Compartmentation of Folate Metabolism in Rat Pancreas: Nitrous Oxide Inactivation of Methionine Synthase Leads to Accumulation of 5-Methyltetrahydrofolate in Cytosol1,2

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ABSTRACT Folate-dependent one-carbon metabolism and methylation reactions have been implicated in the secretory function of the pancreas. Because vitamin B-12 deficiency perturbs folate metabolism, we determined the effects of nitrous oxide inactivation of methionine synthase on the compartmentation of folate metabolism in rat pancreas. Rats were exposed to an atmosphere of nitrous oxide and oxygen (80 and 20%, respectively) for 18 h; control rats breathed air. Folate coenzyme concentrations were determined by HPLC and Lactobacillus casei microbiological assay of the cytosolic and mitochondrial fractions of pancreas, which contained 62 and 46%, respectively, of the total folate. In pancreas of control rats, cytosolic folates were 5-methyltetrahydrofolate (31% of total folates), tetrahydrofolate (54%) and 5- and 10-formyltetrahydrofolate (6 and 8%, respectively). In the rats exposed to nitrous oxide, cytosolic 5-methyltetrahydrofolate concentrations were significantly greater (59% of total folates) and tetrahydrofolate concentrations were significantly lower (32%) than in controls; however, total cytosolic folate levels were unaffected by nitrous oxide exposure. In controls, mitochondrial folates were composed of 5-methyltetrahydrofolate (9% of total folates), tetrahydrofolate (60%) and 5- and 10-formyltetrahydrofolate (22 and 10%, respectively). Exposure to nitrous oxide led to significantly lower total mitochondrial folates (1.49 ± 0.18 vs. 0.75 ± 0.29 nmol/g, control vs. nitrous oxide, P < 0.05). This was due to a significantly lower concentration of tetrahydrofolate and 5-formyltetrahydrofolate, but not of 5-methyl- or 10-formyltetrahydrofolate. The activity of methionine synthase was 85% lower (P < 0.001) in pancreatic extracts of rats exposed to nitrous oxide than in controls. These results show that cytosolic folates accumulate in pancreas as the 5-methyl derivative at the expense of the other folates, as happens in liver. However, in contrast to results in liver, the mitochondrial folate concentration was lower in the pancreas of rats exposed to nitrous oxide, and this decline was limited to the 5-formyl- and tetrahydrofolate derivatives. J. Nutr. 127: 1772–1775, 1997

KEY WORDS: • folic acid • nitrous oxide • vitamin B-12 • rats • pancreas mitochondria

Recent findings have implicated one-carbon compounds and methylation in the secretory function of the pancreas. Gilliland and Steer (1980) showed that consumption of a diet deficient in choline and containing 0.5% ethionine led to a failure to discharge the zymogen granules but had no effect on enzyme levels in the granules. Balaghi et al. (1993b) and Balaghi and Wagner (1995) showed that folate deficiency perturbs pancreatic one-carbon metabolism and results in an inhibition of secretion of amylase. They also showed that the methylation ratio (the ratio of S-adenosylmethionine to S-adenosylhomocysteine) was lower in the folate-deficient group [this ratio is thought to regulate many physiologically important methylation reactions (Cantoni et al. 1978)]. These considerations suggest that factors which affect folate-dependent one-carbon metabolism may perturb pancreatic enzyme secretion. Because vitamin B-12 deficiency (either dietary or induced by the anesthetic gas nitrous oxide) perturbs folate metabolism, we decided to determine the effects of nitrous oxide on pancreatic folate coenzyme distribution.

 Exposure to nitrous oxide results in the symptoms of vitamin B-12 deficiency, megaloblastic anemia and neuropathy, in humans (Chanarin 1980, monkeys (Scott et al. 1981) and pigs (Weir et al. 1988). This is due to the inactivation of methionine synthase (5-methyltetrahydrofolate:homocysteine methyltransferase, EC 2.1.1.13) by oxidizing the enzyme-B12 (Co) complex formed during catalysis (Chanarin 1980, Drummond and Matthews 1994). Several investigators have used nitrous oxide as a tool for exploring the folate-vitamin B-12 interrelationships (see Shane and Stokstad 1983 and 1985 for reviews). These studies have shown that nitrous oxide exposure results in an accumulation of folates as the 5-methyltetrahydrofolate derivative at the expense of other folates. This has been called the methyl trap hypothesis (Herbert and Zalusky 1962, Noronha and Silverman 1962), which states that in vitamin B-12 deficiency folates are trapped as 5-methyltetrahydrofolate. This is because the synthesis of 5-methyltetrahy-
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Drofolate is not reversible in vivo (Green et al. 1988) and methionine synthase is inactive due to vitamin B-12 deficiency (either dietary or via inactivation by nitrous oxide). This trapping of folates results in decreased amounts of 10-formyl- and 5,10-methylene tetrahydrofolate (which are necessary for purine and thymidine synthesis, respectively), which results in megaloblastosis.

The major pools of folate in the liver are in the cytosol and mitochondria (Cook and Blair 1979, Corrocher and Hoffbrand 1972, Shin et al. 1976). We found (Horne et al. 1989), in rats exposed to nitrous oxide, that hepatic 5-methyltetrahydrofolate was about twice the concentration (from 45 to 84% of total cytosolic folates, control and nitrous oxide, respectively) and this change was at the expense of 5-formyl-, 10-formyl- and tetrahydrofolate. The folate distribution in the mitochondria from these rats was not affected by nitrous oxide.

In the present study, we examined the compartmentation of and the effect of nitrosative damage on folate metabolites in rat pancreas.

MATERIALS AND METHODS

Materials. Sodium l-ascorbate, HEPES and 2-(N-cyclohexylamino)ethanesulfonic acid (CHES), p-phenylmercuric sulfonamide, and fatty acid-free bovine serum albumin were purchased from Sigma Chemical (St. Louis, MO). 2-Mercaptoethanol was from Eastman Kodak (Rochester, NY); 6-(RS)-5-formyl-H,PreGlut was from Fluka Chemical (Ronkonkoma, NY).

Animals. Male Sprague-Dawley rats (Harlan Sprague Dawley, Indianapolis, IN) weighing ~250 g were used in the studies. Both experimental and control groups had free access to a commercial rodent diet (Teklad F6 Redent Diet; protein 24%, fat 6% and fiber 4.5%; Harlan Teklad, Madison, WI) and tap water. Rats were exposed to nitrous oxide in a chamber through which flowed nitrous oxide (87 and 13 mg/kg body wt, respectively), and the pancreas was excised, trimmed of fat, and homogenized in three volumes (based on original weight of the pancreas) of medium and again centrifuged at 28,000 concentrations of 10-formyl- and 5-methyltetrahydrofolate. These results demonstrate that essentially all pancreatic folate resides in the cytosol and mitochondria.

Effect of nitrosative damage on subcellular distribution of folate derivatives. In the cytosol of control rats, tetrahydrofolate was the major folate derivative (54%), followed by 5-methyltetrahydrofolate (31%), 10-formyltetrahydrofolate (8%) and 5-formyltetrahydrofolate (6%) (Table 1). This distribution was significantly different in rats exposed to nitrous oxide. The concentration of 5-methyltetrahydrofolate was twice as great (59%), and the concentration of tetrahydrofolate was almost 50% lower (32%). The concentrations of 5-formyl- and 10-formyltetrahydrofolate did not differ significantly. The total folate concentration in cytosol (obtained by summing the individual derivatives) did not differ between control and nitrous oxide-exposed rats.

In mitochondria of control rats, tetrahydrofolate was the major folate derivative (54%), followed by 5-methyltetrahydrofolate (31%), 10-formyltetrahydrofolate (8%) and 5-formyltetrahydrofolate (6%) (Table 1). Unlike results in the cytosol, nitrous oxide treatment led to a 50% lower concentration of folates in mitochondria. The concentration of tetrahydrofolate was 62% lower and 5-formyltetrahydrofolate was 45% lower in nitrous oxide–exposed rats. The concentrations of 10-formyl- and 5-methyltetrahydrofolate were not significantly different between groups.

DISCUSSION

We have previously shown (Horne et al. 1989) that nitrosative damage inactivates of methionine synthase in liver results in
a marked redistribution of folate coenzymes in the cytosolic compartment. After 18 h of exposure to nitrous oxide, 5-methyltetrahydrofolate accounted for 84% of cytosolic folates. This accumulation of 5-methyltetrahydrofolate was at the expense of the other folate coenzymes (5- and 10-formyl- and tetrahydrofolate). However, nitrous oxide exposure had no effect on the mitochondrial folate pool. This is in accord with the methyl trap hypothesis (Herbert and Zalusky 1962, Noro- 

Horne et al. 1989). The observation that nitrous oxide exposure resulted in accumulation of 5-methyltetrahydrofolate in the cytosolic compartment, in trapping of 5-methyltetrahydrofolate as the 5-methyl derivative in the cytosol of pancreas; in nitrous oxide–exposed rats, this value was 59% (Table 1). The greater concentration of 5-methyltetrahydrofolate was exclusively at the expense of tetrahydrofolate, which was 54% of total folate in controls and 32% in nitrous oxide–exposed rats, respectively. Neither 5- nor 10-formyl-tetrahydrofolate concentrations differed significantly. In contrast, in liver cytosol all reduced folates other than 5-methyltetrahydrofolate declined during nitrous oxide exposure (Horne et al. 1989). The observation that nitrous oxide exposure resulted in accumulation of 5-methyltetrahydrofolate to only 59% of cytosolic folates in pancreas, whereas in liver 5-methyltetrahydrofolate accumulated to 84% of cyto- 
solic folates, could be due to more pronounced inhibition of methionine synthase in some cell types than in others in pancreas. However, our results showed that methionine synthase activity was about 86% lower in the pancreas of rats breathing nitrous oxide than in controls, and this is similar to the 84% inhibition seen in liver (Horne et al. 1989). In any case, our studies show that folates are trapped as the 5-methyl derivative in the cytosol of pancreas, as in the liver, when methionine synthase is inhibited.

The effect of methionine synthase inactivation on the spectrum of folate coenzymes in mitochondria from pancreas is quite different from that seen in liver. In liver, there was no change in the mitochondrial folate coenzyme distribution or in the total amount of folate in the nitrous oxide–exposed group (Horne et al. 1989). However, in the pancreas, the total mitochondrial folate concentration was about 50% lower in the nitrous oxide–exposed group compared with air-breathing controls. The reason for this difference is unknown; however, the data suggest that polyglutamate turnover may be greater in mitochondria than in the cytosol of pancreas. Folate poly-
glutamates do not readily cross the mitochondrial membrane in liver (Horne et al. 1989, Lin et al. 1993); however, our data suggest that folate polyglutamates may cross the mitochondrial membrane to some extent in pancreas, because the concentra-
tion of folate was lower and the distribution of the coenzymes was altered in nitrous oxide–exposed rats. Further experi-
ments, using radiolabeled folate, would be necessary to deter-
mine whether there is an increased rate of polyglutamate turn-
over in pancreatic mitochondria. In any event, total mitochon-
drial folate was lower and this was due to lower concentrations of 5-formyl- and tetrahydrofolate. The concentrations of 10-
formyl- and 5-methyltetrahydrofolate did not differ in pancre-
atic mitochondria of the nitrous oxide group and controls.

As stated in the introduction, there seems to be a connec-
tion between perturbed pancreatic secretion and folate-depen-
dent one-carbon metabolism, which results in a lower methyla-
tion ratio (the ratio of S-adenosylmethionine to S-adenosylho-
mocysteine) in rats fed folate-deficient diets (Balaghi and Wagner 1995, Balaghi et al. 1993b). We reasoned that other factors that alter folate-dependent one-carbon metabolism, e.g., vitamin B-12 deficiency might perturb pancreatic secretion. We used nitrous oxide exposure to inactivate the vitamin B-12–dependent enzyme methionine synthase. This causes trapping of folate coenzymes as the 5-methyl derivative at the expense of other reduced folates. Because methionine synthase is inactivated, the concentration of methionine, and conse-

TABLE 1
Nitrous oxide inactivation of methionine synthase affects the concentration of folate coenzymes in cytosol and mitochondria in rat pancreas

<table>
<thead>
<tr>
<th>Coenzyme</th>
<th>Cytosol</th>
<th>Mitochondria</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
<td>Nitrous oxide</td>
</tr>
<tr>
<td></td>
<td>28,000 × g pellet</td>
<td></td>
</tr>
<tr>
<td>10-Formyl-H₄PteGlu</td>
<td>0.28 ± 0.07</td>
<td>0.22 ± 0.14</td>
</tr>
<tr>
<td>H₄PteGlu</td>
<td>1.70 ± 0.16</td>
<td>0.92 ± 0.25*</td>
</tr>
<tr>
<td>5-Formyl-H₄PteGlu</td>
<td>0.19 ± 0.08</td>
<td>0.07 ± 0.04</td>
</tr>
<tr>
<td>5-Methyl-H₄PteGlu</td>
<td>0.96 ± 0.11</td>
<td>1.78 ± 0.28*</td>
</tr>
<tr>
<td>Total</td>
<td>3.12 ± 0.14</td>
<td>2.99 ± 0.29</td>
</tr>
</tbody>
</table>

1 Values are means ± SEM, n = 4 separate rats. H₄PteGlu is 5,6,7,8-tetrahydrofolic acid. *P < 0.05 compared with the respective control value.
methyltetrahydrofolate derivative in the cytosol of rat pancreas as it does in liver. However, unlike effects in liver, folate coenzyme concentration was lower in pancreatic mitochondria in rats exposed to nitrous oxide, and the distribution of mitochondrial folate coenzymes was also altered.

**LITERATURE CITED**


