Title : METABOLISM OF NITROUS OXIDE BY HUMAN AND RAT INTESTINAL CONTENTS

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Introduction. Nitrous oxide has long been considered chemically inert in the body. Sawyer et al(1) concluded that nitrous oxide was not metabolized by the liver after a study demonstrating that the fraction of nitrous oxide removed from the blood during a single pass through the liver was only 0.03 ± 0.05 percent at 4.65 ± 0.21 percent alveolar concentration. However, Matsubara and Mori(2) have shown that nitrous oxide is an intermediate in the reduction of nitrite to nitrogen by the soil bacteria Pseudomonas denitrificans. Metabolic reduction of aromatic nitro compounds has also been shown to occur in vitro and in vivo in both mammalian tissue and gastrointestinal microflora. Compounds which are reduced in the intestine were shown to be reabsorbed into the blood and thus may be toxic to the animal(3). Recent evidence that nanomolar amounts of certain nitrogenous substances, such as N-nitroso compounds, may be carcinogenic or teratogenic has suggested that metabolism of nitrous oxide might be potentially toxic even if this were to occur in very small amounts. For these reasons it seemed important to test whether nitrous oxide is metabolized by rats and humans. There are no useful radioisotopes of nitrogen or oxygen, therefore a stable heavy isotopy of nitrogen was used to make nitrous oxide. Possible metabolites were detected and quantitated by isotope ratio mass spectrometry.

Methods. In a typical experiment, a 20 ml homogenate of 2.5 g of intestinal contents was degassed on a vacuum line at room temperature. The 80 ml reaction vessel was then evacuated and either pure $^{15}N_2O$ or a desired mixture of $^{15}N_2O$ and O_2 were introduced until atmospheric pressure was reached. The reaction vessel was sealed and incubated at 37°C for 16 hours. Following the incubation period the reaction vessel was remounted on the vacuum line and the headspace gas that was incondensable at liquid nitrogen temperature was removed by distillation. The nitrogen gas produced was then introduced into a Micromass 602C isotope ratio mass spectrometer to measure the amount of $^{15}N^{15}N$ produced by the homogenate in comparison with the N_2 internal standard.

Results. The amounts of $^{15}N^{15}N$ gas produced by 20 ml homogenates of rat intestinal content, human feces, and intestinal contents from rats pretreated with antibiotics are given below.

Incubation Conditions	Human Large Intestinal Contents	Rat Intestinal Contents
0% O_2	561 ± 35 (5)	118 ± 14 (8)
5% O_2	103 ± 17 (4)	47 ± 13 (6)
10% O_2	-	6 ± 4 (3)
20% O_2	-	6 ± 5 (3)
Antibiotics	-	0.1± 0.4(3)
Boiled	0.3 ± 0.2 (3)	0.0± 0.3(3)

The data indicate that both rat intestinal contents and human feces actively metabolize nitrous oxide. Metabolism is greatly inhibited by 10 and 20 percent oxygen and partially inhibited by five percent oxygen. This finding is consistent with a reductive route of nitrous oxide metabolism and with the previous studies on the soil bacteria Pseudomonas dentrificans(2).

Discussion. Mason and Holtzman(4) have reported that enzymatic reduction of drugs containing nitro groups and other nitro aromatic compounds produce anion free radicals. It is conceivable that the reduction of nitrous oxide may also proceed through such a single-electron transfer process. If this were so, the pathway of nitrogen production may initiate free radical reactions even though the end-metabolite is inert. It is well established that free radicals are important in the etiology of carcinogenesis, teratogenesis and associated tissue damage. In future studies it will be important to search for these possible radical intermediates.

References

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