The Influence of DL-Methionine on the Metabolism of S-Adenosylethionine in Rats Chronically Treated with DL-Ethionine

Zbynek Brada, Stephan Bulba, and Norman H. Altman

Papanicolaou Cancer Research Institute at Miami [Z. B., S. B., N. H. A.], and Department of Pathology, University of Miami [Z. B., N. H. A.], Miami, Florida 33136

SUMMARY

The concentration of S-adenosylmethionine in the liver of ethionine-fed rats was increased gradually during the process of carcinogenesis. This increase may have been due to the decreased capacity of the treated rats to acetylate ethionine sulfoxide. Ethionine sulfoxide is considered as the main reserve pool of ethionine for the synthesis of S-adenosylmethionine. When the ethionine diet was supplemented by DL-methionine (0.3 to 0.9%), the increase in the concentration of S-adenosylmethionine during the period of observation (28 to 150 days) was lower and the acetylation of ethionine sulfoxide was significantly higher. The concentration of the total S-adenosyl compounds in the liver of rats on a diet supplemented with DL-methionine was increased over the concentration of S-adenosylmethionine in rats fed ethionine alone, and the S-adenosylmethionine portion of this fraction was only about 30% lower. The supplementation of the diet with methionine restored the diurnal oscillation of adenosine 5'-triphosphate in the liver, which had been absent in rats ingesting only ethionine.

INTRODUCTION

It is well known that the administration of ethionine (an ethyl homolog of methionine) to rats produces a variety of morphological and biochemical lesions in the liver and other organs and induces hepatoma (8, 9, 31). Methionine reverses many biochemical and morphological effects of ethionine (11, 13, 26, 28, 34) and prevents the induction of liver carcinoma in rats (10). Assuming that ethionine interferes with some methionine pathways, it is of considerable importance to pinpoint the interaction sites of the metabolism of both compounds. Stekol et al. (32) suggested and Shull (24) has experimentally proven that the administration of ethionine to rats leads to the formation of S-AE² accompanied by a rapid decrease in the concentration of ATP in liver. It was indicated (25), on the basis of a study of the acute effect of ethionine administration, that the primary effect of ethionine is the trapping of the adenosine moiety resulting from the interaction between ethionine and ATP. The administration of methionine prevented the synthesis of S-AE and simultaneously increased the level of ATP in the liver. The timing of methionine administration was apparently of decisive importance; methionine had to be administered a few hours after ethionine to exercise the inhibitory activity. S-AE has been considered as an essential metabolite leading to tumor formation through ethylation of cellular macromolecules (15, 20, 30). This study was carried out in an effort to examine the effect of methionine on the metabolism of S-AE in the liver of rats chronically intoxicated with ethionine. In chronic experiments, the timing of the methionine intake is regulated by biorhythmic food consumption and is therefore synchronized with the ethionine intake.

MATERIALS AND METHODS

CFN female rats were purchased from Carworth, Division of Charles River Breeding Laboratories, Wilmington, Mass. The rats were fed ad libitum C-24, a semisynthetic diet (34), and were housed in stainless steel cages with wire mesh bottoms at 22° and 50% relative humidity. The windowless room was illuminated from 6 a.m. to 6 p.m. Each group of rats was weighed weekly and sacrificed at different stages during the ethionine treatment. One group of rats was fed C-24 only, the 2nd group was fed C-24 + 0.30% DL-ethionine (Nutritional Biochemicals Corp., Cleveland, Ohio), the 3rd group was fed C-24 + 0.30% DL-ethionine + 0.30% DL-methionine (Nutritional Biochemicals Corp., Cleveland, Ohio), and the 4th group was fed C-24 + 0.30% DL-ethionine + 0.90% DL-methionine. The compounds added to the basal diet replaced a corresponding amount of sucrose.

At the termination of each experiment, the rats were initially anesthetized with ether and a blood sample was obtained by cardiac puncture. A portion of liver was quickly removed, frozen in liquid nitrogen and homogenized in 10-fold volume (w/v) of cold 3% perchloric acid in a Waring Blender. After the homogenates were centrifuged in the cold for 10 min at 2000 rpm, the resulting precipitate was washed twice with 3% HClO₄, once with 95% ethanol containing 10% potassium acetate, once with absolute ethanol, twice with a mixture of ethanol:diethyl ether (3:1) (once at 60° and once at room temperature), and twice with diethyl ether; they were then dried in a vacuum to constant weight (dry fat-free substance). The assay of S-AE (or S-AM or S-AC) was done by the method proposed by Schlenk and

1 Supported by USPHS Grant Ca-11271 from the National Cancer Institute, NIH. This work was presented in preliminary form at the FASEB meeting (1).

2 The abbreviations used are: S-AE, S-adenosylmethionine; S-AC, S-adenosylhomocysteine.

Received August 18, 1975; accepted January 20, 1976.

MAY 1976
DePalma (23) for determination of S-AM in yeast, which was modified for S-AE determination in the liver of rats by Stekol (30). For the determination of S-AE in the presence of other S-AC, the ethionine in the diet was labeled with DL-[ethyl-1-14C]ethionine (Schwarz/Mann, Orangeburg, N. J.; specific activity, 1.0 μCi/25 mg ethionine). Before the experiment, the diet containing cold ethionine was replaced for 24 hr by the diet without ethionine. At 5 p.m. the next day, the diet was supplemented with [14C]ethionine (0.3 g/100 g diet). The rats were kept on the radioactive diet for 72 to 90 hr, depending on the time of sacrifice during the day cycle. This time was considered as sufficient for the replacement of cold ethionine in view of: (a) a rapid excretion of ethionine metabolites into urine (in 12 hr more than 50%; in 24 hr, 70 to 80%); (b) the metabolic inertness of the main metabolic product N-acetylhistidine sulfoxide (2); and (c) a high metabolic turnover of S-AE (25). According to Shull et al. (25), the administration of methionine did not change the turnover of S-AE. The total S-AC was isolated as described above and its radioactivity was determined. The S-AC fraction isolated from liver of ethionine + methionine-fed rats does not contain any detectable amount of ethyl-1-14C-containing material except S-AE, as demonstrated by a chromatographic analysis. In a control experiment, with rats fed only ethionine, the specific activity of isolated S-AE was close to the expected calculated values.

The urine was collected in a BioNuclear glass metabolic cage. Before the experiments, 1 ml n HCl was added for preservation and to avoid the decomposition of S-AE. The volume of the urine was adjusted to 50 ml and an aliquot was used for the chromatographic analysis. The analysis of ethionine and its metabolites in urine was performed chromatographically (2) on an AG 50W-X12 column, 200 to 400 mesh (Bio-Rad Laboratories, Richmond, Calif.). The radioactivity was determined in an aliquot of the fractions in a mixture with Bray’s solution in a Packard spectrometer after a proper quenching correction. A 2nd aliquot of perchloric acid extract was neutralized by 5 N KOH, and ATP was determined by a method proposed by Cohn (4) and modified by Siekevitz and Potter (27) and Stekol. Liver ATP was separated on AG 1-X8 column (Bio-Rad Laboratories) and hydrolyzed, and the liberated adenosine was isolated on the same exchange material and its concentration was subsequently determined spectrophotometrically (about the details and limitations of this method, see Ref. 2). The hemoglobin concentration in blood was determined spectrophotometrically as CN-ferrihemoglobin by the method of Heilmeyer (17), seromucoid concentration was determined by the method of Winzler (36), and total serum proteins were determined by Lowry’s method, modified by Oyama and Eagle (21).

For light microscopic observations, the tissues were fixed in 10% neutral-buffered formalin, and routine paraffin sections were stained with hematoxylin and eosin.

RESULTS

The growth curves of the rats in the experiment, in which 2 supplementary levels of methionine were used, are shown in Chart 1. It is evident that supplementation of methionine to an ethionine diet increases the body weight of the rats slightly, but the increase does not restore the total decrease due to ethionine. This is not surprising in view of the known toxicity of larger doses of methionine (34). Grossly, the liver of all rats fed only ethionine had typical changes, while the liver of rats fed ethionine supplemented with methionine was pale tan but otherwise unremarkable. Microscopically, characteristic hepatic changes were seen in the ethionine-fed rats (ductular proliferation, cholangiointerstitial fibrosis, megacytosis, and nodule formation) but not in the rats supplemented with 0.30% or 0.90% methionine in agreement with previously published observations (11). The effect of methionine supplementation on the toxicity of ethionine is presented in Table 1, which shows that the decrease of hemoglobin in blood is only partially restored, total serum proteins are normalized, and the seromucoid, which is decreased during the ethionine intoxication, is not corrected. The seromucoid fraction represents a complicated mixture of glycoproteins and mucoproteins synthesized almost exclusively in the liver and subsequently released into circulation (6).

Table 2 presents the effect of methionine on ATP and on S-AC in the liver of rats ingesting ethionine. The analytical method used in our studies does not discriminate between S-AE, S-AM, and S-AHCy. In the absence of methionine supplementation, the observed values correspond practically only to S-AE, because the amount of S-AM in the liver differs from S-AE by several orders of magnitude. Food was removed from the cages at 5 p.m. the day before sacrifice, which took place at 9:00 a.m. the following day. We expected that, after 16 hr of starvation, the levels of S-AM and of S-AHCy would be negligible in relationship to the S-AE concentration. The results presented in Table 2 demonstrate that the values of hepatic S-AE following an ethionine ingestion had a tendency to rise during the period of observation. The cause of this increase could be the change in
Effect of dl-methionine on rats fed dl-ethionine

The assay and procedure for the treatment of the animals are described in "Materials and Methods." The data were statistically analyzed by Student's t test.

<table>
<thead>
<tr>
<th>Treatmenta</th>
<th>No. of rats</th>
<th>Days fed</th>
<th>Body wt (g)</th>
<th>Liver wt in % of body wt</th>
<th>Hemoglobin (%)</th>
<th>Serum proteins (%)</th>
<th>Seromucoid (mg/100 ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>4</td>
<td>28</td>
<td>205 ± 7b</td>
<td>2.52 ± 0.13</td>
<td>15.7 ± 0.4</td>
<td>6.40 ± 0.16</td>
<td>20.5 ± 0.9</td>
</tr>
<tr>
<td>0.3% ethionine</td>
<td>4</td>
<td>28</td>
<td>165 ± 5'</td>
<td>2.97 ± 0.18</td>
<td>14.1 ± 0.44</td>
<td>5.58 ± 0.09</td>
<td>14.2 ± 0.65</td>
</tr>
<tr>
<td>0.3% ethionine + 0.3% methionine</td>
<td>4</td>
<td>28</td>
<td>167 ± 3</td>
<td>2.92 ± 0.09</td>
<td>13.9 ± 0.6</td>
<td>6.21 ± 0.16</td>
<td>12.7 ± 0.63</td>
</tr>
<tr>
<td>0.3% ethionine + 0.9% methionine</td>
<td>4</td>
<td>28</td>
<td>179 ± 5</td>
<td>3.70 ± 0.16c</td>
<td>14.9 ± 0.4</td>
<td>6.64 ± 0.12</td>
<td>15.0 ± 1.6</td>
</tr>
<tr>
<td>Control</td>
<td>4</td>
<td>55</td>
<td>229 ± 2</td>
<td>2.77 ± 0.25</td>
<td>15.5 ± 0.3</td>
<td>6.61 ± 0.14</td>
<td>20.5 ± 0.5</td>
</tr>
<tr>
<td>0.3% ethionine</td>
<td>4</td>
<td>55</td>
<td>186 ± 8'</td>
<td>3.89 ± 0.32</td>
<td>13.9 ± 0.3'</td>
<td>5.86 ± 0.10</td>
<td>13.9 ± 0.3'</td>
</tr>
<tr>
<td>0.3% ethionine + 0.3% methionine</td>
<td>4</td>
<td>55</td>
<td>193 ± 2</td>
<td>3.85 ± 0.31</td>
<td>14.3 ± 0.7</td>
<td>6.05 ± 0.14</td>
<td>14.3 ± 0.7</td>
</tr>
<tr>
<td>0.3% ethionine + 0.9% methionine</td>
<td>4</td>
<td>55</td>
<td>200 ± 4</td>
<td>3.94 ± 0.17</td>
<td>15.1 ± 0.4</td>
<td>6.47 ± 0.08</td>
<td>15.1 ± 0.4</td>
</tr>
</tbody>
</table>

Effect of dl-methionine on ATP and S-AC concentration in liver of rats fed dl-ethionine

The assay and procedure for the treatment of the animals are described in "Materials and Methods." The data were statistically analyzed by Student's t test.

<table>
<thead>
<tr>
<th>Treatmenta</th>
<th>No. of rats</th>
<th>Days fed</th>
<th>ATP (µmoles/g DFFS)</th>
<th>S-AC (µmoles/g DFFS)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>4</td>
<td>28</td>
<td>5.10 ± 0.23c</td>
<td>NA</td>
</tr>
<tr>
<td>0.3% ethionine</td>
<td>4</td>
<td>28</td>
<td>3.11 ± 0.20d</td>
<td>2.17 ± 0.09</td>
</tr>
<tr>
<td>0.3% ethionine + 0.3% methionine</td>
<td>4</td>
<td>28</td>
<td>2.91 ± 0.13</td>
<td>1.69 ± 0.06</td>
</tr>
<tr>
<td>0.3% ethionine + 0.9% methionine</td>
<td>4</td>
<td>28</td>
<td>4.43 ± 0.11f</td>
<td>2.14 ± 0.22</td>
</tr>
<tr>
<td>Control</td>
<td>4</td>
<td>55</td>
<td>5.68 ± 0.07</td>
<td>NA</td>
</tr>
<tr>
<td>0.3% ethionine</td>
<td>4</td>
<td>55</td>
<td>3.60 ± 0.12d</td>
<td>3.90 ± 0.09</td>
</tr>
<tr>
<td>0.3% ethionine + 0.3% methionine</td>
<td>4</td>
<td>55</td>
<td>4.37 ± 0.40</td>
<td>3.37 ± 0.15</td>
</tr>
<tr>
<td>0.3% ethionine + 0.9% methionine</td>
<td>4</td>
<td>55</td>
<td>5.12 ± 0.40</td>
<td>3.11 ± 0.08</td>
</tr>
<tr>
<td>Control</td>
<td>4</td>
<td>146</td>
<td>5.92 ± 0.28</td>
<td>NA</td>
</tr>
<tr>
<td>0.3% ethionine</td>
<td>2</td>
<td>146</td>
<td>3.51 ± 0.08d</td>
<td>5.23 ± 0.20</td>
</tr>
<tr>
<td>0.3% ethionine + 0.3% methionine</td>
<td>2</td>
<td>146</td>
<td>4.28 ± 0.11f</td>
<td>3.15 ± 0.03</td>
</tr>
<tr>
<td>0.3% ethionine + 0.9% methionine</td>
<td>2</td>
<td>146</td>
<td>5.23 ± 0.15f</td>
<td>2.95 ± 0.12</td>
</tr>
</tbody>
</table>

- The rats were fasted 16 hr prior to sacrifice.
- Mean ± S.D.
- Significantly different from control group.
- Significantly different from ethionine group.

The time of food intake, in the extent of S-AE synthesis, or in the rate of S-AE catabolism. In these experiments, only a slight modification in the pattern of food intake was observed (see Chart 2). The synthesis of S-AE depends on the availability of ethionine, which is regulated by the formation of ethionine sulfide. Ethionine sulfide represents the main reserve pool of ethionine in the organism of the rat (2). Acetylated ethionine sulfide is more or less excluded from active ethionine metabolism. The acetylation of ethionine sulfide decreases gradually during the progress of chronic liver intoxication (experiments not presented here). Methionine supplementation partially abolished the increase in S-AE concentration, as observed on the 55th day and the 146th day of the experiment. This effect can be explained by the increased capacity of ethionine-treated rats to acetylate ethionine sulfide after methionine supplementation. Alternatively, the increase in the S-AE level can be the result of its diminished catabolism. So far, no data are available on the degradation pathway of S-AE with the exception of the transfer of the ethyl groups.

To elucidate the effect of the pattern of food intake on ethionine metabolism, we investigated the S-AC and ATP concentration during the 24-hr cycle (Chart 2). The chart demonstrates that the ATP concentration in the liver oscillates in agreement with our previous observation (3). The ATP concentration in the liver of ethionine-ingesting rats was almost constant during the 24-hr cycle. The supplementation with methionine increased the concentration of ATP and restored its biorhythmical fluctuation. The concentration of S-AC (equal to S-AE) reached a maximum at 6 a.m.
and did not exhibit greater changes during the 24-hr cycle. In the presence of added methionine, there was an increase in the concentration of S-AC (representing a mixture of S-AE, S-AM, and S-AHcy). Because the S-AC and S-AE values showed a convergent trend after the maximum was reached, we can assume that the values presented in Table 2 represent almost exclusively the concentration of S-AE.

Expressing the ratio between the concentration of S-AC and the decrease of ATP (ΔATP) as a function of time during the 24-hr cycle, we obtained different values for rats ingesting ethionine only and rats supplemented with methionine (Chart 3). This relationship is affected by an error produced by different amounts of diets consumed by the control group (6.04 g per 100 g body weight per 24 hr) and the experimental animals (ethionine, 4.35 g per 100 g body weight per 24 hr; ethionine + methionine, 5.41 g per 100 g body weight per 24 hr). However, the differences between the ratio of both experiments surpass considerably the expected error. We can conclude from these results that the ATP fall in ethionine-treated rats is in good relationship with the S-AC concentration. Although the administration of methionine significantly increased the amount of trapped adenosine in S-AC, the increase in the ATP concentration shows that the decisive factor determining the ATP level in chronic experiments is the turnover of ATP and not the trapping effect. The excessive diurnal fluctuation of the ratio of S-AC/ΔATP in methionine-supplemented rats demonstrates the difference in the ATP generation during the diurnal cycle. As shown in Chart 2, methionine supplementation increased the concentration of S-AC in the liver; however, under the conditions of this experiment, it was not clear to what extent S-AM and S-AE contributed to this increase. In order to clarify that point, we fed the rats a diet containing labeled DL-[ethyl-1-14C]ethionine and cold DL-methionine. The concentration of S-AC was established by measuring the radioactivity of the isolated S-AC fraction, and the difference from the total S-AC expressed the concentration of the sum of S-AM + S-AHcy. Chart 4 shows clearly that the simultaneous application of ethionine and methionine resulted only in a partial decrease in the S-AE concentration in liver. The decrease represented on the average of about 1/3 of the concentration of S-AE that would be expected in rats treated with the same amount of ethionine but without methionine supplementation.

**Excretion of Ethionine Metabolites into Urine.** The analysis of metabolites of ethionine from rats treated for 23 days with ethionine or with ethionine + methionine is presented in Table 3. The ratio of acetylated to nonacetylated ethionine sulfoxide was increased as a result of supplementation with methionine. In addition, a compound appearing between the peaks attributed to free ethionine and S-AE was
The effect of DL-methionine on the excretion of ethionine metabolites in the urine of DL-ethionine-ingesting rats

The rats were fed a diet containing 0.3% cold DL-ethionine for 20 days. The cold DL-ethionine was then replaced for 3 days by DL-[ethyl-1-14C]ethionine (specific activity, 1 μCi/25 mg) as described in "Materials and Methods." On the day of the experiment the rats were placed in a metabolic cage at 6 a.m. and given no food. The urine was collected within a period of 27 hr. An aliquot of the urine was analyzed by chromatography on AG 50W column (see "Materials and Methods"). The figures represent an average of 2 independent experiments and are given in percentage of total radioactivity in urine. Two rats were used in every group.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Acetylated ethionine metabolites (%)</th>
<th>Ethionine sulfoxide (%)</th>
<th>Ethionineb (%)</th>
<th>S-AE (%)</th>
<th>Others (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>DL-Ethioninea</td>
<td>37.3 (34.1-40.5)</td>
<td>40.2 (45.8-34.6)</td>
<td>7.5 (6.6-8.4)</td>
<td>14.9</td>
<td>0.0</td>
</tr>
<tr>
<td>DL-Ethionine + DL-</td>
<td>59.7 (62.3-57.1)</td>
<td>19.2 (22.0-16.4)</td>
<td>3.6 (4.2-3.0)</td>
<td>14.1</td>
<td>3.3</td>
</tr>
<tr>
<td>methioninea</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

a The fraction contained substances not absorbed on the chromatographic column. In acute experiments with L-ethionine, this fraction was designated as N-acetylation sulfoxide (2). In chronic experiments the composition of this fraction was more complex but the acetylated ethionine metabolites represented the major components.
b This fraction contained free ethionine and some unidentified minor components.
c A new fraction located between the peaks of ethionine and S-AE.
d The total radioactivity found in the urine of both experimental groups was similar when expressed as a percentage of L-[ethyl-1-14C]ethionine ingested within 24 hr prior to urine collection.
e Numbers in parentheses, results of individual determinations.
f DL-Methionine was supplied to the diet in the concentration of 0.9%.

Table 3

observed for the 1st time. This compound has not yet been identified.

DISCUSSION

DL-Ethionine metabolism proceeds in rats in 4 basic metabolic pathways, the roles of which in the mechanism of carcinogenesis are not completely established: (a) the oxidation of ethionine to ethionine sulfoxide and subsequent acetylation (a predominant pathway representing about 50% of metabolized ethionine in acute experiments); (b) the activation of ethionine to S-AE (representing 30 to 40% of metabolized ethionine in acute experiments) with subsequent transethylation; (c) the oxidative pathway of the ethyl group of ethionine to CO2 via α-keto-γ-ethyl-butynic acid; (d) the inversion of D-ethionine into L-ethionine.

Initial investigations of the 1st pathway are only now taking place (2, 29). Nevertheless, it was suggested (2) that ethionine sulfoxide (which is easily reduced to ethionine in vivo) might represent the most important pool of ethionine for the S-AE synthesis and, specifically, for the maintenance of a high level of this compound in the liver a long time after ethionine administration. The 2nd pathway (via S-AE activation) has been the principal point of attack by those interested in the mechanism of ethionine carcinogenesis. The 3rd pathway has not been studied directly as yet, but recently, new evidence was presented by T. M. Farber (14), who demonstrated the formation of acetaldehyde from the ethyl group of ethionine by liver microsomal fraction. The inversion of D-ethionine was suggested many years ago (31), and direct evidence was presented recently (2).

Our study is limited with the pathway of S-AE activation, as affected by methionine feeding, although this regime may also influence the other pathways. S-AE serves as a donor of ethyl groups that can be transferred to many low molecular and high molecular substances in cells. The ethylation of cellular macromolecules (proteins and nucleic acids) is considered to be a decisive part of the carcinogenic action of ethionine (8, 9, 19). This hypothesis is based on logical molecular-biological speculation, but direct evidence is lacking. The main support for this hypothesis was contributed by the observation that the addition of DL-methionine to DL-ethionine (3:1) in the carcinogenic diet almost completely inhibited the development of early pathological changes in liver and prevented the formation of hepatoma (10). It was suggested that the protective effect is due to the competitive inhibition of S-AE synthesis, and this idea found a strong experimental support from the observations of Shull et al. (25) in acute experiments. In their study the importance of the exact timing of the DL-methionine administration was also stressed. To suppress the S-AE concentration in the liver for 24 hr, 2 injections of methionine were needed. The simultaneous administration of methionine with ethionine resulted in only a temporary (not exceeding 5 hr) decrease of S-AE concentration. The authors concluded that the effect of methionine was rather a result of the inhibition of formation of S-AE than of the acceleration of its breakdown.

In our experiments, the effect of methionine supplementation on the growth rate and on the pathological changes in liver of rats ingesting ethionine are in agreement with previous observations (7, 10, 35).

In chronic experiments, methionine intake was not uniform during the 24-hr cycle and was determined by the characteristic diurnal food intake pattern, which was only slightly modified by the presence of ethionine. Despite the fact that 50% of the food was consumed between 6 p.m. and...
midnight and 75% during the night (6 p.m. to 6 a.m.), the S-AE concentration in liver was considerably stable. The supplementation of DL-methionine increased the level of the total S-AC, which oscillated characteristically during the 24 hr. The increase in the S-AC concentration was due to elevation of S-AM and perhaps S-AHCy, which are subject to a stronger diurnal variation. The effect of DL-methionine on the S-AE formation is weaker than expected from the observation in acute experiments (25). This may be due partially to the increase in the food and ethionine intake resulting from the improved condition of the rats despite the presence of ethionine. Whether the observed decrease of S-AE concentration in the liver is a sufficient explanation for the protective effect of DL-methionine remains an open question. On the other hand, the presence of large amounts of S-AM (and perhaps of S-AHCy) in the liver suggests that ethionine, in chronic experiments, has a distinct effect on the metabolism of these compounds. The concentration of S-AHCy has not yet been studied systematically, but it is believed (18) to be in the range of the level of S-AM under physiological conditions. However, in ethionine systems it is not known whether the concentration of S-AHCy is also related to S-AM. Stekol has found in acute experiments (unpublished results) that the concentration of S-AM after an i.p. injection of L-methionine (50 mg/100 g body weight) reached its maximum in 40 to 50 min. Compared with the same dose of ethionine, S-AM represented only 20% of the concentration of S-AE at the time of its maximum (4 hr after administration). Farber et al. (12) observed even larger differences in a similar experiment. The amount of S-AM can be more than sufficient to restore the normal methylation of tRNA, which is undermethylated in the presence of ethionine (16). The concentration of S-AHCy in liver after ethionine feeding has not been studied as yet, but since this compound was recognized as a potent inhibitor of transmethylations (5, 22, 37), in the case of its increased concentration, the transalkylation could be influenced.

In our experiments the S-AE concentration in liver of ethionine-fed rats starved for 16 hr tends to increase. This increase did not take place to the same extent when the ethionine diet was supplemented with methionine. We suggest tentatively that the increase itself is due to the decreased capacity of the organism to acetylate ethionine sulfoxide. The acetylation of ethionine sulfoxide is not reversible (29). Since methionine prevents the decrease of the capacity of the rats to acetylate ethionine sulfoxide, less ethionine is available for S-AE synthesis and thus partially decreases the S-AE concentration in the liver of rats starved for 16 hr. The effect of methionine on the restoration of acetylation of ethionine sulfoxide is probably indirect (through the protection of hepatocytes).

Every molecule of ethionine that is activated to S-AE requires 1 molecule of ATP. Because the metabolism of S-AE appears to be at a much slower rate than that of S-AM, a significant amount of adenosine is tied up in this compound. This adenosine trapping effect suggested by Stekol (32) was experimentally confirmed by Shull (24), and it indicated that the administration of ethionine to rats resulted in a biologically significant drop in the level of liver ATP. Methionine reversed the suppression of ATP produced by ethionine. If the decrease in ATP concentration is a direct consequence of adenosine trapping in S-AE, a close inverse relationship should be found between the levels of ATP and S-AE as a function of time (after administration) and of dosage of ethionine. Shull et al. (25) have shown in acute experiments that when the concentrations of S-AE and ATP are plotted against time, the curves formed are essentially mirror images of each other. This was not true when ATP and S-AE concentrations were compared with respect to the administered dose. After administration of a given amount of ethionine (50 mg or more per 100 g body weight), the S-AE concentration reached its maximum (4 hr after the injection) while the amount of ATP was still decreasing. Shull et al. (25) suggested that a major difference between the consequences of small or large doses of ethionine reflected the extent of the synthesis of adenine nucleotide de novo. With small amounts of ethionine, the synthesis de novo remained operating and compensated for the trapping effect of S-AE. Larger doses produced an inhibition of adenosine synthesis.

In our experiments the oscillation of ATP concentration in liver in control rats varied with the food intake (Chart 4). Forced feeding during the daylight hours or a time change of exposure to light completely reversed the pattern demonstrated in this chart. In ethionine-ingesting rats, the oscillation of ATP concentration within a 24-hr period was insignificant. The supplementation of the diet with DL-methionine restored the diurnal fluctuation. The ATP concentration in the liver was also significantly increased, despite the fact that the amount of trapped adenosine in S-AC was increased in comparison with findings in the experiment with rats ingesting only ethionine. This is considered as evidence that, contrary to the observation in acute experiments (25), the lower rate of ATP synthesis is responsible for the ATP drop in chronic experiments rather than the trapping effect of S-AC. This would be in agreement with the observation of Stekol et al. (33) that an important part of the ethionine action in chronic experiments is an inhibition of mitochondrial oxidation and of oxidative phosphorylation, resulting in the concomitant decrease of ATP synthesis.

ACKNOWLEDGMENTS

The authors wish to express their thanks to J. Blicharska and W. Prince for their excellent technical assistance.

REFERENCES


The Influence of dl-Methionine on the Metabolism of S-Adenosylethionine in Rats Chronically Treated with dl-Ethionine

Zbynek Brada, Stephan Bulba and Norman H. Altman


Updated version
Access the most recent version of this article at:
http://cancerres.aacrjournals.org/content/36/5/1573

E-mail alerts
Sign up to receive free email-alerts related to this article or journal.

Reprints and Subscriptions
To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.

Permissions
To request permission to re-use all or part of this article, use this link http://cancerres.aacrjournals.org/content/36/5/1573. Click on "Request Permissions" which will take you to the Copyright Clearance Center's (CCC) Rightslink site.