups A and B, respectively. Similar sharp increases in taurine excretion were observed after cysteine administration as in sulfate excretion. Total excretions in groups A and B were 1127.5 ± 120.2 (n = 8) and 1128.3 ± 73.8 (n = 10), respectively. The average increase in taurine excretion compared to the previous day (μmol/kg of body weight per day, mean ± SD of days 15 and 22) was 818.6 ± 117.9 (n = 8) and 587.8 ± 94.0 (n = 10) in groups A and B, respectively. The increase in group A was significantly larger than that in group B (P < 0.001).

Fig. 4 shows the ratio of excretions of total taurine and total sulfate. The average taurine/sulfate ratio in group A was 0.30 ± 0.08 (range after the adaptation to the synthetic diet, 0.25-0.35) and that in group B was 0.22 ± 0.07 (range after adaptation to the synthetic diet, 0.16-0.24). The taurine/sulfate ratio after the cysteine administration was 0.28 ± 0.05 and 0.19 ± 0.03 in groups A and B, respectively. The ratio in group B was significantly lower (P < 0.001) than that in group A before and after the cysteine administration, and there were no significant differences between the ratios before and after the cysteine administration in either group A or group B. These results seem to indicate that the ratio of taurine and sulfate excretion in each group is held in a relatively narrow range as shown in Fig. 4, suggesting that the ratio of in vivo production of taurine and sulfate is also held in a certain range, in other words, in a steady state. Moreover, this ratio changed only slightly when a single administration of an excess of L-cysteine was given.

The present results also show that an increase in daily protein intake resulted in a significant decrease in taurine/sulfate ratio (compare groups A and B), suggesting that the increase in the rate of in vivo sulfate production was greater than that of taurine production when dietary protein was increased. This increased ratio of sulfate production was maintained at this level within a relatively narrow range, namely, in a steady state as mentioned above, and was affected only slightly by the single administration of a high dose of L-cysteine.

Table 1 summarizes food intake, steady-state excretion of sulfate and taurine, and increased excretion of these metabolites after L-cysteine injection. In accordance with the growth curves shown in Fig. 1, there was no significant difference between the assumed value of sulfur retained in the body of rats of both groups. Moreover, there was no difference in the sum of sulfate and taurine excretion after L-cysteine injection between these groups.

<table>
<thead>
<tr>
<th>Table 1</th>
<th>Intake of diet and urinary excretion of sulfate and taurine</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Group</td>
</tr>
<tr>
<td>Diet</td>
<td>25% casein</td>
</tr>
<tr>
<td>Intake of diet&lt;sup&gt;a&lt;/sup&gt;</td>
<td>84.1 ± 8.8</td>
</tr>
<tr>
<td>Intake of Met and Cys&lt;sup&gt;b&lt;/sup&gt;</td>
<td>4.02</td>
</tr>
<tr>
<td>Excretions&lt;sup&gt;c&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>Sulfate&lt;sup&gt;d&lt;/sup&gt;</td>
<td>0.94 ± 0.14</td>
</tr>
<tr>
<td>Taurine&lt;sup&gt;e&lt;/sup&gt;</td>
<td>0.28 ± 0.09</td>
</tr>
<tr>
<td>Sulfate + Taurine</td>
<td>1.22 ± 0.22</td>
</tr>
<tr>
<td>(% of ingested sulfur as Met + Cys)</td>
<td>(30.4)</td>
</tr>
<tr>
<td>(Sulfur retained&lt;sup&gt;f&lt;/sup&gt;)</td>
<td>(2.80)</td>
</tr>
<tr>
<td>Increased excretions&lt;sup&gt;g&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>Sulfate&lt;sup&gt;d&lt;/sup&gt;</td>
<td>2.91 ± 0.11</td>
</tr>
<tr>
<td>Taurine&lt;sup&gt;e&lt;/sup&gt;</td>
<td>0.82 ± 0.12</td>
</tr>
<tr>
<td>Sulfate + taurine</td>
<td>3.74 ± 0.27</td>
</tr>
<tr>
<td>(% of sulfur injected as Cys)</td>
<td>(74.8)</td>
</tr>
</tbody>
</table>

<sup>a</sup>: g/kg of body weight, mean ± SD of days 6-14.<n>sup>b</sup>: Sum of L-methionine (Met) and L-cysteine (Cys), calculated from their contents in casein and corrected for water content in the diets, mmol/kg of body weight per day.<n>sup>c</sup>: mmol/kg of body weight per day, mean ± SD of days 6-14.<n>sup>d</sup>: Total sulfate (see text).<n>sup>e</sup>: Total taurine (see text).<n>sup>f</sup>: Calculated assuming that the sulfur in L-methionine and L-cysteine was metabolized completely to sulfate and taurine, and the recoveries of these metabolites were 100%.<n>sup>g</sup>: Increased excretions after L-cysteine administration, mean ± SD of days 15 and 22.
References


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