assay for cholinesterase, suitable for multiple determinations. Anal. Biochem. 64: 229–238

Kawabuchi, M., Boyne, A. F., Deshpande, S. S., Cintra, W. M., Brossi, A., Albuquerque, E. X. (1988) Enantiomer (+)physostigmine prevents organophosphate-induced subjunctional damage at the neuromuscular synapse by a mechanism not related to cholinesterase carbamylation. Synapse 2: 139–147

Lennox, W. J., Harris, L. W., Talbot, B. G., Anderson, D. R. (1985) Relationship between reversible acetylcholinesterase inhibition and efficacy against soman lethality. Life. Sci. 37: 793–798

Litchfield, J. T., Wilcoxon, F. (1949) A simplified method of evaluating dose-effect experiments. J. Pharmacol. Exp. Ther. 96: 99–113

Main, A. R. (1979) Mode of action of anticholinesterases. Pharmacol. Ther. 6: 579–628

Rosecrans, J. A., Domino, E. F. (1974) Comparative effects of physostigmine and neostigmine on acquisition and performance of a conditioned avoidance behavior in the rat. Pharmacol. Biochem. Behav. 2: 67–72

Silver, A. (1974) The Biology of Cholinesterases. North Holland, Amsterdam

Stephens, R. J., Waterfall, J. F., Franklin, R. A. (1978) A review of the biological properties and metabolic disposition of the new analgesic agent, meptazinol. Gen. Pharmacol. 9: 73–78

Taylor, P. (1985) Anticholinesterase agents. In: Gilman, A. G., Goodman, L. S., Rall, T. W., Murad, F. (eds) The Pharmacological Basis of Therapeutics. 7th edn, Macmillan, New York, pp 110–129

The effect of nitrous oxide on S-adenosylmethionine levels in mouse brain

ROY L. DORRIS, Department of Pharmacology, Baylor College of Dentistry, Dallas, Texas 75246, USA

Abstract—Mice were exposed to nitrous oxide (50%) for up to 24 h and S-adenosylmethionine (SAMe) levels measured in corpus striatum and cerebellum, areas with high and low catecholamine turnover rates, respectively. After 4 h, levels were 21 and 8% and after 6 h, 33 and 14% lower than controls in striatum and cerebellum, respectively. Thus, the effect was more pronounced in corpus striatum, the area with the presumed higher rate of catecholamine O-methylation. With continued exposure to nitrous oxide SAMe concentrations in the two areas returned to nearly normal at 24 h. The observation that levels did not continue to decline, and even returned towards control levels, while animals were still in the presence of the gas suggests that a mechanism other than that of methionine synthase inhibition may have been responsible for the initial effect. Alternatively, some other source of SAMe may have become available to compensate for the inhibition of the enzyme.

Although methionine is an essential amino acid, an interference with the contribution provided by the enzymatic conversion of homocysteine to methionine by methionine synthase (EC 2.1.1.13) can result in a decrease in tissue levels of the amino acid. Thus, nitrous oxide (N_2O) exposure has been shown to decrease the activity of methionine synthase in rodents and result in diminished tissue levels of methionine (Koblin et al 1981; Lumb et al 1983; Brennt & Smith 1989). The enzyme requires cobalamin I (vitamin B_{12}) as a cofactor and the mechanism of its inhibition by N_2O is thought to result from oxidation of cobalamin I to cobalamin III, a form of the coenzyme that is inactive (Banks et al 1968).

S-Adenosylmethionine (SAMe) is derived from methionine and exposure to N_2O in some studies has resulted in its diminution in liver (Lumb et al 1983) and whole brain (Vina et al 1986).

SAMe is a methyl donor in a variety of transmethylation reactions, including the O-methylation of the catechol neurotransmitters. Under normal circumstances, the biosynthesis of SAMe keeps pace with its utilization. However, there is evidence that demand for the methyl donor can outstrip its supply. For example, rats treated for 1 h with haloperidol, a dopamine receptor blocking drug that is known to reflexly increase dopamine turnover in the dopamine-rich corpus striatum, had

approximately a 30% decrease in SAMe levels in that brain area (Waldmeier & Feldtrauer 1987). Thus it seemed possible that, since N_2O can inhibit methionine synthesis, and even possibly lower whole brain SAMe levels, the agent might have a greater effect on SAMe levels in corpus striatum than in cerebellum, an area with relatively little catecholamine content (Heffner et al 1980). This possibility was investigated.

Materials and methods

Male mice, 30–40 g, HSD: (ICR) BR were exposed to $N_2O:O_2$ using 4 L filtration flasks as exposure chambers and a Quanitiflex MDM, N_2O machine. The gas mixture (50:50) was delivered at 4 L min^{-1} via a glass tubing which passed through a rubber stopper in the opening of each flask and extended to within 5 cm of the bottom. Gases exited via the flask side arm and were vented into a fume hood. Two flasks, each containing two mice, received the gases simultaneously by using a split connector in the line. Two other flasks were similarly arranged but with $N_2:O_2$ (50:50) serving as control gases.

The animals were killed by exsanguination under chloroform or ether anaesthesia. Brains were chilled in cold saline and placed on a chilled plate for dissection of corpus striatum and a portion of cerebellum approximating the weight of the corpus striatum. Tissue SAMe concentrations were determined using a method based on that of Wagner et al (1984). This method has a detection limit of approximately 1 pmol which far exceeds the sensitivity needed in the present study. Thus, after tissues were blotted and weighed, they were homogenized in 1 mL 0·2 M perchloric acid and centrifuged at 4°C. The supernatants were filtered through 0·2 μm cellulose filters in a centrifugal filtering apparatus. Portions of the filtrates were subjected to HPLC, and absorbance was measured at 254 nm. The HPLC mobile phase consisted of 850 mL 0·15 M NaH_2PO_2, 150 mL acetonitrile and 1·73 g sodium octanesulphonic acid. The pH was adjusted to 3·0 with 3 M phosphoric acid. Early in the study it was noticed that absolute control values seemed to vary (though not consistently) with the time of day. For that reason, controls were always included in each experiment and results expressed as percentage change from these controls. Statistical differences between means were determined by Tukey's test.

Results

Exposure to N$_2$O for 2 h resulted in striatal SAMe levels that were marginally lower than controls. Cerebellar levels were not significantly altered by the 2 h treatment (Fig. 1). After 4 h striatal levels were approximately 21% lower than controls and cerebellar levels approximately 8% lower than controls. After 6 h levels were 33 and 14% lower than controls in the two respective areas. At both 4 and 6 h, the changes in striatum were statistically significantly greater than those in cerebellum. With continued exposure, levels did not continue to decline but returned to nearly normal at 24 h.
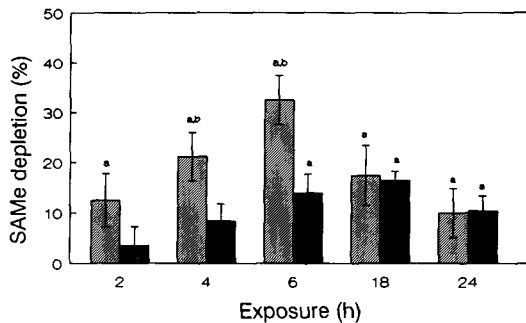

Fig. 1. Effects of nitrous oxide on striatal and cerebellar SAMe concentrations. Mice were exposed to 50% nitrous oxide or nitrogen for the indicated times. Except at 18 h (n = 4), each of the other data sets represent at least 8 animals. Bars are means ± s.e. of percent change from control values obtained from animals run in parallel at each time. Absolute control values (nM g^{-1}) for striatum and cerebellum respectively were: 2 h–31·2 ± 1·9 and 37·1 ± 2·0; 4 h–34·0 ± 0·7 and 41·2 ± 0·6; 6 h–30·0 ± 0·6 and 38·5 ± 1·3; 18 h–31·5 ± 1·0 and 43·1 ± 0·4; 24 h–36·0 ± 2·6 and 46·2 ± 2·7. Striatum, left column; cerebellum, right column. $^a P < 0.05$ compared with control; $^b P < 0.05$ compared with cerebellum.

Discussion

Other investigators have reported that short-term exposure of rodents to 50–80% N$_2$O depressed methionine synthase activity in several tissues (Koblin et al 1981; Lumb et al 1983; Brennt & Smith 1989). Interference with this enzymatic source by N$_2$O exposure has been shown to decrease tissue methionine levels (Lumb et al 1983; Vina et al 1986). Because SAMe is derived from methionine, it would seem that a decrease in tissue levels of methionine, particularly in tissues with a high rate of methyl group utilization, could eventually lead to diminished levels of this methyl donor. Even though one report indicated that whole brain levels of SAMe were decreased by N$_2$O, the results of its influence on brain SAMe levels are not as clear as those on its precursor, methionine. However, in the present investigation, a short exposure to 50% N$_2$O clearly resulted in striatal and cerebellar SAMe concentrations that were lower than controls. Furthermore, as predicted, the influence of the gas was greater in

the dopamine-rich corpus striatum than in the cerebellum, an area with a minuscule catecholamine content.

The observation that SAMe levels did not continue to decline throughout the 24 h exposure time and even reverted toward normal, suggests that there is much more to be learned about the influence of N$_2$O on brain SAMe metabolism. As SAMe levels decrease to a certain level, another source of methionine and/or SAMe may become available. In this regard, it has been shown that there is a nearly coincidental increase in betaine methyltransferase activity with the decrease in methionine synthase activity in livers of rats under N$_2$O exposure (Lumb et al 1983). This enzyme is another source of methionine and its activation may be to compensate for conditions in which methionine synthase becomes deficient. Although betaine methyltransferase is not found in brain and, thus, cannot serve locally in that tissue to offset deficiencies of methionine synthase activity, it might be that the methionine formed in liver by the enzyme is diverted to the brain at the expense of an adequate amount for the synthesis of liver SAMe. Consistent with this suggestion is the observation by Lumb et al (1983) that, after continuous exposure (several days) to N$_2$O, liver SAMe levels were markedly lowered while those of whole brain were within the normal range. In the same studies, after an initial decline, liver and brain methionine was maintained at nearly normal levels.

References

Banks, R. G. S., Henderson, R. J., Pratt, J. M. (1968) Reactions of gasses in solution. Part III. Some reactions to nitrous oxide with transition-metal complexes. J. Chem. Soc. (A): 2886–2889

Brennt, C. E., Smith, J. R. (1989) The inhibitory effects of nitrous oxide and methylmercury in vivo on methionine synthase (EC 2.1.1.13) activity in the brain, liver, ovary and spinal cord of the rat. Gen. Pharmacol. 20: 427–431

Heffner, T. G., Hartman, J. A., Seiden, L. S. (1980) A rapid method for the regional dissection of the rat brain. Pharmacol. Biochem. Behav. 13: 453–456

Koblin, D. D., Watson, J.E., Deady, J. E., Stokstad, E. L. R., Eger, E. I. (1981) Inactivation of methionine synthetase by nitrous oxide in mice. Anesthesiology 54: 318–324

Lumb, M., Sharer, N., Deacon, R., Jennings, P., Purkiss, P., Perry, J., Chanarin, I. (1983) Effects of nitrous oxide-induced inactivation of cobalamin on methionine and S-adenosylmethionine metabolism in the rat. Biochim. Biophys. Acta 756: 354–359

Vina, J. R., Davis, D. W., Hawkins, R. A. (1986) The influence of nitrous oxide on methionine, S-adenosylmethionine and other amino acids. Anesthesiology 64: 490–495

Wagner, J., Claverie, N., Danzin, C. (1984) A rapid high-performance liquid chromatographic procedure for the simultaneous determination of methionine, ethionine, S-adenosylmethionine, S-adenosylethionine and the natural polyamines in rat tissues. Anal. Biochem. 140: 108–116

Waldmeier, P. C., Feldtrauer, J.-J. (1987) On the role of O-methylation in the metabolism of S-adenosylmethionine in rat brain. Biochem. Pharmacol. 36: 2855–2861