Neither Methionine nor Nitrous Oxide Inactivation of Methionine Synthase Affect the Concentration of 5,10-Methylenetetrahydrofolate in Rat Liver

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ABSTRACT 5,10-Methylenetetrahydrofolate occupies a key position in folate-dependent one-carbon metabolism. It is involved directly in the biosynthesis of deoxythymidine, it can be converted to 10-formyltetrahydrofolate for purine synthesis and it may be reduced to 5-methyltetrahydrofolate for methylation of homocysteine to methionine. We have developed a HPLC method for measuring 5,10-methylenetetrahydrofolate in liver and we have used this method to investigate the control of one-carbon metabolism: 1) administration of methionine and 2) administration of the anesthetic gas, nitrous oxide (N\(_2\)O). Rats were given 1.3 mmol/kg of N\(_2\)O. Rats were given 1.3 mmol/kg of methionine, and accumulation of 5-methyltetrahydrofolate at the expense of other folate coenzymes appeared to revert to control values. There was no apparent change in the concentration of 5,10-methylenetetrahydrofolate. Exposing rats to an atmosphere containing N\(_2\)O results in inactivation of methionine synthase on the levels of 5,10-methylenetetrahydrofolate and 5,10-methyltetrahydrofolate.

KEY WORDS: • folic acid • one-carbon metabolism • 5,10-methylenetetrahydrofolate • methionine administration • nitrous oxide

MATERIALS AND METHODS

Materials. Sodium L-ascorbate, 6-(RS)-5-formyltetrahydrofolate, l-methionine, Hepes and 2-(cyclohexylamino)ethanesulfonic acid (Ches) buffers were purchased from Sigma Chemical (St. Louis, MO), 2-mercaptoethanol was from Acros Organics USA (Fairlawn, NJ), tetrabutylammonium hydroxide and folic acid (5-CH\(_3\)-H\(_4\)PteGlu) were from Fisher Chemical (Atlanta, GA). Lyophilized cultures of Lactobacillus rhamnosus (ATCC 7469) were from the American Type Culture Collection (Rockville, MD). The concentration of folic acid was determined by a colorimetric method that used a spectrophotometer to measure the absorbance at 450 nm.

Folate deficiency is associated with an increased plasma concentration of homocysteine and increased risk of cardiovascular disease, cancer and neural tube defects (1–3). Elevated homocysteine also is associated with polymorphisms in the 5,10-methylenetetrahydrofolate reductase (MTHFR) gene and results in a variety of vascular and neurological symptoms (4).

A key intermediate in folate coenzyme metabolism is 5,10-methylenetetrahydrofolate (5,10-CH\(_2\)-H\(_4\)PteGlu). It can be synthesized from 5,6,7,8-tetrahydrofolic acid (H\(_2\)PteGlu) and formate via the trifunctional enzyme, C\(_1\)-tetrahydrofolate synthase, and by the reaction of serine with H\(_2\)PteGlu via serine hydroxymethyltransferase. 5,10-CH\(_2\)-H\(_4\)PteGlu may be converted to 10-formyltetrahydrofolic acid (10-HCO-H\(_4\)PteGlu) which provides one-carbon units for purine synthesis. 5,10-CH\(_2\)-H\(_4\)PteGlu also provides the methyl group and reducing equivalents for the synthesis of dTMP from dUMP. 5,10-CH\(_2\)-H\(_4\)PteGlu may be reduced by MTHFR to 5-methyltetrahydrofolic acid (5-CH\(_3\)-H\(_4\)PteGlu), which is utilized by the vitamin B-12–dependent enzyme, methionine synthase, to methylate homocysteine and regenerate methionine and H\(_2\)PteGlu.

Administration of methionine results in a redistribution of folate coenzymes such that the level of 5-CH\(_3\)-H\(_4\)PteGlu decreases and the level of H\(_2\)PteGlu increases (5–7). This is due to the inhibition of MTHFR by S-adenosylmethionine (AdoMet) whose level increases with methionine administration (8). In addition, inactivation of the B-12–dependent enzyme, methionine synthase, by nitrous oxide (N\(_2\)O) results in trapping of folates as 5-CH\(_3\)-H\(_4\)PteGlu. This is because only two enzymes use 5-CH\(_3\)-H\(_4\)PteGlu, i.e., methionine synthase, which is inactivated, and MTHFR, which is irreversible in vivo (10).

Because of the key role 5,10-CH\(_2\)-H\(_4\)PteGlu plays in the metabolism of folate coenzymes, we devised a HPLC method for measuring this derivative in liver (11). We have used this procedure to determine the effects of methionine administration and inactivation of methionine synthase on the levels of 5,10-CH\(_2\)-H\(_4\)PteGlu in rat liver.

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3 Abbreviations used: AdoMet, S-adenosyl-L-methionine; Ches, 2-(cyclohexylamino)ethanesulfonic acid (Ches) buffers were purchased from Sigma Chemical (St. Louis, MO), 2-mercaptoethanol was from Acros Organics USA (Fairlawn, NJ), tetrabutylammonium hydroxide and folic acid (5-CH\(_3\)-H\(_4\)PteGlu) were from Fisher Chemical (Atlanta, GA). Lyophilized cultures of Lactobacillus rhamnosus (ATCC 7469) were from the American Type Culture Collection (Rockville, MD). The concentration of 5,10-methylenetetrahydrofolate reductase.

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Folate distribution after administration of methionine. Methionine was administered to the rats and folate coenzymes were determined at various times. At 30 and 60 min after administration, the level of 5-CH$_2$H$_4$PteGlu appeared to be greatly decreased (Fig. 1). This decrease was reflected by increases in the levels of 10-HCO- and H$_4$PteGlu. After 120 min, the level of 5-CH$_2$- and 5-formyltetrahydrofolic acid (5-HCO-H$_4$PteGlu) had returned to normal and the level of H$_4$PteGlu continued to decrease. The amount of 5,10-CH$_2$-H$_4$PteGlu was apparently unchanged at all time intervals.

Effect of N$_2$O on hepatic folate coenzymes. In air-breathing control rats, H$_4$PteGlu represented 24% of total hepatic folates; 5,10-CH$_2$H$_4$PteGlu, ~30%; 5-CH$_3$H$_4$PteGlu, ~15% and the 5- and 10-HCO-H$_4$PteGlu, ~30% (Table 1). As expected, after exposure for 18 h to an atmosphere containing N$_2$O, 5,10-CH$_2$H$_4$PteGlu levels increased at the expense of H$_4$PteGlu; no H$_4$PteGlu was detected in the N$_2$O-treated rats. The levels of 5- and 10-HCO-H$_4$PteGlu were not changed by exposure to N$_2$O. The level of 5,10-CH$_2$H$_4$PteGlu also was not affected (P > 0.18).

DISCUSSION

The level of 5-CH$_2$H$_4$PteGlu decreased at 30 and 60 min after methionine administration, whereas the levels of 10-HCO- and H$_4$PteGlu increased. At 120 min, the levels of 5-CH$_2$H$_4$PteGlu and 10-HCO-H$_4$PteGlu returned to control values and H$_4$PteGlu was decreasing. The level of 5,10-CH$_2$-H$_4$PteGlu was unchanged by the administration of methionine to rats. Administration of methionine leads to a rapid increase in the hepatic levels of AdoMet and the levels return to control values after 2–4 h (6,7,15,16). This increased AdoMet concentration leads to decreased levels of 5-CH$_2$H$_4$PteGlu and increased levels of the other reduced folate coenzymes (6,7) because the enzyme that reduces 5,10-CH$_2$H$_4$PteGlu to 5-CH$_3$H$_4$PteGlu, MTHFR, is inhibited by AdoMet (8). Thus, although 5-CH$_2$H$_4$PteGlu is being utilized to methylate homocysteine, thereby reducing the 5-CH$_3$H$_4$PteGlu pool, its synthesis is inhibited by the high level of AdoMet. It might be suspected that the inhibition of MTHFR would result in increased levels of 5,10-CH$_2$H$_4$PteGlu. Unlike MTHFR, which is irreversible in vivo, the trifunctional enzyme, C$_1$-PteGlu synthetase, is reversible and may convert 5,10-CH$_2$H$_4$PteGlu to 5,10-methenyl-H$_4$PteGlu and then to 10-HCO-H$_4$PteGlu, which may be converted to H$_4$PteGlu and CO$_2$ via 10-HCO-H$_4$PteGlu dehydrogenase (17); however, conversion

TABLE 1

<table>
<thead>
<tr>
<th>Folate coenzyme</th>
<th>Control</th>
<th>Nitrous oxide</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>nmol/g</td>
<td>%</td>
</tr>
<tr>
<td>10-HCO-H$_4$PteGlu</td>
<td>5.37 ± 1.14</td>
<td>20.7</td>
</tr>
<tr>
<td>H$_4$PteGlu</td>
<td>6.17 ± 0.28</td>
<td>23.7</td>
</tr>
<tr>
<td>5-HCO-H$_4$PteGlu</td>
<td>2.55 ± 0.23</td>
<td>9.8</td>
</tr>
<tr>
<td>5-CH$_3$H$_4$PteGlu</td>
<td>3.86 ± 0.11</td>
<td>14.9</td>
</tr>
<tr>
<td>5,10-CH$_2$H$_4$PteGlu</td>
<td>8.04 ± 0.27</td>
<td>30.9</td>
</tr>
</tbody>
</table>

$^1$ Values are means ± SEM, n = 3 for control and n = 4 for nitrous oxide.

$^2$ Not Detected. * Different from control, P < 0.03.
of 5,10-CH$_2$-H$_4$PteGlu to 5,10-methenyl-H$_4$PteGlu requires NADPH and the ratio of NADPH/NADP$^+$ of 4 in normal liver (18) would argue against C$_5$-H$_4$PteGlu synthethase playing a major role in regulating the amount of 5,10-CH$_2$-H$_4$PteGlu. Alternatively, 5,10-CH$_2$-H$_4$PteGlu may be converted to H$_4$PteGlu via cytosolic serine hydroxymethyltransferase, which utilizes 5,10-CH$_2$-H$_4$PteGlu and glycine to synthesize serine (19). The fact that we found a large increase in the amount of H$_4$PteGlu and a rather small increase in the amount of 10-HCO-H$_4$PteGlu (Fig. 1) after administration of methionine would support the role of cytosolic serine hydroxymethyltransferase in maintaining the concentration of 5,10-CH$_2$-H$_4$PteGlu in the liver under these conditions.

Earlier studies showed that inactivation of methionine synthase or severe vitamin B$_12$ deficiency leads to an accumulation of folates as 5-CH$_3$-H$_4$PteGlu. This is called the methyl trap hypothesis and was first described by Noronha and Silverman (20) and Herbert (21). 5-CH$_3$-H$_4$PteGlu accumulates because in vitamin B$_12$ deficiency (either dietary or induced by N$_2$O), the B$_12$-dependent enzyme methionine synthase is inactive and the enzyme that catalyzes the synthesis of 5-CH$_1$-H$_4$PteGlu, MTHFR, is irreversible in vivo (10). Many studies have confirmed this hypothesis (5,6,22,23). However, no information is available concerning the effects of B$_12$ deficiency on the level of 5,10-CH$_2$-H$_4$PteGlu. Therefore, we exposed rats to N$_2$O and measured the distribution of hepatic folate coenzymes. As expected, N$_2$O exposure resulted in an increase in the amount of hepatic 5,10-CH$_2$-H$_4$PteGlu compared with air-breathing control rats. The amounts of 5- and 10-HCO-H$_4$PteGlu were unchanged. The amount of 5,10-CH$_2$-H$_4$PteGlu was not affected. Surprisingly, we detected no H$_4$PteGlu in livers from the N$_2$O-exposed rats. All previous studies, including ours, have shown that H$_4$PteGlu levels decreased in the N$_2$O group (5,6,22,23). However, all of these studies employed tissue extraction procedures performed at pH <9.5 and/or with 2-mercaptoethanol, which would lead to dissociation of 5,10-CH$_2$-H$_4$PteGlu to H$_4$PteGlu and formaldehyde (11,24). Thus, it appears that there is little H$_4$PteGlu present in livers of rats exposed to N$_2$O.

In conclusion, we investigated whether two conditions known to perturb folate coenzyme metabolism altered the concentration of 5,10-CH$_2$-H$_4$PteGlu in liver. 1) Administration of methionine might be expected to increase the concentration of 5,10-CH$_2$-H$_4$PteGlu because methionine raises the concentration of AdoMet and inhibits the utilization of 5,10-CH$_2$-H$_4$PteGlu for the biosynthesis of 5,10-CH$_2$-H$_4$PteGlu. 2) Inactivation of methionine synthase, which results in the trapping of folates as 5-CH$_3$-H$_4$PteGlu, might be expected to result in a decrease in the concentration of 5,10-CH$_2$-H$_4$PteGlu because it is being reduced to 5-CH$_1$-H$_4$PteGlu by MTHFR and the resulting 5-CH$_1$-H$_4$PteGlu is trapped. However, neither of these conditions resulted in changes in the concentration of 5,10-CH$_2$-H$_4$PteGlu. These results may be explained by the reversible activities of the C$_5$-H$_4$PteGlu synthethase to 5,10-methenyl-H$_4$PteGlu and 10-HCO-H$_4$PteGlu and/or by the activity of cytosolic serine hydroxymethyltransferase, thus maintaining a relatively constant concentration of this key folate coenzyme.

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LITERATURE CITED