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COMMUNICATIONS

Locomotor effects of nitrous oxide in mice: requirement of newly-synthesized and main intraneuronal storage pools of dopamine

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Abstract—Nitrous oxide increases locomotor activity in mice. Other locomotor stimulants are thought to act via central dopaminergic mechanisms and can be divided into two groups as determined by their antagonism by tyrosine hydroxylase inhibitors or by reserpine pretreatment. The purpose of the present study was to determine if nitrous oxide fits one or the other of the groups. Mice were acclimatized for 1 h to exposure chambers (4 L filtration flasks), in air, delivered at 4 L min^{-1} and then exposed to $N_2O:O_2$ (50:50), also delivered at 4 L min^{-1}. Locomotor activity was evaluated at 10 min intervals throughout the experiment. Racemic α-methyltyrosine methyl ester HCl (200 mg kg^{-1}), administered at the beginning of acclimatization, almost totally eliminated the nitrous oxide effect but not that of methylphenidate HCl (20 mg kg^{-1}). Reserpine pretreatment (5 mg kg^{-1}, 18 h) totally eliminated the nitrous oxide effect but not that of amphetamine (5 mg kg^{-1}). The results suggest that nitrous oxide requires both the newly synthesized and the main storage pools of dopamine and do not allow assignment of the agent, specifically, to either of the groups.

Locomotor stimulants exert their effects by an action on brain catecholaminergic neurons. However, the precise mechanism by which they act is quite different and the agents can be divided into two groups in accordance with their actions (Braestrup 1977). Thus, one group (e.g. methylphenidate, amfonelic acid, cocaine, pipradol) is distinguished by the fact that its constituents are antagonized by reserpine pretreatment. Members of the other group (e.g. amphetamine, methamphetamine, phenmetrazine) are not antagonized by reserpine but are antagonized by α-methyltyrosine, an inhibitor of catecholamine biosynthesis—the antagonism occurring at a time when a large part of the main storage pool of dopamine is still present (Javoy & Glowinski 1971). Thus, it appears that the methylphenidate-like drugs depend on the main storage pool of intraneuronal catecholamines and the amphetamine-like drugs on the newly synthesized pool, for effects on behaviour.

When mice are exposed to 50% nitrous oxide they respond with an increase in locomotor activity (Hynes & Berkowitz 1979) reminiscent of that produced by classical locomotor stimulants (personal observations). The action would appear to involve the catecholamines (particularly dopamine) in that it was blocked by haloperidol (Hynes & Berkowitz 1983). The present study was undertaken to determine if the mechanism of locomotor action of nitrous oxide is like that of either of the two groups of locomotor stimulants discussed above.

Materials and methods

Male mice (HSD:ICR BR), 35–50 g, were used in these studies. Two mice were placed into each of two 4 L filtration flasks located within the field of an Opto-Varimex-Minor-s activity monitor. The flasks were equipped with rubber stoppers through which gases were delivered at approximately 4 L min^{-1} and exited into a fume hood. Following an acclimatization period of 1 h, in an atmosphere of air, a switch was made to a mixture of nitrous oxide:oxygen (50:50) and exposure continued for another hour. Locomotor activity readings were taken at 10 min intervals throughout the acclimatization and nitrous oxide exposure periods. Experiments were generally performed at approximately the same time of day. However, in some cases, one experiment was done before noon and another in the afternoon. In these cases, the experimental and control groups

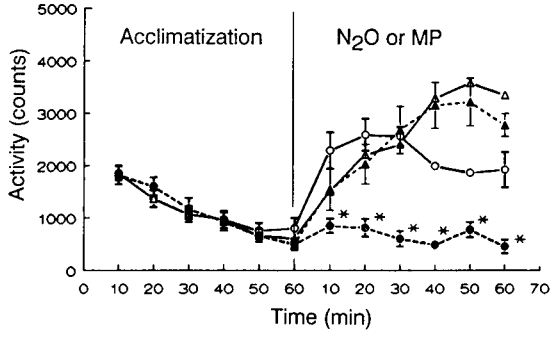

FIG. 1. Effect of α-MT on nitrous oxide (N_2O)- or methylphenidate (MP)-induced locomotor activity. α-MT was administered (200 mg kg^{-1}) at the beginning of the acclimatization period. Some animals were injected with saline in lieu of α-MT. Methylphenidate HCl was administered (20 mg kg^{-1}) following the 1 h acclimatization period and air was the gaseous environment during both 1 h periods. In the experiments with nitrous oxide, its administration followed the 1 h acclimatization period and was continued throughout the second hour. Values from animals treated only with methylphenidate, represent activities of 4 animals (n = 1). All other values are means of at least 5 experiments with 4 animals each. Vertical bars represent s.e.m. $*P < 0.005$ compared with animals receiving nitrous oxide without α-MT pretreatment. o—o N_2O, ●– –● α-MT + N_2O, Δ—Δ MP, ▲– – –▲ α-MT + MP.

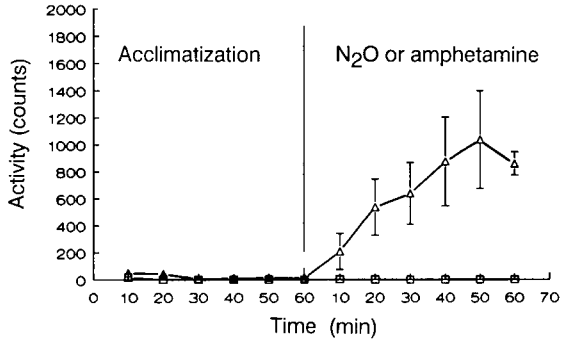

FIG. 2. Locomotor response to nitrous oxide (N_2O) or amphetamine after reserpine pretreatment. Reserpine (5 mg kg^{-1}) was administered intraperitoneally 18–20 h before amphetamine or nitrous oxide. Amphetamine sulphate (5 mg kg^{-1}, i.p.) or nitrous oxide (50%) was administered following the 1 h acclimatization period. Values from amphetamine-treated animals are means ± s.e.m. of 5 experiments (20 mice), from nitrous oxide-treated, 3 experiments (12 mice). □—□ N_2O, Δ—Δ amphetamine.

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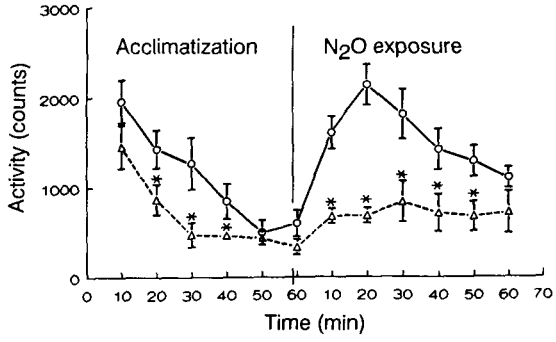

FIG. 3. Effect of haloperidol on nitrous oxide (N_2O)-induced locomotor activity. Haloperidol HCl (0·1 mg kg^{-1}, s.c.) was administered just before the period of acclimatization. Controls were injected with saline. Values are means of 5 experiments of 4 animals each. $*P < 0.025$ compared with animals not treated with haloperidol. O—O Control, Δ- - - Δ haloperidol.

were counterbalanced with equal numbers at each time of day. Animals were killed by exposure to 100% carbon dioxide. Catecholamines were assayed using standard isolation methodology consisting of alumina extraction followed by HPLC and electrochemistry. (±)-α-Methyltyrosine methyl ester HCl (α-MT), methylphenidate HCl and amphetamine sulphate were dissolved in water. Haloperidol HCl was dissolved in a small amount of 0·01 M HCl and diluted more than a hundredfold with water. Reserpine was a commercial, injectable preparation (Serpasil, Ciba Pharmaceutical Co.) and was diluted with water. All drugs were prepared at concentrations such that injection volumes were 0·1 mL/10 g. All drug doses refer to the salt forms. Data were evaluated using Student's *t*-test.

Results

The administration of α-MT just before acclimatization resulted in a marked attenuation of the nitrous oxide-induced locomotor effect (Fig. 1). The inhibitory effect occurred at a time when brain noradrenaline and dopamine concentrations were still 76±4 and 68±4% (±s.e.m) of mean concentrations of four untreated controls (noradrenaline, 412±17; dopamine, 1378±28 ng g^{-1}). In contrast to the observation with nitrous oxide, α-MT had no observable effect on the locomotor response produced by methylphenidate. Reserpine obliterated the locomotor effect of nitrous oxide but not that of amphetamine (Fig. 2). In confirmation of the report of Hynes & Berkowitz (1983), there was a marked antagonism of nitrous oxide by haloperidol (Fig. 3).

Discussion

The present report corroborates that of Hynes & Berkowitz (1979) showing locomotor stimulation in mice exposed to nitrous oxide. In confirmation of the same report, the effect was virtually eliminated by the dopamine antagonist, haloperidol, suggesting that dopamine release is involved in the mechanism of action.

As pointed out in the introduction, locomotor stimulants can be generally divided into two groups: those that are inhibited by reserpine pretreatment (i.e. they require the main storage pool of dopamine); and those that can be inhibited by the tyrosine hydroxylase inhibitor, α-MT (i.e. they require the pool of newly synthesized dopamine). In the present study the effect of nitrous oxide was almost completely eliminated by α-MT pretreatment—suggesting that the gas, like amphetamine, acts via the newly synthesized pool of dopamine in producing its stimulatory effects. However, reserpine also prevented the effect. This observation suggests that nitrous oxide has a methylphenidate-like mechanism in that it appears to require the main intraneuronal storage pool of dopamine. The ability of either agent to antagonize the effect would suggest that both the newly synthesized and the main storage pools of dopamine are essential to the locomotor action of the gas. This being the case, the mechanism of locomotor stimulation by nitrous oxide appears to be more complex than for the classical stimulants and its description requires further investigation.

It should be mentioned that Hynes & Berkowitz (1983) also investigated the effects of α-MT on the nitrous oxide-induced increase in locomotor activity. They reported only a small reduction in the effect by the dopamine synthesis inhibitor. However, in their study α-MT was administered 16 h before exposure to the gas, a pretreatment time that was shown by Weissman et al (1966) to also have very little influence on the action of amphetamine. According to the report of Spector et al (1965), there was very little α-MT present in the brain 16 h after administration of the drug.

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