Title: DIMETHYLTHIOUREA IMPEDES THE INACTIVATION OF METHIONINE SYNTHASE BY NITROUS OXIDE

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Introduction. Nitrous oxide inactivates the vitamin B12-dependent enzyme methionine synthase and may produce hematologic and neurological abnormalities in certain patients. Recent biochemical studies utilizing the purified enzyme suggest that a free hydroxyl radical is formed on reaction of the cobalt atom in vitamin B12 with nitrous oxide according to the following scheme:\(^2\)

\[
\text{cob(I)alamin} + N_2O \rightarrow \text{cob(II)alamin} + N_2 + \text{OH}
\]

It was suggested that the hydroxyl radical could attack amino acid residues near the active site of the enzyme and lead to an irreversible inactivation of methionine synthase.\(^3\) If this suggestion is correct, then a compound that scavenges free hydroxyl radicals should be able to protect methionine synthase from inactivation by nitrous oxide; provided this compound could penetrate to the site of formation of the hydroxyl radical, we tested this hypothesis by measuring the ability of the potent and highly cell permeable hydroxyl radical scavenger, dimethylthiourea (DMTU),\(^4\) to attenuate the inactivation of methionine synthase produced by nitrous oxide.

Methods. A total of 40 adult male ICR mice were examined. Mice were given intraperitoneal injections of 0.5, 1.0, 2.0, or 4.0 mg DMTU/g body weight, or an equal volume of 0.9% NaCl. One hour after the injections, DMTU-injected (4 mice for each dose of DMTU) and saline-injected mice were placed in a 20 l chamber and exposed to 66% \(N_2O/34\% \text{O}_2\) for 1 hr. Following \(N_2O\) exposure, mice were killed with 100% \text{CO}_2, and livers, kidneys, and brains were removed, homogenized in 0.01 M phosphate buffer (pH 7.3), and the homogenate was centrifuged at 20,000 g for 80 min. Methionine synthase activity in these organs was measured by incubating an aliquot of the supernatant with \([\text{CH}_3\text{]tetrahydrofolate and homocysteine, and quantitating the amount of radioactive methionine produced.}\(^5\) In addition, methionine synthase activities were measured in 4 mice given 2.0 mg/g DMTU but not exposed to \(N_2O\), and in 4 mice not given DMTU and not exposed to \(N_2O\). Statistical comparisons between the DMTU- and saline-injected groups were performed with an unpaired \(t\)-test.

Results. Methionine synthase activities in kidneys of mice injected with DMTU were significantly higher (\(p<0.001\) for 0.5, 2.0, and 4.0 mg DMTU/g, and \(p<0.05\) for 1.0 mg DMTU/g) than those in saline-injected mice after exposure to 66% \(N_2O/34\% \text{O}_2\) for 1 hr (Figure 1). Similar findings were obtained in liver and brain, although the differences in enzyme activities between the DMTU- and saline-injected animals were less in these organs. The higher doses of DMTU (2.0 and 4.0 mg/g) produced marked sedation. In mice not exposed to nitrous oxide, renal methionine synthase activity in animals given 2.0 mg/g DMTU (298 ± 30.3 nmol methionine hr\(^{-1}\) g\(^{-1}\)) did not differ from the activity (264 ± 27.8 nmol methionine hr\(^{-1}\) g\(^{-1}\)) in those not treated with DMTU (mean values ± S.D.).

Discussion. The present findings show that the hydroxyl radical scavenger DMTU protects against the \(N_2O\)-induced inactivation of methionine synthase. Although it is possible that DMTU acts by prolonging the time required for equilibration between organs and inspired gas, the results are consistent with the hypothesis that a hydroxyl radical is produced when \(N_2O\) reacts with the Co\(^{3+}\) atom of vitamin B12 and that the formation of this free radical is associated with the inactivation of methionine synthase. Damage produced by a free radical near the active site of methionine synthase could explain the irreversible loss of enzyme activity that follows exposure to \(N_2O\). If humans possessed a higher cytoplasmic concentration of intrinsic hydroxyl radical scavengers than rodents, these experiments also provide one possible explanation for the slower rate of onset of \(N_2O\)-induced inactivation of methionine synthase activity in humans than in rodents.

References.
1. Nunn JF: Clinical aspects of the interaction between nitrous oxide and vitamin B\(_{12}\). Anesthesiology 59: 3-13, 1987

Figure 1. Methionine Synthase Activities in Kidneys of Mice Exposed to 66% \(N_2O\) for 1 hr (mean values ± S.D.)