NITROUS OXIDE AND NEUTROPHIL CHEMOTAXIS IN MAN


SUMMARY

Inhalation of 60% nitrous oxide in oxygen by seven adult male volunteers for 60 min was found to increase significantly polymorphonuclear leucocyte (PMN) chemotaxis, but not to influence total white or PMN blood cell counts. Also, the addition of morphine 0.2 mg kg\(^{-1}\) i.v. during the breathing of nitrous oxide did not alter any variable. These results indicate that nitrous oxide and nitrous oxide in combination with morphine do not depress PMN chemotaxis.

Previous studies from this laboratory have demonstrated that halothane impairs neutrophil chemotaxis (the ability of the polymorphonuclear leucocyte to move towards a bacterial chemotactic stimulus) in volunteers (Hill et al., 1977) and in patients before and during operation (Stanley et al., 1976). In volunteers, nitrous oxide partially reverses halothane-induced depression of neutrophil chemotaxis (Hill et al., 1977). The influence of nitrous oxide alone on neutrophil chemotaxis is unknown and was investigated in the following study.

METHODS

Written informed consent was obtained from seven adult male volunteers (aged 17–24 yr). Each volunteer came to the operating room between 9 and 11 a.m. after a minimum of 12 h of fasting. No premedication was administered. An i.v. infusion was commenced and a catheter placed in a radial artery. The subject remained supine throughout the study. After a blood sample (control sample) had been obtained with the subject breathing room air (9.5 ml of venous blood collected in a syringe containing 0.5 ml of a solution of beef lung heparin 1000 units ml\(^{-1}\)), the subject breathed 60% nitrogen in oxygen for 20 min and a second blood sample was obtained. The inspired gas mixture was then changed to 60% nitrous oxide in oxygen and after 20 min a second blood sample was obtained. After nitrous oxide had been administered for 60 min, morphine sulphate 0.2 mg kg\(^{-1}\) was administered i.v. and 20 min later another blood sample was obtained.

The inspired gases were administered via a semi-closed circle system with carbon dioxide absorption and a total fresh gas inflow of 5–6 litre min\(^{-1}\). Nitrous oxide and nitrogen were introduced into the anaesthetic circuit from recently calibrated flowmeters.

All blood specimens were examined for polymorphonuclear leucocyte (PMN) chemotaxis, PMN random motion and total white blood cell (WBC) and PMN counts. Data were analysed for statistical significance using Student’s paired \(t\) tests.

Leucocyte-function tests

Chemotaxis. The \textit{in vitro} method used for studying chemotaxis was that of Hill and co-workers (1975). Leucocyte-rich plasma was obtained by allowing the erythrocytes in the 10-ml heparinized blood samples to settle over a period of 1 h. After determination of the WBC and PMN counts in the leucocyte-rich plasma, using a standard haemocytometer, 0.1 ml of the suspension was diluted to 0.4 ml with tissue-culture medium 199, and the cells were deposited on one side of a 5-\(\mu\)m pore size millipore filter utilizing a Shandon cyto-centrifuge (Shandon Scientific Co., Sewickley, Pennsylvanian). The filters were placed immediately in a modified Boyden Chamber (Neuroprobe Corporation, Bethesda, Maryland) and a chemotactic stimulus was added to the attractant side. After incubation for 3 h at 37 °C, the number of cells that had migrated through the filter within 10 random fields was counted (using a 10× ocular, 45× objective and 5 mm × 5 mm photographic reticule). A chemotactic index was calculated by dividing the number of PMN that had migrated through the filter within the reticule in 10 random
fields by the total number of PMN \( (\times 10^6) \) delivered to the starting side of the filter. This method of calculating the chemotactic index takes into account only the number of PMN delivered to the filter and the number moving completely through the filter. The percentage of the total number of mononuclear cells in the suspension does not affect the chemotactic index and there is no association between the number of PMN delivered to the filter and the chemotactic index. Chemotactic assays were performed in triplicate.

Chemotactic factor. A bacterial chemotactic factor was prepared from a culture filtrate of *Escherichia coli* grown in medium 199 for 24 h at 37 °C. After passage through a 0.2-μm pore size millipore filter, the bacterial chemotactic factor was frozen at −70 °C in 1-ml ampoules. For each experiment an ampoule was thawed and diluted in medium 199 so that each millilitre of the solution was added to the bottom or attractant side of the chemotactic chamber for each analysis. The same filtrate was used throughout the study.

Random motion. Random motion of leucocytes was measured by determining the chemotactic index when the bacterial factor was omitted from the attractant side of the modified Boyden Chamber.

**RESULTS**

Sixty per cent nitrogen in oxygen did not change significantly the PMN chemotactic index, random motion or the total WBC and PMN counts (figs 1–4). Random motion and total WBC and PMN counts were also unchanged by 60% nitrous oxide in oxygen before and after the addition of morphine. In contrast, the substitution of nitrous oxide for nitrogen significantly \( (P<0.05) \) increased the PMN chemotactic index. The addition of morphine during nitrous oxide breathing did not alter the chemotactic index.

**DISCUSSION**

Early migration of neutrophils to an area of bacterial invasion has been demonstrated to be a critical factor in the prevention of infection (Mile, Mile and Burke, 1957). Neutrophil chemotaxis is an important mechanism attracting PMN to bacteria (Marsh et al., 1967). Any factor which reduces the effectiveness of this mechanism may increase the possibility of bacterial infection. Halothane 0.5–2.0% and high doses of morphine (0.5–1.1 mg kg\(^{-1}\)) in combination with 60% nitrous oxide significantly depress PMN chemotaxis. The results of the present study demonstrate that nitrous oxide alone stimulates neutrophil chemotaxis (not as a result of reduction in inspired oxygen concentration) and that the addition of small amounts of morphine (0.2 mg kg\(^{-1}\)) does not further influence chemotaxis during nitrous oxide breathing.
The mechanism of anaesthetic-induced alteration of neutrophil chemotaxis remains unclear, but it may be related to anaesthetic-induced alteration of circulating catecholamines. Previous studies have demonstrated that alpha-adrenergic stimulating agents (noradrenaline) enhance chemotaxis, while beta-adrenergic stimulating drugs (isoprenaline) inhibit chemotaxis (Hill et al., 1976). By subsequent alteration of intracellular cyclic nucleotide concentrations (increased cyclic AMP with beta-adrenergic stimulation and increased cyclic GMP with alpha-adrenergic stimulation) anaesthetic agents may alter neutrophil chemotaxis, as do alpha- and beta-adrenergic drugs. Eisele and Smith (1972) have demonstrated in human volunteers a consistent increase in plasma noradrenaline concentrations during the inhalation of 40% nitrous oxide. The same workers also reported significant increases in peripheral vascular resistance in their subjects. Smith and co-workers (1970) found that the plasma noradrenaline concentration increased when nitrous oxide was given to volunteers breathing halothane. It seems possible, therefore, that the effects of nitrous oxide on neutrophil chemotaxis observed in this and our previous study may be related to increased plasma concentrations of noradrenaline. Unfortunately, plasma noradrenaline was not measured in this or our previous study.

The absence of impaired neutrophil chemotaxis during anaesthesia with nitrous oxide and low doses of morphine in comparison with a significant depression of chemotaxis during anaesthesia with halothane, enflurane or nitrous oxide and large doses of morphine (Stanley et al., 1976) implies that the former technique may be more desirable in patients susceptible to infection. However, carefully controlled clinical investigation will be required to determine if these laboratory findings have any clinical significance.

REFERENCES


**LE PROTOXYDE D’AZOTE ET LA CHIMIOTAXIE NEUTROPHILE CHEZ L’HOMME**

On a trouvé que l’inhalation d’oxygène contenant 60% de protoxyde d’azote, par sept volontaires adultes du sexe masculin, pendant 60 min, augmentait d’une manière significative la chimiotaxie des leucocytes polymorphonucléaires (PMN), mais tout de même pas au point d’influencer la numération des globules blancs ou des PMN. L’addition de morphine par voie intraveineuse à raison de 0,2 mg kg⁻¹ pendant la respiration de protoxyde d’azote, n’a, de son côté, modifié aucun des éléments variables. Ces résultats indiquent que le protoxyde d’azote et le protoxyde d’azote allié à la morphine n’affaiblissent pas la chimiotaxie des PMN.

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**STICKOXYD UND NEUTROPHILE CHEMOTAXIS BEIM MENSCHEN**

ZUSAMMENFASSUNG

Die Inhalation von 60% Stickoxyd in Sauerstoff bei sieben erwachsenen männlichen Freiwilligen für eine Dauer von 60 min führte zu einem wesentlichen Anstieg der polymorphonuklearen Leukozyten-Chemotaxis, aber nicht zur Beeinflussung der Gesamtmenge der weissen oder der polymorphonuklearen Blutzellen. Auch die Beifügung von 0,2 mg kg⁻¹ Morphium auf intravenösem Wege während der Einatmung von Stickoxyd führte nicht zu Veränderungen in den variablen Werten. Diese Resultate zeigen, dass Stickoxyd allein oder kombiniert mit Morphium die polymorphonukleare Chemotaxis nicht unterdrückt.

**OXIDO NITROSO Y QUIMIOTAXIS NEUTROFILA EN EL HOMBRE**

SUMARIO

Se descubrió que la inhalación de un 60% de óxido nitroso en oxígeno por 7 voluntarios masculinos adultos durante 60 min aumentaba significativamente la quimiotaxis de leucocitos polimorfonucleares (PMN), pero no influyó el conteo total de células blancas ni de PMN en la sangre. Además, la adición de morfina 0,2 mg kg⁻¹ i.v. durante la respiración de óxido nitroso no alteró ninguna de las variables. Estos resultados indican que el óxido nitroso y óxido nitroso en combinación con morfina no deprimen la quimiotaxis PMN.