Anesthesiology
56:45-48, 1982

Nitrous Oxide Decreases Motility of Human Neutrophils In Vitro

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The effect of 80 per cent nitrous oxide on motility of human neutrophils obtained from six volunteers was studied *in vitro*. In every case, random migration through the pores of 3 μm-millipore filter paper was reduced (mean value 16 per cent). Chemotaxis toward casein also was reduced in every case (mean value 22 per cent). These results contrast with a previous study in which mean rates of migration were found to increase following exposure to 2 per cent halothane. (Key words: Anesthetics, gases: Nitrous oxide. Blood: Leukocytes. Cells: Chemotaxis; motility; neutrophils.)

IN 1970, Nunn *et al.*[1] demonstrated an approximately sixfold reversible reduction in the velocity of random movement of mammalian lymphocytes in culture when exposed to medium equilibrated with 2 per cent halothane. Rather unexpectedly, we found no such effect with human neutrophils: motility and various other functions (including phagocytosis) *in vitro* were not impaired by halothane in concentrations up to 2 per cent in the gas phase.[2] It was therefore surprising that Tannières‡ reported significant decreases in random motility of neutrophils *in vitro* during exposure to nitrous oxide (25 and 50 per cent), although the relative anesthetic potency of these concentrations of nitrous oxide is barely one sixth that of concentrations of halothane which caused no decrease in neutrophil motility in our studies.

It is now clear that nitrous oxide has chemical effects which distinguish it from other anesthetics[3] and we therefore planned a more detailed investigation of any effect of nitrous oxide on neutrophil motility, including a study of chemotaxis toward casein.

Methods

Venous blood was obtained from six healthy volunteers and placed in heparin 10 IU ml^{-1}. Erythrocytes underwent sedimentation for 45 min at room temperature with an approximately equal volume of 6 per cent dextran (Lomodex R 70, Fisons Ltd.). The resulting supernatant fluid was layered on top of Ficol-Paque (Pharmacia, Piscataway, New Jersey) medium and centrifuged at 4500 rpm for 20 min.

The plasma and suspended lymphocytes were removed, leaving a pellet composed of mostly neutrophils. The pellet was resuspended in hypertonic saline solution to lyse remaining erythrocytes. Then water was added to restore isotonicity. The suspension was centrifuged a second time to yield a pellet consisting entirely of neutrophils which were then resuspended in Hanks balanced saline medium to a concentration of 2×10^6 cells/ml divided into two aliquots of 2 ml each. Eighty per cent nitrous oxide and 20 per cent oxygen were bubbled through one aliquot in a conical glass test tube suspended in a water bath maintained at 37° C. The second aliquot was treated similarly but room air was used instead of the nitrous oxide/oxygen mixture. The glass tubes had previously been treated with a 2 per cent solution of dimethyldichlorosilane in carbontetrachloride (Repelcote, Hopkin and Williams, London) to prevent the neutrophils from adhering to the wall of the test tube. After 15 min bubbling, the tubes were transferred to a hot room maintained at 37°C. The control tube was exposed to air but the tube equilibrated with nitrous oxide was immediately transferred to a glove box within the hot room. The glove box had previously been flushed with a humidified gas mixture of 80 per cent nitrous oxide and 20 per cent oxygen.

Random movement and chemotaxis toward casein were determined by measuring the migration of neutrophils in 0.25 ml of the suspension through a millipore membrane of 3 μm-pore size, using a modification of the method of Aggett *et al.*[4] Casein (BDH Ltd.) was dissolved in alkali at a concentration of 1 mg/ml and then neutralized to a *p*H of 7.4. Special glass chambers were constructed to avoid diffusion of nitrous oxide into the plastic chambers that are normally used (fig. 1). Migration was measured by the "leading front" method[5] taking the mean of five readings on each filter paper, duplicates being set up for both test and control. After two hours incubation, the membrane was washed free of any remaining erythrocytes, fixed in isopropyl alcohol, washed, stained with hemotoxylin, dehydrated, cleared and mounted on slides. The distance advanced by the "leading

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Received from the Clinical Research Centre, Harrow, Middlesex, England. Accepted for publication July 14, 1981.

‡ Tannières M-L: Action des facteurs physiques et des gaz anésthésiques sur la mobilité des polynucleaires neutrophiles. M.D. Thesis, Librairie Arnette, Paris, 1974.

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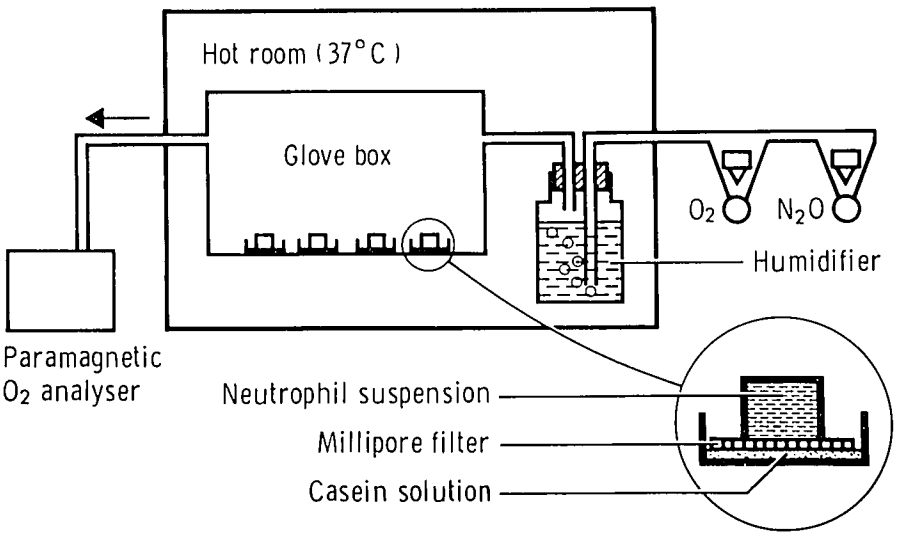

FIG. 1. Arrangement of glass chambers used to measure motility.

front" was then determined by light microscopy measuring the difference with the fine focusing control of the microscope.

For both test and control, duplicate chambers were assembled with casein below the membrane (to measure chemotaxis) and a further pair of chambers was assembled with Hanks medium below the membrane (to measure random motility). There were thus a total of eight chambers containing neutrophil suspensions from each volunteer. The suspensions that had been equilibrated with nitrous oxide were subsequently handled and incubated entirely in an atmosphere of 80 per cent nitrous oxide and 20 per cent oxygen. All materials used in assembly of the migration chambers had remained in the glove box for 20 min to become equilibrated with the gas mixture prior to assembly.

Effluent gas from the glove box was continuously monitored with a Servomex DCL 101 paramagnetic gas analyzer and the oxygen concentration was held at 19 to 22 per cent oxygen, the remainder being nitrous oxide.

The significance of the results was assessed by a paired t test, one-tail being appropriate for confirmation of the

TABLE 1. Leading Front Migration of Neutrophils (μM in two hs): Paired Results for Six Subjects (Mean ± SE)

	Control (Air)		80 percent N_2O/20 percent O_2	
Subject	Number of Measurements	Movement	Number of Measurements	Movement
Random Movement to Hanks Solution				
1	5	36.8 (±3.0)	9	24.2 (±3.8)
2	10	23.4 (±3.0)	10	21.6 (±4.4)
3	10	31.8 (±4.6)	10	29.2 (±6.2)
4	11	19.6 (±4.8)	10	19.0 (±1.94)
5	10	24.0 (±1.64)	10	21.6 (±2.28)
		20.2 (±1.76)		15.8 (±1.48)
6	10		10	
		$\bar{x}$ 25.96		$\bar{x}$ 21.9*
Chemotaxis to Casein Solution				
1	5	69.6 (±6.0)	10	57.6 (±8.0)
2	5	49.6 (±3.2)	5	44.8 (±3.4)
3	10	43.4 (±5.8)	10	32.8 (±4.8)
4	5	30.4 (±0.9)	5	27.6 (±2.6)
5	10	51.8 (±6.7)	10	29.2 (±3.2)
		30.0 (±0.94)		21.8 (±1.8)
6	10		10	
		$\bar{x}$ 45.8		$\bar{x}$ 35.6†

Paired t test: * P = 0.072 (two-tail); 0.036 (one-tail). † P = 0.016 (two-tail); 0.008 (one-tail).

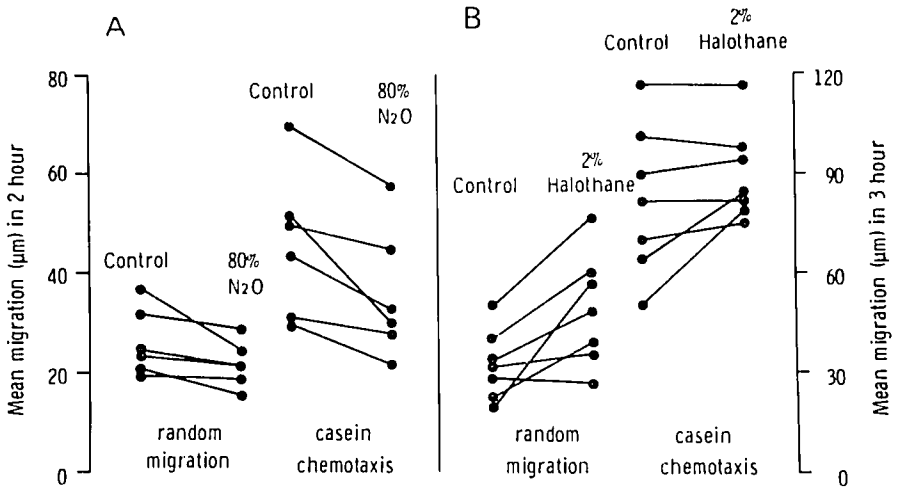

FIG. 2. (*A*) Changes in random migration and casein chemotaxis with 80 per cent nitrous oxide (present study). (*B*) Changes with 2 per cent halothane using similar techniques.[2]

study of Tannières and two-tail being appropriate for comparison with halothane.

Results

Control preparations showed a mean migration of 26.0 μm in 2 h in the presence of Hanks solution but this was increased to a mean value of 45.8 μm during chemotaxis towards casein (table 1 and fig. 2). Equilibration with nitrous oxide reduced the random migration below the level of the corresponding control in every case. The mean value was 21.9 μm, a reduction of 16 per cent (paired t test: $P = 0.072$ (two-tail) or $P = 0.036$ (one-tail). Equilibration with nitrous oxide reduced the migration toward casein below the level of the corresponding control in every case. The mean value was 35.6 μm, a reduction of 22 per cent which was significant (paired t test: $P = 0.016$ (one-tail) or $P = 0.008$ (two-tail).

Discussion

The results obtained in the present study were compared with a similar study using 2 per cent halothane for 3 h instead of nitrous oxide 80 per cent for 2 h, undertaken by the same team using similar methods[2] (fig. 2). Two per cent halothane caused an increase in mean values for both random and chemotactic migration, the former being statistically significant ($0.02 > P > 0.01$). Duncan and Cullen[6] also reported an increase in chemotaxis in the presence of halothane. The results for nitrous oxide thus appear to be opposite to those of halothane.

We are unaware of any previous *in vitro* study of the effect of nitrous oxide on chemotaxis of neutrophils but Tannières reported a decrease in random movement (measured on glass) of the order of 30 per cent after equilibration with 25 and 50 per cent nitrous oxide for 30 min. Further evidence for the lack of effect of halothane on neutrophil function has been adduced.[6,7]§ In contrast to these studies, Moudgil and his colleagues[8] found that halothane (1 or 2 per cent) inhibited neutrophil chemotaxis towards casein by at least 50 per cent.

In terms of the action of nitrous oxide as an anesthetic, it appears remarkable that nitrous oxide should decrease the motility of neutrophils while halothane, acting at a relative anesthetic potency six times greater, should actually cause an increase. It is therefore tempting to suggest that this effect of nitrous oxide is not related to any property that it shares with other anesthetics but that it might be related to a chemical action such as the oxidation of a metalloporphyrin compound. Such an effect is known to occur with vitamin B_{12}[9] although it is not possible at present to explain the effect we have observed in terms of inactivation of B_{12}. It would seem that effects of anesthesia on neutrophil function should now be reexamined in the light of an effect of nitrous oxide which might have been overlooked in view of the lack of effect of 2 per cent halothane.

Finally, these observations may have relevance to the rapid fall in neutrophil count when patients breathe nitrous oxide for more than 24 h. Extravascular stores of mature neutrophils should be sufficient for 72 h and would not be affected by inhibition of DNA synthesis which is known to result from exposure of patients to nitrous oxide for 24 h.[10] It is possible that nitrous oxide

§ Rosenbaum KJ, Orkin F: The effect of halothane on in vitro phagocytosis. Abstracts of Scientific Papers, American Society of Anesthesiologists. 1973, p 71.

may affect the process of diapedesis from the extravascular stores into the venous sinuses of the bone marrow, although margination also must be considered as a possible explanation of the rapid fall in neutrophil count.

The authors thank Mr. N. Sharer, Mr. N. Luff, and Miss B. Dobson for their assistance.

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