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EFFECT OF NITROUS OXIDE-INDUCED INACTIVATION OF VITAMIN B_{12} ON GLYCINAMIDE RIBONUCLEOTIDE TRANSFORMYLASE AND 5-AMINO-4-IMIDAZOLE CARBOXAMIDE TRANSFORMYLASE

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Exposure to nitrous oxide (N_2O) in vivo is accompanied by oxidation of cob[I]-alamin to the inactive cob[III]alamin [1]. There is loss of methionine synthetase activity [2] and evidence of depressed supply of single carbon units at the formate level of oxidation [3,4,5]. We measured the effect of inactivation of B_{12} on the folate-dependent transformylases concerned in purine synthesis. After 24 h exposure to N_2O there was a significant fall in glycinamide ribonucleotide transformylase (EC 2.1.2.2) and a significant increase in 5-amino-4-imidazole carboxamide transformylase (EC 2.1.2.3).

Exposure to the anesthetic gas, N_2O, leads to rapid oxidation of vitamin B_{12} from the active reduced cob[I]alamin form to the inactive cob[III]alamin form [1]. This also occurs in the intact animal [2,3,4,6] and in man [7,8,9]. There is impairment of methionine synthetase activity [2]. There is no folate polyglutamate synthesis from either H_4PteGlu or 5-CH_3-H_4PteGlu, but normal synthesis when the substrate carries a formyl substituent such as 5-CHO-, 10-CHO- and 5,10-CH=H_4PteGlu [4]. Further the N_2O-treated animal cannot use deoxyuridine normally for thymidine synthesis and this impairment is not improved by the addition to the incubation mixture of H_4PteGlu and 5-CH_3-H_4PteGlu but is improved with 5-CHO-H_4PteGlu [4]. It was suggested that methionine was the major source of formyl groups via a pathway involving S-adenosylmethionine, decarboxylated S-adenosylmethionine, 5-methylthioadenosine and 5-methylthioribose [5,10,30].

Abbreviations: Nitrous oxide, N_2O; glycinamide ribotide, GAR; 5-amino-4-imidazole carboxamide, AICAR; tetrahydropteroylglutamic acid, H_4PteGlu; 5-methyltetrahydropteroylglutamic acid, 5-CH_3-H_4PteGlu; 10 formyltetrahydropteroylglutamic acid, 10-CHO-H_4PteGlu; 5,10-methenyltetrahydropteroylglutamic acid, 5,10-CH=H_4PteGlu; 5-formyltetrahydropteroylglutamic acid, 5-CHO-H_4PteGlu.

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Carbons 2 and 8 of the purine nucleus are both donated as formyl groups through 10-formyltetrahydrofolate [11,12]. The purpose of this study was to assess the effect N_2O-induced inactivation of cobalamin on these 2 reactions by measuring the activity of glycinamide ribotide (GAR) transformylase and 5-amino-4-imidazole carboxamide (AICAR) transformylase.

MATERIALS AND METHODS

Animals: Male, Sprague-Dawley, 80-120 g rats were used. At the end of the study they were given an injection of sodium pentobarbitone and killed by exsanguination by cardiac puncture. Livers were removed and processed immediately. Animals exposed to N_2O were kept in a perspex chamber in which a mixture of N_2O (50%)/ oxygen (50%) was passed and CO_2 and humidity controlled. Control animals were left in air.

Reagents: AICAR, ATP, azaserine, folinic acid, ribose-5-phosphate, 3-phosphoglycerate, ammonium sulfamate (0.5% w/v), naphthylethylene dihydrochloride were obtained from Sigma. Dowex-50W, hydrogen form, 8% cross-linked, 200-400 dry mesh (Sigma) was converted to the ammonium form with 1MNH_4OH. AG 1-X8 acetate form, 100-200 mesh was obtained from Bio-Rad. [1-^{14}C]glycine (51.2 mCi/mmol) was obtained from Amersham International.

Liver extract: Liver (+ 50 mg/ml) was homogenized in a glass hand homogenizer in cold, 0.03 M potassium phosphate buffer, pH 7.0. The homogenate was centrifuged at 3000 g for 45 m at 4°C and the supernatant used for assay of GAR and AICAR transformylase activity.

5,10-CH=H_4PteGlu and 10-CHO-H_4PteGlu: These compounds were prepared from folinic acid (5-CHO-H_4PteGlu) [13]. Conversion on acidification was monitored by the appearance of an absorption peak at 348 nm. On neutralization, the formation of 10-CHO-H_4PteGlu was indicated by the disappearance of the peak at 348 nm and the presence of a peak at 258 nm.

GAR: This was prepared from acetone-dried chicken liver powder [14,15]. Formyl-GAR and GAR were identified by the incorporation of [1-^{14}C]glycine, a positive orcinol test for pentose [16] and by a specific assay for each of the ribotides using the ammonium sulphate fraction (0-60%) of acetone-dried chicken liver as enzyme source [15,17].

Protein: This was measured by the method of Lowry et al. [18].

AICAR transformylase: The method was that described by Flaks and colleagues [17,19]. The reaction was terminated by the addition of 0.4 ml 10% trichloracetic acid and 0.1 ml acetic anhydride was added to 0.5 ml aliquots of the supernatant. After 20 m the remaining non-acetylated diazotizable amine was measured by the Bratton-Marshall reaction [20]. AICAR transformylase activity was calculated by measuring the disappearance of AICAR at 540 nm using a molar extinction coefficient of 26,400.

GAR transformylase: The method used was that of Warren and Buchanan [17,21] using 0.2 M maleate buffer pH 6.8 [22]. The reaction was terminated by the addition of 0.1 ml 30% trichloracetic acid and 0.4 ml aliquots measured for diazotizable amine by the Bratton-Marshall reaction [20]. GAR transformylase activity was calculated from the amount of p-aminobenzoic glutamate (derived from H_4PteGlu produced) at 540 nm using a molar extinction coefficient of 40,500.

Table: GAR transformylase and AICAR transformylase activity in rat liver of controls and of animals exposed to N_2O/O_2 (v/vl/l) for 24 h.

	Controls			N_2O-treated		
	Number	Activity (Mean)	S.D.	Number	Activity (Mean)	S.D.
GAR transformylase (nmol H_4PteGlu formed/mg protein)	8	1.52	0.27	9	0.82	0.30
AICAR transformylase (nmol AICAR utilized/mg protein)	9	7.57	0.73	9	9.23	1.43

RESULTS

GAR-transformylase activity in livers of 8 air-breathing rats was 1.52 (S.D. 0.27) nmol H_4PteGlu formed per mg protein. Following exposure of 9 rats to N_2O for 24 h this fell to 0.82 (S.D. 0.30) nmol H_4PteGlu formed per mg protein (Table, Fig. 1). The fall was highly significant ($p< 0.001$).

AICAR transformylase activity in livers of 9 air-breathing rats was 7.57 (S.D. 0.73) nmol per mg protein and in 9 N_2O-breathing animals after 24 h the

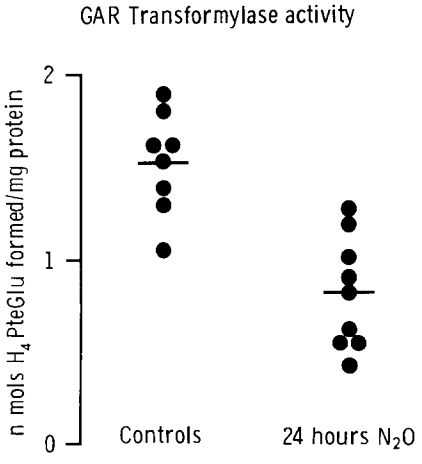

Figure 1. GAR transformylase activity in livers of rats breathing air (controls) and of rats breathing N_2O/O_2 (v/v, 1/1) for 24 h expressed as nmol H_4PteGlu produced per mg protein.

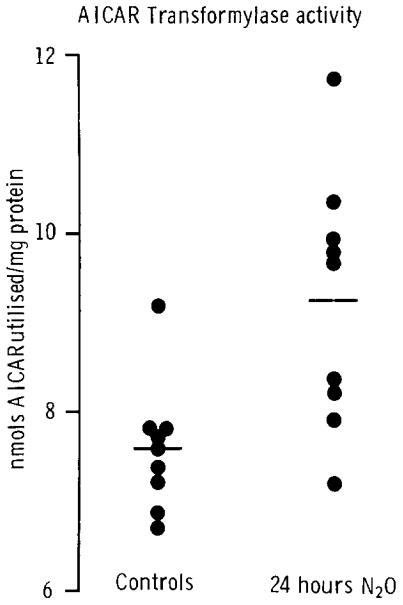

Figure 2. AICAR transformylase activity in livers of rats breathing air (controls) and of rats breathing N_2O/O_2 (v/v, 1/1) for 24 h expressed as nmol AICAR utilized per mg protein.

value was 9.23 (S.D. 1.43) nmol per mg protein. This increase was significant ($p < 0.01$) (Table and Fig. 2).

DISCUSSION

Cobalamins are not involved directly in the folate-dependent transformylases concerned with purine synthesis. Nevertheless increased urinary excretion of AICAR has been reported in patients with cobalamin as well as folate-deficient megaloblastic anaemia [23,24,25]. The role of cobalamin is presumably through its effect on folate metabolism. It was thus not surprising to find that inactivation of cobalamin by N_2O has significant effects on purine synthetic pathways involving folate. In terms of the methylfolate trap hypothesis this would be explained by unavailability of H_4PteGlu which is trapped in the methyl-form [26]. However, there is no evidence of methylfolate trapping in the N_2O-treated rat [27]. Nor does this hypothesis explain why H_4PteGlu is not utilized in the N_2O-treated rat [4,5], nor why the serine transhydroxymethylase reaction remains intact [28]. Exposure to N_2O causes a marked increase in activity of formyltetrahydrofolate synthetase [29] and, coupled with the evidence that formyl-H_4PteGlu analogues overcame the N_2O-induced block, we have proposed that the effect of

N_2O is to limit the supply of single carbon units at the formate level of oxidation. As the primary effect of N_2O is on methionine synthesis we have proposed that methionine is a precursor of formate and the likely pathway is through S-adenosylmethionine and 5-methylthioadenosine which yields formate [10,30].

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