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Effects of Nitrous Oxide and Methotrexate Administration on Hepatic Methionine Synthetase and Dihydrofolate Reductase Activities, Hepatic Folates, and Formate Oxidation in Rats

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SUMMARY

Rats are generally not susceptible to methanol poisoning, but the administration of nitrous oxide (N_2O) to methanol-treated rats leads to the development of formic acidemia and metabolic acidosis, a clinical state observed only in methanol-poisoned monkeys and humans. Formate, the metabolite associated with the toxicity of methanol, is oxidized to carbon dioxide by a tetrahydrofolate (H_4folate)-dependent pathway. The role of two hepatic H_4folate-generating enzymes, 5-methyltetrahydrofolate:homocysteine methyltransferase (methionine synthetase, EC 2.1.1.13) and dihydrofolate (H_2folate) reductase (EC 1.5.1.3), in the regulation of hepatic folates and the rate of formate oxidation *in vivo* in rats was studied after the administration of N_2O, an inhibitor of methionine synthetase, and methotrexate (MTX), an inhibitor of H_2folate reductase. Exposure of rats to N_2O produced an inhibition of hepatic methionine synthetase activity which was dependent upon the duration of exposure. N_2O exposure also produced duration-dependent decreases in hepatic H_4folate and the rate of formate oxidation *in vivo* and an increase in hepatic 5-methyltetrahydrofolate (5-CH_3-H_4folate). The effects of N_2O on hepatic folates and the rate of formate oxidation followed time courses similar to that observed for the inhibition of methionine synthetase activity. Maximal effects had occurred by 4 hr of N_2O exposure, at which time hepatic methionine synthetase activity was inhibited by 90%, hepatic H_4folate was reduced by 50%, and rate of formate oxidation was decreased by 50%. When rats were exposed to N_2O and then allowed to recover in room air, the activity of hepatic methionine synthetase activity gradually increased and reached the control level following 48 hr of recovery. Hepatic H_4folate, 5-CH_3-H_4folate, and the rate of formate oxidation *in vivo* also gradually returned to control values during the recovery period, following time courses similar to the recovery of hepatic methionine synthetase activity. Administration of MTX produced a greater than 95% inhibition of hepatic H_2folate reductase activity, but had no effect on hepatic folates or the rate of formate oxidation in either air-breathing or N_2O-exposed rats. These results indicate that hepatic methionine synthetase activity is a key regulator of hepatic H_4folate and H_4folate-dependent processes, such as the oxidation of formate to CO_2, whereas hepatic H_2folate reductase plays no apparent role in the regulation of these factors. The generation of H_4folate by methionine synthetase would appear to be a major factor in determining a species' sensitivity to methanol.

INTRODUCTION

Formate accumulates in the blood of methanol-poisoned humans and monkeys, and appears to be associated with the development of the major signs and symptoms of methanol poisoning, such as metabolic acidosis and blindness (1–6). Administration of methanol to rats produces only central nervous system depression and no accumulation of formate in the blood (2). The species difference is explained by a difference in the rate at which various species oxidize formate to CO_2. Over a wide range of dose levels, the rate at which rats oxidize formate to CO_2 is at least twice the rate observed in monkeys (5). Therefore, investigations into the regulation of formate oxidation by various species should provide a better understanding of methanol poisoning.

In both rats and monkeys, formate is metabolized to CO_2 by a folate-dependent pathway (5, 7) (Fig. 1). For-

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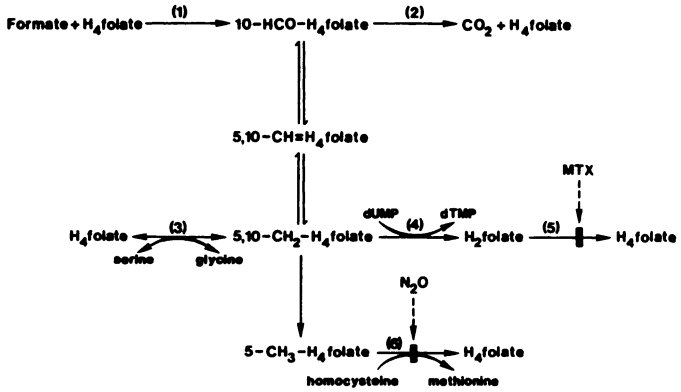

FIG. 1. *Pathways of tetrahydrofolate generation and folate-dependent formate metabolism*

The reactions discussed in the text are catalyzed by (*1*) 10-HCO-H_4folate synthetase, (*2*) 10-HCO-H_4folate dehydrogenase, (*3*) serine transhydroxymethylase, (*4*) thymidylate synthetase, (*5*) H_2folate reductase, and (*6*) methionine synthetase. Only folate-dependent reactions directly pertinent to the present study are shown.

mate combines with H_4folate[2] in a reaction catalyzed by 10-HCO-H_4folate synthetase (EC 6.3.4.3) (8) (Fig. 1, Reaction 1). The product of the reaction is 10-HCO-H_4folate, which, in turn, is converted to H_4folate and CO_2 through the action of 10-HCO-H_4folate dehydrogenase (EC 1.5.1.6) (9) (Fig. 1, Reaction 2). Although catalase (EC 1.11.1.6) can mediate the oxidation of formate *in vitro* (10), this enzyme plays no apparent role in the oxidation of formate *in vivo* in monkeys or control rats (5, 7). A role for catalase as a mediator of formate oxidation is uncovered in folate-deficient or N_2O-exposed rats (7, 11).

Enzymes which catalyze the formation of H_4folate may possibly regulate formate oxidation. One such enzyme is H_2folate reductase (EC 1.5.1.3) (Fig. 1, Reaction 5), which catalyzes the reduction of H_2folate to H_4folate and is irreversibly inhibited by the cancer chemotherapeutic agent, MTX (8). 5-Methyl-tetrahydrofolate:homocysteine methyltransferase (methionine synthetase, EC 2.1.1.13) (Fig. 1, Reaction 6) is another enzyme which is believed to play a key role in the regulation of tissue levels of H_4folate (12–14). This vitamin B_{12}-dependent enzyme catalyzes the transfer of a methyl group from 5-CH_3-H_4folate to homocysteine, resulting in the formation of methionine and H_4folate (15). Exposure of rats to the anesthetic gas, N_2O, produces an inhibition of hepatic methionine synthetase activity which appears to be irreversible (16). N_2O reacts *in vitro* with vitamin B_{12} to cause an oxidation of the cobalt moiety (17), and this is presumed to be the mechanism by which N_2O inhibits methionine synthetase *in vivo*.

Eells *et al.* (11) have shown that exposure of rats to N_2O induces susceptibility to methanol toxicity. Rats which were exposed to 4 hr of N_2O/O_2 (1:1) and administered methanol accumulated formate in the blood and developed metabolic acidosis. The rates of formate oxidation to CO_2 and formate disappearance from the blood were one-half of those observed in control animals. N_2O exposure produced an accumulation of methyl-folates at the expense of nonmethyl forms. These results are consistent with an action of N_2O on hepatic methionine synthetase.

Previous studies on the effects of N_2O exposure on rat liver folates have used time-consuming column chromatographic methods (18) or indirect techniques based upon the ability of various folates to differentially support the growth of several species of microorganisms (11, 19, 20). A significant advancement in the measurement of tissue folates was recently developed by McMartin *et al.* (21). This HPLC method allows the separation and direct determination of monoglutamate forms of the various folate derivatives and was used in the present investigation to explore directly the effects of N_2O exposure and MTX administration on hepatic folates.

The goal of this study was to investigate the possible regulatory roles of hepatic methionine synthetase and H_2folate reductase activities on the levels of various hepatic folates and the rate of formate oxidation *in vivo* in rats. The results indicate that hepatic methionine synthetase activity plays a major role in the regulation of the level of hepatic H_4folate and formate oxidation in rats, whereas hepatic H_2folate reductase activity does not. Thus, factors which affect the levels of hepatic methionine synthetase activity or H_4folate could affect a species' susceptibility to the toxic effects of methanol.

EXPERIMENTAL PROCEDURES

Materials. [5-^{14}C]CH_3-H_4 folic acid, barium salt (>45 mCi/mmole) was obtained from Amersham Corporation (Arlington Heights, Ill.). [^{14}C]Formic acid, sodium salt (40–60 mCi/mmole) was purchased from New England Nuclear Corporation (Boston, Mass.). Preblend 3a70B liquid scintillation cocktail was obtained from Research Products International (Elk Grove, Ill.). 5-CH_3-H_4folic acid, barium salt; folic acid, 5-HCO-H_4folate, calcium salt; H_4folic acid, and AT were purchased from Sigma Chemical Company (St. Louis, Mo.). MTX was obtained from Lederle Laboratories (Pearl River, N. Y.). Folate derivatives were prepared and purified as previously described (21). *Lactobacillus casei* (7469) was obtained from the American Type Culture Collection. Folic acid casei media (0822) and Lactobacilli broth AOAC (0901) were obtained from Difco Laboratories, Inc. (Detroit, Mich.). All other reagents employed in this study were of the highest available purity.

Animals. Male Sprague-Dawley rats (200–325 g) were obtained from Bio-Labs (Madison, Wisc.) and were housed in wire-bottomed cages. Temperature, humidity, and lighting were controlled. Purina laboratory chow and water were provided ad libitum.

Enzyme assays. A minor modification of the radiochemical method of Billings *et al.* (14) was used to determine methionine synthetase activity present in rat liver cytosol. The changes employed were the omission of ammonium sulfate fractionation, use of 0.1 M Tris-HCl (pH 7.4) as the buffer, adjusting the concentration of dithiothreitol and *S*-adenosylmethionine to 10 mM and 0.5 mM, respectively, and increasing the duration of incubation to 30 min. H_2folate reductase activity present in rat liver cytosol was determined by the use of the spectrophotometric method of Hillcoat and Blakley (22). The amount of H_2folate reduced was quantitated using a millimolar extinction coefficient of 12.3 (23). Protein was determined by the method of Lowry *et al.* (24), using bovine serum albumin as the standard.

Measurement of hepatic folates. The reversed-phase, paired-ion HPLC method of McMartin *et al.* (21) was used to measure directly

[2] The abbreviations used are: H_2folate, dihydrofolate; H_4folate, tetrahydrofolate; 5-HCO-H_4folate, 5-formyl-tetrahydrofolate; 10-HCO-H_4 folate, 10-formyl-tetrahydrofolate; 5-CH_3-H_4folate, 5-methyltetrahydrofolate; 5,10-CH_2-H_4folate, 5,10-methylenetetrahydrofolate; 5,10-CH=H_4folate, 5,10-methenyltetrahydrofolate; N_2O, nitrous oxide; MTX, methotrexate; AT, 3-amino-1,2,4-triazole; HPLC, high-pressure liquid chromatography.

the levels of the various folate derivatives found in rat liver. Liver extracts were treated with a partially purified hog kidney polyglutamate hydrolase (EC 3.4.22.12) preparation to convert folate polyglutamates to the monoglutamate derivatives (21). Two minor modifications of the original method were employed: (a) the amount of each folate present was quantitated by the ability of fractions collected during HPLC to support the growth of *L. casei* alone since the particular μBondapak C_{18} column (Waters Associates, Milford, Mass.) employed completely separated 5,10-CH=H_4folate, 10-HCO-H_4folate, H_4folate, 5-HCO-H_4folate, H_2folate, 5,10-CH_2-H_4folate, 5-CH_3-H_4folate, and folic acid; and (b) H_4folate was used as the standard when quantitating H_4folate. 5-HCO-H_4folate was used as the standard when all other folate derivatives were measured. All folate monoglutamates except H_4folate supported the growth of *L. casei* equally well. The precautions taken to prevent the oxidative degradation of reduced folate derivatives have been previously reported (21). Using these techniques, no folic acid, H_2folate, 5,10-CH_2-H_4folate, or 5,10-CH=H_4folate were detected in rat liver. The sum of 10-HCO-H_4folate and 5-HCO-H_4folate amounted to 5% or less of the total hepatic folate, and the percentage was not changed by any of the treatments employed; thus, data regarding these derivatives are not presented. MTX did not coelute with any endogenous folate derivatives and thus did not interfere with the measurement of hepatic folates in MTX-treated animals.

Formate metabolism studies. Sodium [^{14}C]formate (40,000 dpm/mg) was administered i.p. at a dose of 500 mg/kg. This dose of formate produces a maximal rate of formate oxidation in the rat (7). Rats were placed in glass metabolic chambers, and $^{14}CO_2$ present in expired air was trapped in 2 N NaOH and analyzed as previously described (2). In some studies, the catalase inhibitor, AT, was administered (1 g/kg, i.p.) 2 hr before formate. This dose of AT reduces hepatic catalase activity to less than 10% of the control value (25). In experiments where MTX was used, it was administered i.p. as a 0.05 mg/ml solution in 0.9% saline. Control animals received an equivalent volume of 0.9% saline.

Nitrous oxide exposure. Two to four rats were placed in a 35-liter metabolic chamber (Plas Labs, Inc., Lansing, Mich.) and were exposed to N_2O/O_2 (1:1) for various durations. The total flow rate of gas was 8 liters/min. In control studies, rats were exposed to room air or nitrogen/O_2 (1:1). In formate metabolism experiments, animals were exposed to the various gases before the injection of [^{14}C]formate and breathed room air during $^{14}CO_2$ collection.

Statistical analysis. Data were evaluated by one-way analysis of variance followed by application of the Student-Newman-Keuls multiple-comparison procedure, or by two-way analysis of variance. If only two treatment groups were present in an experiment, the data were analyzed by the Student's *t*-test for unpaired data. Rates of formate oxidation were determined by linear regression analysis using the least-squares fit method. The curves representing time courses were fit with the assistance of a computer operating in an iterative mode using the nonlinear regression program, NLIN, of the Statistical Analysis System (26). Probability values < 0.05 were considered statistically significant.

RESULTS

Effect of N_2O and 3-amino-1,2,4-triazole on formate oxidation in vivo. An experiment was carried out to determine the route of formate oxidation in N_2O-treated rats. Animals were exposed to N_2O/O_2 (1:1) for 4 hr immediately prior to the injection of [^{14}C]formate (500 mg/kg). N_2O exposure produced a significant ($p < 0.01$) decrease in the rate of formate oxidation (Fig. 2). This duration of exposure to N_2O produces a maximal inhibition of formate oxidation without imposing any demonstrable behavioral changes in the animals (11). The administration of AT, a catalase inhibitor, caused a further reduction ($p < 0.05$) in the rate of formate oxidation in N_2O-treated rats. The rate of formate oxidation in animals receiving both N_2O and AT was approximately 50% of the rate measured in air-breathing control rats. AT had no effect on the rate of formate oxidation in air-breathing animals. Also, the administration of AT (1 g/kg) had no effect on hepatic methionine synthetase activity, total hepatic folate, or the level of any of the individual folate derivatives (data not shown). Thus, the catalase peroxidative pathway played no apparent role in the oxidation of formate in air-breathing rats, but did function to a slight degree in the oxidation of formate in N_2O-exposed rats. Therefore, in all subsequent formate metabolism experiments reported here, rats were pretreated with AT so that only H_4folate-dependent formate oxidation was measured.

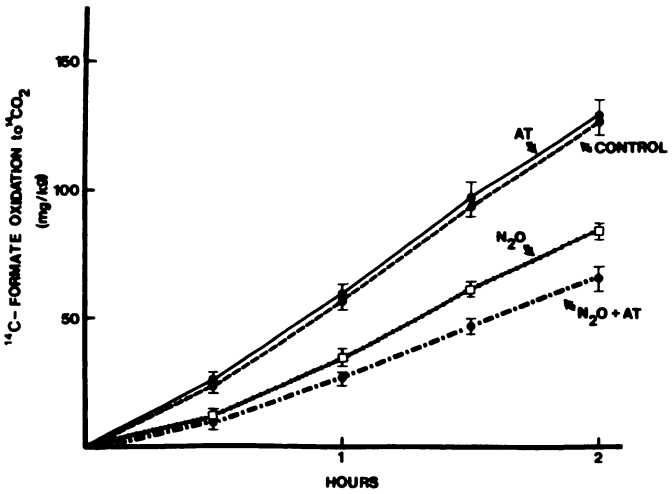

FIG. 2. *Effects of N_2O and aminotriazole on [^{14}C]formate oxidation in vivo*

[^{14}C]formate (500 mg/kg, i.p.) was administered as a 20% (w/v) solution at time zero. Rats were exposed to N_2O/O_2 (1:1) or room air for 4 hr prior to formate, and breathed room air during $^{14}CO_2$ collection. Some animals received AT (1 g/kg, i.p.) 2 hr prior to formate. Each value is the mean ± standard error obtained from at least four rats.

Effect of nitrogen/O_2 (1:1) on hepatic methionine synthetase activity and formate oxidation in vivo. In a control experiment, rats were exposed to nitrogen/O_2 (1:1) for 4 hr immediately preceding the determination of hepatic methionine synthetase activity or the rate of formate oxidation *in vivo*. Exposure of rats to nitrogen/O_2 (1:1) for 4 hr did not significantly affect hepatic methionine synthetase activity ($p > 0.20$), although a slight, but significant, decrease in the rate of formate oxidation was observed (Table 1). The effect on formate oxidation was much less than that observed in AT-treated rats exposed to N_2O/O_2 (Figs. 2 and 3).

Effect of duration of N_2O exposure on hepatic methionine synthetase activity, hepatic folates, and the rate of formate oxidation. Rats were exposed to N_2O/O_2 (1:1) for various durations, ranging from 0.25 hr to 4 hr. At the end of the exposure period, hepatic methionine synthetase activity, hepatic folates, or the rate of formate oxidation were determined.

N_2O exposure produced an inhibition of hepatic methionine synthetase activity which was dependent upon the duration of exposure (Fig. 3A). The inhibition became significant following 0.5 hr of exposure and reached a maximum after 2 hr of exposure, at which time the methionine synthetase activity had declined to approximately 10% of the control value, a level which approached the limit of sensitivity of the assay.

TABLE 1

Effects of nitrogen/O_2 on hepatic methionine synthetase activity and formate oxidation in vivo

Rats were exposed to nitrogen/O_2 (1:1) for 4 hr prior to the administration of [^{14}C]formate (500 mg/kg, i.p.) or the determination of hepatic methionine synthetase activity. In formate metabolism experiments, AT (1 g/kg, i.p.) was administered 2 hr prior to formate. Each value represents the mean ± standard error obtained from four rats.

Treatment	Formate oxidation to CO_2	Methionine synthetase activity
	mg formate oxidized/kg/hr	*pmoles/min/mg protein*
Control (room air)	66.8 ± 1.7	82.0 ± 13.6
Nitrogen/O_2 (4 hr)	56.4 ± 0.7[a]	60.0 ± 8.4[b]

[a] Significantly different from control ($p < 0.01$).

[b] Not significantly different from control ($p > 0.20$).

N_2O exposure also produced a duration-dependent alteration in the distribution of hepatic folates (Fig. 3B). 5-CH_3-H_4folate accounted for approximately 40% of the total hepatic folate in air-breathing animals and increased in a duration-dependent manner following N_2O exposure. This effect became statistically significant following 2 hr of N_2O exposure, and after 4 hr 5-CH_3-H_4folate accounted for approximately 70% of the total hepatic folate. The increase in hepatic 5-CH_3-H_4folate following N_2O exposure was reflected by a decrease in hepatic H_4folate. H_4folate accounted for approximately 55% of the total hepatic folate in air-breathing animals and decreased in a duration-dependent manner following N_2O exposure. The decline in hepatic H_4folate became statistically significant following 0.5 hr of exposure; after 4 hr, H_4folate accounted for approximately 25% of the total hepatic folate. N_2O exposure did not affect the total amount of hepatic folate, which was 18.7 ± 1.2 nmoles/g of liver (mean ± standard error; $n = 24$).

Figure 3A also shows that N_2O exposure produced a duration-dependent decrease in the rate of formate oxidation which followed a time course similar to those observed for the effects on methionine synthetase activity and hepatic folates. The effect on the rate of formate oxidation became significant following 0.25 hr of N_2O exposure and reached a maximum after 1 hr, at which time the rate had declined to approximately 50% of the control value.

Effect of duration of recovery in room air on hepatic methionine synthetase activity, hepatic folates, and the rate of formate oxidation in N_2O-exposed rats. Rats were exposed to N_2O/O_2 (1:1) for 4 hr and used either immediately or after a recovery period in room air, the duration of which ranged from 2 to 96 hr. Hepatic methionine synthetase activity gradually increased after rats were removed from N_2O and placed in room air (Fig. 4A). The hepatic methionine synthetase activity became significantly elevated following 12 hr of recovery and reached the control level after 48 hr.

The distribution of hepatic folates also displayed a gradual change during the recovery period (Fig. 4B). 5-CH_3-H_4folate accounted for approximately 75% of the total hepatic folate in rats sacrificed immediately after N_2O exposure. During the recovery period, the level of 5-CH_3-H_4folate gradually decreased. Following 48 hr of recovery, the level of 5-CH_3-H_4folate was significantly

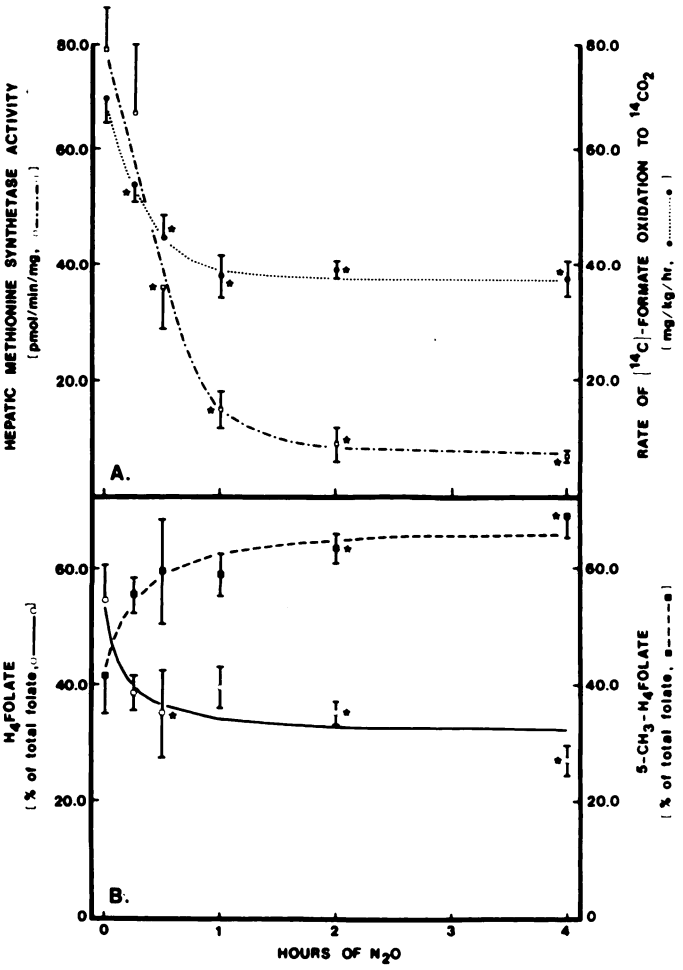

FIG. 3. *Effects of duration of N_2O exposure on hepatic methionine synthetase activity, hepatic folates, and the rate of formate oxidation in vivo*

Rats were exposed to N_2O/O_2 (1:1) for various durations as indicated along the abscissa. Each value represents the mean ± standard error obtained from at least four rats. An *asterisk* indicates that the value is significantly different ($p < 0.05$) from that observed in air-breathing rats (zero-time values).

A. Immediately following the exposure period, rats were administered [^{14}C]formate (500 mg/kg, i.p.) or were killed and used for the determination of hepatic methionine synthetase activity. In the formate metabolism experiments, rats received AT (1 g/kg, i.p.) 2 hr prior to formate and breathed room air during $^{14}CO_2$ collection.

B. Immediately following the exposure period, rats were killed and used for the determination of hepatic folates. Values are expressed as the percentage of the total hepatic folate represented by each folate derivative. N_2O exposure had no effect on total hepatic folate.

less than that observed in animals not allowed to recover and was not different from that observed in control animals. The hepatic H_4folate level changed in a manner reciprocal to that observed for 5-CH_3-H_4folate. In rats killed immediately after N_2O exposure, H_4folate represented approximately 25% of hepatic folate. The level of H_4folate gradually increased following the removal of rats from N_2O and after 12 hr became significantly elevated over the level found in rats sacrificed immediately after N_2O exposure. The level of hepatic H_4folate in animals allowed to recover from N_2O exposure for 48 hr was not significantly different from that observed in control animals. The duration of recovery from N_2O

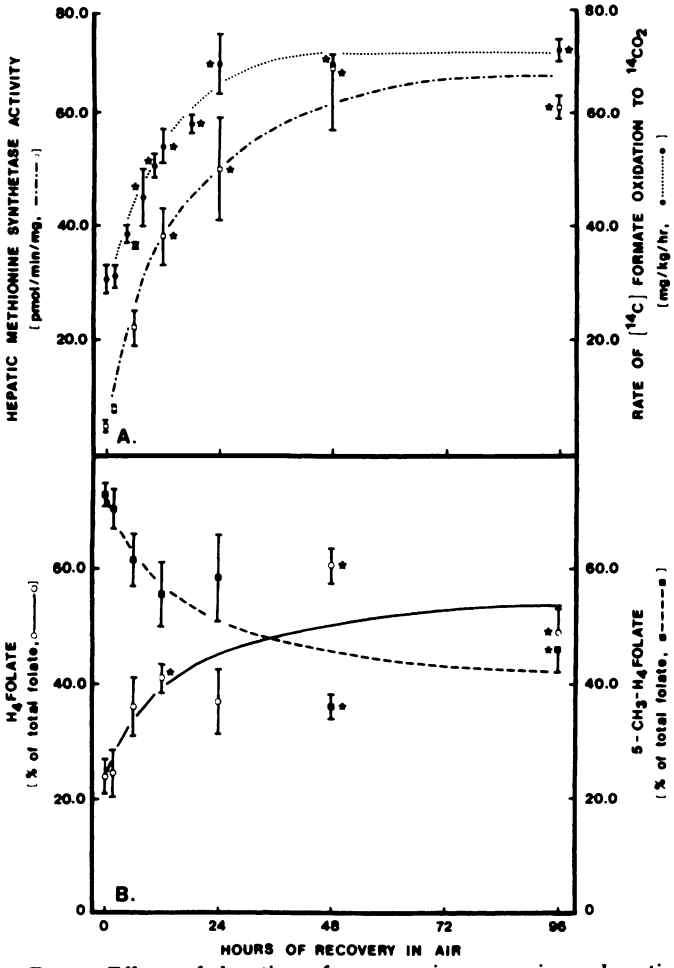

FIG. 4. *Effects of duration of recovery in room air on hepatic methionine synthetase activity, hepatic folates, and the rate of formate oxidation in vivo in N_2O-exposed rats*

Rats were exposed to N_2O/O_2 (1:1) for 4 hr and then removed to a room air atmosphere for various durations as indicated along the abscissa. Each value represents the mean ± standard error obtained from at least three rats. An *asterisk* indicates that the value is significantly different ($p < 0.05$) from that observed in rats not allowed to recover from N_2O (zero-time values).

A. Immediately following the recovery period, rats were administered [^{14}C]formate (500 mg/kg, i.p.) or were killed and used for the determination of hepatic methionine synthetase activity. In formate metabolism experiments, AT (1 g/kg, i.p.) was administered 2 hr prior to formate.

B. Immediately following the recovery period, rats were killed and used for the determination of hepatic folates. Data are expressed as described in the legend to Fig. 3.

exposure had no effect on the total hepatic folate, which was 25.5 ± 1.0 nmoles/g of liver (mean ± standard error, $n = 26$).

The rate of formate oxidation also gradually increased during the recovery period and followed a time course similar to those observed for changes in hepatic methionine synthetase activity and hepatic folates (Fig. 4A). The rate of formate oxidation in rats allowed to recover from N_2O for 10 hr was significantly elevated above the rate in rats not allowed to recover. Following 24 hr of recovery, the rate of formate oxidation reached the rate observed in control animals.

Effect of MTX on hepatic H_2folate reductase activity, hepatic folates, and the rate of formate oxidation. MTX was used to determine the role of hepatic H_2folate reductase in the regulation of hepatic folates and the rate of formate oxidation. Although Makar and Tephly (27) have shown that MTX administration is relatively ineffective in inhibiting the oxidation of formate in air-breathing rats [a result which is consistent with the low rate of H_2folate production by the thymidylate synthetase reaction in liver (8)], it was thought that H_2folate reductase might become important in the supply of hepatic H_4folate in N_2O-exposed rats in response to the inhibition of methionine synthetase activity. The administration of MTX (0.1 mg/kg, i.p.) reduced hepatic H_2folate reductase activity to less than 5% of the control value, which was 18.2 ± 2.3 nmoles/min/mg (mean ± standard error, $n = 4$), for at least 4 hr but did not affect the rate of formate oxidation when administered 1 hr prior to formate in either air-breathing or N_2O-exposed rats. The rates of formate oxidation were 64.1 ± 3.7 (mean ± standard error, $n = 4$), 62.7 ± 8.2, 28.5 ± 3.4, and 28.5 ± 2.3 in rats receiving air + saline, air + MTX, N_2O + saline, and N_2O + MTX, respectively. Likewise, MTX did not affect the level of any of the hepatic folate derivatives or the total hepatic folate in either air-breathing or N_2O-exposed rats (data not shown).

DISCUSSION

This study was undertaken to determine the roles of hepatic methionine synthetase and H_2folate reductase activities in the regulation of hepatic folates and formate oxidation *in vivo* in rats. The results clearly indicate that hepatic methionine synthetase activity plays a major role in the generation of hepatic H_4folate and the regulation of H_4folate-dependent processes, such as the oxidation of formate to CO_2. This was shown by the similarity between the time courses of the changes in hepatic methionine synthetase activity, hepatic folates, and the oxidation of formate *in vivo* during exposure to, and recovery from, N_2O. The results also indicate that hepatic H_2folate reductase activity apparently does not play a role in the regulation of hepatic H_4folate and formate oxidation *in vivo*, since nearly complete inhibition of hepatic H_2folate reductase activity by MTX produced no effect on the distribution of hepatic folates or the rate of formate oxidation *in vivo*.

Factors which affect the activity of hepatic methionine synthetase or the level of hepatic H_4folate would be expected to affect a species' sensitivity to methanol. This would be due to a change in the rate of elimination of formate, the metabolite associated with toxic effects of methanol (1–3, 5, 6). Indeed, N_2O exposure (11) or induction of folate deficiency (28) leads to formic acidemia and metabolic acidosis following methanol administration to rats, a species which normally does not exhibit the toxic effects of methanol. Folates have also been shown to play a role in determining the monkey's sensitivity to methanol. The toxicity of methanol is potentiated in folate-deficient (5) or N_2O-exposed (29) monkeys, whereas the administration of 5-HCO-H_4folate or sodium folate both prevents and reverses the development of methanol poisoning in monkeys (30). As it appears that the monkey is a good model of human methanol poisoning, these results

are probably relevant to methanol poisoning in humans. The nutritional status of patients should be considered in cases of human methanol poisoning, and the administration of N_2O to such patients would be expected to potentiate the toxicity of methanol since N_2O exposure has been shown to inhibit the methionine synthetase activity of human liver (31).

The accumulation of hepatic 5-CH_3-H_4folate in response to N_2O exposure, which is reported here, agrees with the results reported by Lumb *et al.* (20) and Eells *et al.* (11), in which N_2O exposure was continued for 8 hr or less; but Brody *et al.* (18) observed no such effect following a similar exposure of rats to N_2O. The reason for this discrepancy is not apparent. When N_2O exposure is continued for longer than 24 hr, the accumulation of 5-CH_3-H_4folate is no longer observed and is accompanied by a decrease in total hepatic folate (19, 20).

The mechanism of the effect of nitrogen/O_2 (1:1) on formate oxidation is unclear since exposure of rats to this mixture for 4 hr did not significantly affect methionine synthetase activity but is probably due to the high concentration of oxygen employed. It could be that longer exposures would lead to a significant inhibition of the enzyme. Deacon *et al.* (16) observed a 50% decrease in methionine synthetase activity following exposure of rats to 100% O_2 for 6 hr, but Koblin *et al.* (32) observed a variable effect of nitrogen/O_2 exposure on hepatic methionine synthetase activity in mice. Although the effect of nitrogen/O_2 requires further study, the effect of N_2O/O_2 on formate oxidation appears to be primarily due to the presence of N_2O since 4 hr of exposure to N_2O/O_2 produced a 50% inhibition of formate oxidation, whereas a similar exposure to nitrogen/O_2 produced only a 15% inhibition.

The effects of N_2O exposure on one-carbon metabolism do not appear to be mediated by an anesthetic action of the gas. The concentration of N_2O used in the present study is subanesthetic (33), and the N_2O-exposed animals exhibited normal behavior. The administration of phenobarbital to rats at a dose which produces profound central nervous system depression has no effect on the rate of formate oxidation, and exposure of rats to N_2O/O_2 (1:1, 4 hr) does not affect total respiratory CO_2 (11). Although Deacon *et al.* (16) reported a moderate inhibition of hepatic methionine synthetase activity following the exposure of rats to a concentration of halothane which was equi-anesthetic to the concentration of N_2O employed, the results of the study by Koblin *et al.* (32) showed that exposure of mice to xenon, halothane, enflurane, or isoflurane, at concentrations which were equi-anesthetic to the concentration of N_2O employed, produced no effect on hepatic methionine synthetase activity.

In the present study, N_2O/O_2 (1:1) exposure of rats for 4 hr produced a 90% inhibition of hepatic methionine synthetase activity but reduced both hepatic H_4folate and the rate of formate oxidation *in vivo* by only 50%. The ability of N_2O-exposed rats to oxidize formate appears to be primarily folate-dependent since inhibition of catalase, the only other known route of formate oxidation, by AT produced only a 20% decrease in the rate of formate oxidation. Also, the rate of formate oxidation in folate-deficient rats treated with AT and N_2O is approximately 5% of the control rate.[3] One explanation for these results is that, once animals are exposed to N_2O, H_4folate-generating enzymes other than methionine synthetase may become important in supplying H_4folate for formate oxidation. Hepatic H_2folate reductase apparently plays no such role, since MTX administration to N_2O-exposed rats had no effect on the rate of formate oxidation. Possibly serine transhydroxymethylase (EC 2.1.2.1) (Fig. 1, Reaction 3) plays a role in the supply of H_4folate in N_2O-exposed rats, but this enzyme is thought to regulate primarily the entry of one-carbon units into the folate biochemical pathway rather than regulate tissue levels of H_4folate (8). An alternative explanation would be that there are different pools of H_4folate, at least one of which is regulatable by methionine synthetase and one or more which serve to function in formate oxidation in N_2O-treated rats. Such pools might represent intracellular compartmentation of H_4folate.

Although no role of H_2folate reductase in the supply of hepatic H_4folate was demonstrated, these results do not rule out such a role for this enzyme in rapidly turning over tissues, such as bone marrow or certain tumors which, unlike liver, possess high rates of DNA synthesis. Such tissues possess relatively high rates of generation of H_2folate through the action of thymidylate synthetase (EC 2.1.1.45) (8) (Fig. 1, Reaction 4). The importance of H_2folate reductase in such tissues is demonstrated by the efficacy of MTX in the treatment of certain malignancies.

In summary, the results of the present study demonstrate the important role which hepatic methionine synthetase plays in the regulation of H_4folate levels and H_4folate-dependent processes, such as the oxidation of formate to CO_2. This knowledge should lead to a better understanding of methanol poisoning and the metabolism of certain drugs and other compounds through one-carbon pathways.

[3] J. T. Eells and T. R. Tephly, unpublished observation.

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