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EFFECTS OF GASEOUS ANAESTHETICS AND INERT GASES ON THE PHASE TRANSITION IN SMECTIC MESOPHASES OF DIPALMITOYL PHOSPHATIDYLCHOLINE

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Summary

The phase transition in smectic mesophases of dipalmitoyl phosphatidylcholine was studied under high pressures of helium (340 atm), nitrogen (340 atm), nitrous oxide (43 atm), cyclopropane (4.4 atm) and *n*-propane (8.2 atm), using a turbidimetric technique. Helium and nitrogen increased the transition temperature by 0.021 and 0.006°C/atm, respectively, compared with 0.024°C/atm for hydrostatic pressure. Nitrous oxide reduced the transition by 0.58°C/atm. The hydrocarbon gases spread the transition width and lowered the transition temperature with increasing effect at higher doses. Comparisons with other membrane probes are made and the concentration of gases in the bilayer which lower the transition temperature by 1°C are estimated, in mol%: He, 10.2; N_2, 13.2; N_2O, 9.04; *n*-C_3H_8, 6.3 and cyclopropane, 12.8.

Introduction

Inert gases (nitrogen, nitrous oxide) exert interesting narcotic effects, apparently by a mechanism similar to that of the more potent inhalational general anaesthetics. The narcotic potency of inert gases is related to their solubility in hydrophobic solvents and in some cases their effects may be reversed by hydrostatic pressure. A narcotic dose of inert gas, such as nitrogen, comprises a significant hydrostatic pressure in conjunction with the perturbation caused by the dissolved gas molecules [1]. The least soluble of the inert gases, helium, fails to narcotise animals, merely acting as a pressure-transmitting fluid. However, higher pressures of helium (>175 atm) have been shown to exert narcotic-like effects in cells by means of experiments which distinguish

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between the hydrostatic pressure and dissolved gas effects [2,3].

Multilamellar liposomes made from dipalmitoyl phosphatidylcholine or other phospholipids have been extensively used to demonstrate the structural perturbations which anaesthetics cause in bilayers [4,5]. The endothermic phase transition temperature (T_m) is lowered by anaesthetics (e.g., methoxyflurane, diethyl ether) which also broaden the thermal range (width) over which the transition proceeds [3,5]. More hydrophilic substances (C_3—C_5 alcohols) also lower the T_m but have little effect on transition width.

In contrast, high hydrostatic pressure raises T_m in accordance with the Clapeyron-Clausius equation, with no effect on transition width [6,7]. This paper examines the phase transition of dipalmitoyl phosphatidylcholine liposomes under the influence of high pressures of some inert gases and weak gaseous anaesthetics. These are small molecules and we were particularly interested to learn if a given gas would lower or raise the T_m.

Materials and Methods

Dipalmitoyl phosphatidylcholine multilamellar liposomes (Koch Light Ltd., UK, unsonicated) were prepared in 100 mM NaCl solution by a conventional method [8]. The T_m was detected by measuring the change in absorbance of a suspension (0.25 mg/ml) at 450 nm wavelength while the temperature was changed at a rate of 0.34°C/min. Absorbance and sample temperature were measured with, respectively, a Unicam SP6-200 spectrophotometer and a bead thermistor, both connected to a Bryans 2900 X-Y recorder. Pressurised gas (99.9% pure) was applied to a 2 ml suspension in a steel pressure cuvette fitted with 1 cm diameter sapphire windows, an external heat-exchange coil and an internal thermistor casing. Pressures in excess of the proprietary cylinder pressure were generated by a hydraulic intensifier [2]. Equilibration of the liposome suspension with the gas partial pressure was achieved by shaking the pressure cuvette for 4 min. Because the solubility of gases changes with temperature, equilibration was carried out close to the T_m previously estimated from preliminary experiments. Hydrostatic pressure was generated by a hydraulic pump and applied to the suspension in the absence of the gas phase through a long clean capillary tube. Both gas and hydrostatic pressures were measured with calibrated gauges to ±1%.

Results

Transition temperatures were read from the mid-point of each transition (Fig. 2). Hydrostatic pressure caused a linear increase in the T_m (dT/dP = 0.024°C/atm) with no effect on transition width (Figs. 1 and 2). Helium and nitrogen pressures increased the T_m less than the corresponding hydrostatic pressure, 0.021 and 0.006°C/atm, respectively. The more soluble gases all reduced the T_m. Nitrous oxide lowered the T_m without affecting transition width, even at the remarkably low temperature of T_m = 21°C, whereas cyclopropane and *n*-propane increased transition widths below T_m = 35—36°C (Fig. 2). Liposomes composed of cholesterol and dipalmitoyl phosphatidyl-

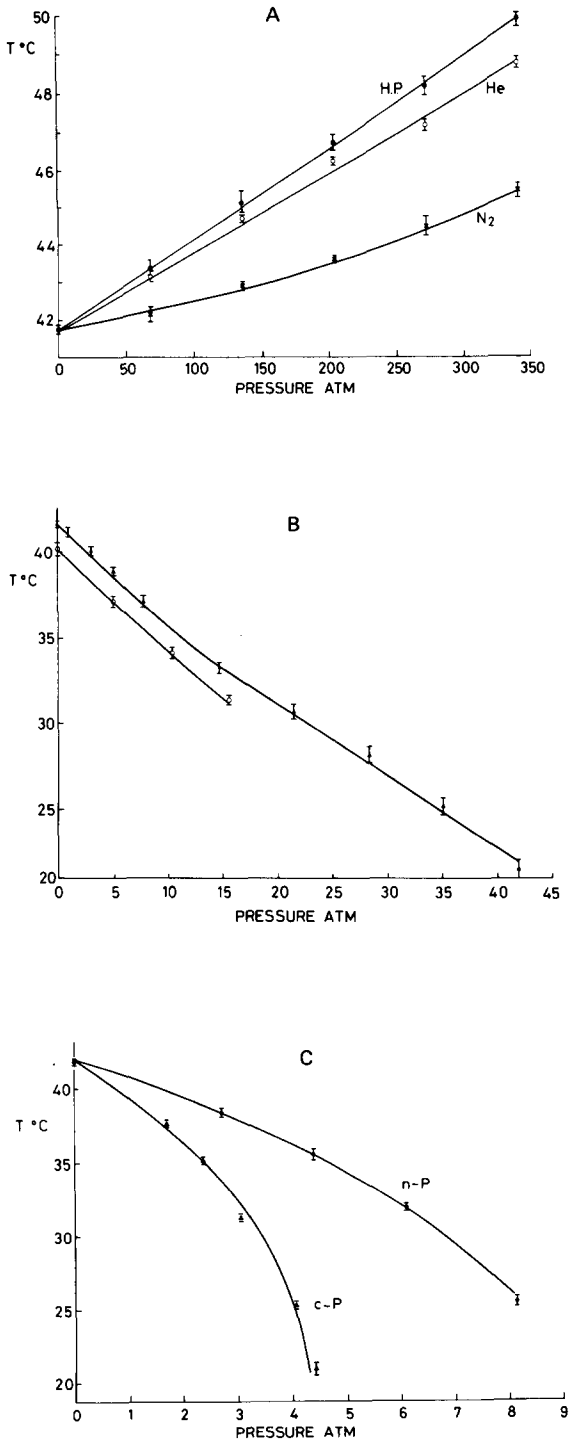

Fig. 1. Effects of inert gases and gaseous anaesthetics on the phase transition of dipalmitoyl phosphatidylcholine multilamellar liposomes. (A) N_2 (X) and He (○) compared with hydrostatic pressure (●). (B) N_2O, (▲); N_2O on cholesterol-dipalmitoyl phosphatidylcholine liposomes in the mole ratio of 16.5 : 100, (○). (C) cyclopropane and *n*-propane. All points are mean ± S.D. and the lines are drawn by inspection.

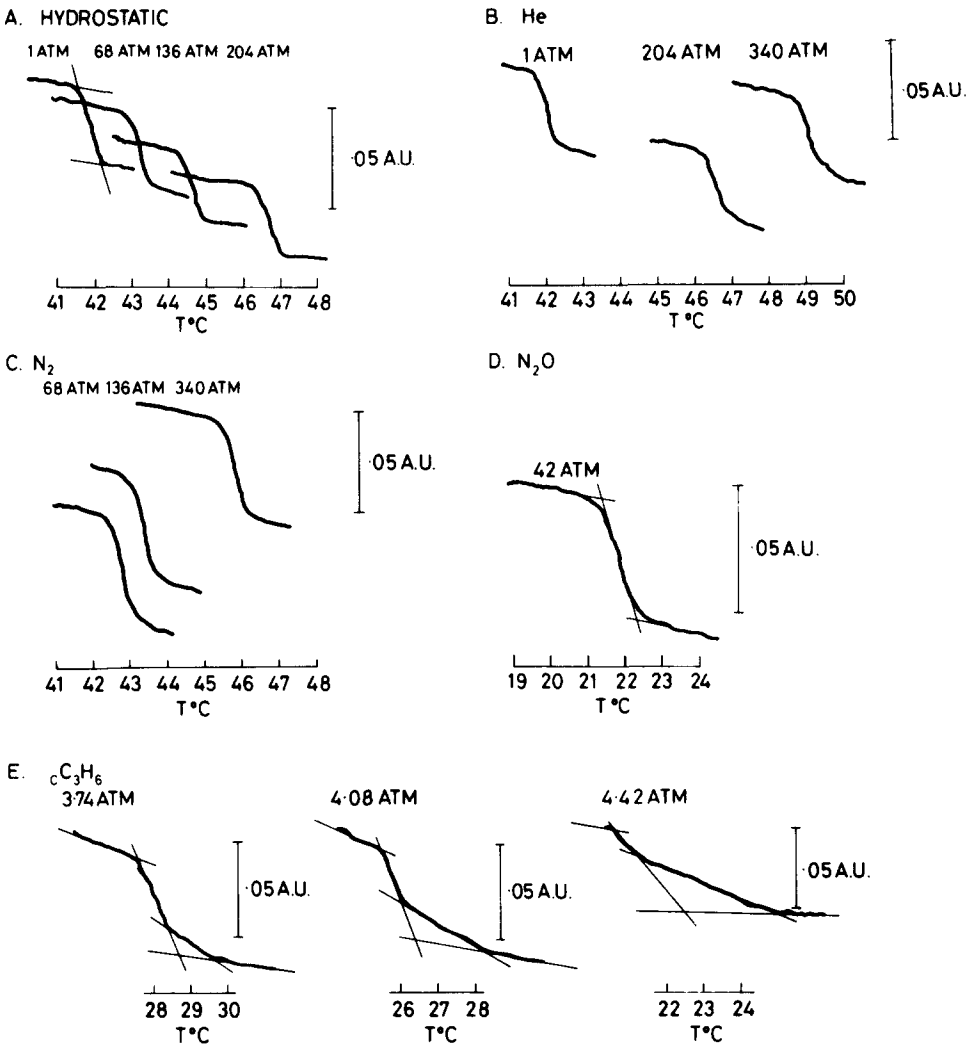

Fig. 2. The change in absorbance in a suspension of dipalmitoyl phosphatidylcholine multilamellar liposomes at the phase transition when equilibrated at the stated pressures. (A) hydrostatic pressure, (the construction lines on the 1 atm curve indicate the method of measuring T_m and transition width, see text); (B) helium; (C) nitrogen; (D) nitrous oxide; (E) cyclopropane. The lipid concentration was 0.25 mg/ml.

choline in a mole ratio of 16.5 : 100 exhibited the same response to nitrous oxide as pure dipalmitoyl phosphatidylcholine liposomes (Fig. 1).

Discussion

The dT/dP value reported here for hydrostatic pressure agrees with data obtained with ESR, light-scattering and dilatometry methods [6,7,9,10]. It also agrees with the value obtained from a sample of dipalmitoyl phosphatidylcholine liposomes which was analyzed by differential scanning calorimetry whilst pressurised by, but perhaps not equilibrated with, 136 atm helium [11].

As dT/dP is unaffected by the presence of anaesthetics [7,10], inert gas effects may, as a first approximation, be interpreted as the net result of pressure linearly increasing the T_m and the dissolved gas molecules lowering the T_m. Hydrostatic pressure has only a slight effect on the solubility coefficient of the gases used [14]. Helium would appear to offset part of the hydrostatic pressure effect in a linear fashion, without affecting transition width. The experiments show that helium pressure may be distinguished from hydrostatic pressure at 150 atm or more. Spin-labelled phospholipid bilayers exhibit a slight fluidising effect of 100 atm helium which partially offsets the ordering effect of hydrostatic pressure [12] and other experiments with growing cells reveal a distinction between helium and hydrostatic pressure at 175 atm [2].

Nitrogen offsets the effect of pressure on T_m far less than was expected from its narcotic potency [1] (Table I). Generally, the results imply that hyperbaric nitrogen favours the existence of a gel phase at temperatures higher than the normal T_m for dipalmitoyl phosphatidylcholine liposomes. However, spin-labelled phosphatidylcholine-cholesterol liposomes pressurised by 100 atm nitrogen reveal a fluidising effect of nitrogen which more than offsets the ordering effect of the hydrostatic pressure at 20°C [12]. Erythrocyte membranes spin-labelled with fatty acids and exposed to 100 atm nitrogen at 37°C are slightly ordered in the polar, peripheral region of the bilayer, slightly disordered in the intermediate region and unaffected in the hydrophobic interior [13]. Nitrogen does not affect the width of the phase transition in liposomes (Fig. 2), but its elevation of the T_m is non-linear, showing a slight upswing (Fig. 1), which is the reverse of the trend seen in its disordering effect in other spin-labelled liposomes [12].

Table I shows that the estimated concentration of gases dissolved in the bilayers which lower the T_m by 1°C differ widely from the theoretical 4.4% [15], probably because bulk solvent solubility coefficients are generally too high to be applied accurately to a bilayer. Thus, use of solubility data obtained for *n*-propane in a bilayer [18] gives the best aggrement with colligative theory (Table I).

The dose-response relationships for cyclopropane and *n*-propane show a slight downward curve in Fig. 1. Both gases spread the transition width at

TABLE I

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Gas	Partial pressure abolishing righting reflex in mice (atm) [19]	Gas pressure causing 1°C lowering of T_m [15] (atm)	Bunsen solubility coefficient, 37°C in bulk solvent	Estimated membrane concentration, for 1°C lowering of T_m (mol%)
He	non-narcotic	315.7	0.0154 [16]	10.2 *
N_2	35.0	68.0	0.0655 [16]	13.2 *
N_2O	1.5	1.72	1.23 [17]	9.04
C_3H_8	—	0.75	2.82 ** [18]	6.3
Cyclopropane	0.11	0.435	10.12	12.8

* Corrected for pressure effect on gas solubility according to Ref. 14, but see Refs. 7, 10 and 11.

** Solubility coefficient for liposomes.

similar transition temperatures, suggesting that their molecular shape has little influence on the transition.

The less hydrophobic gas, nitrous oxide, depresses the T_m without affecting transition width. It exerts the same effect in bilayers containing cholesterol (16.5 : 100, mole ratio) and we concluded that cholesterol does not affect the solubility of nitrous oxide in the bilayer. By increasing the width of the transition the hydrocarbon gases resemble other hydrophobic substances such as methoxyflurane, trichloroethylene, *N*-phenyl-1-naphthylamine and octanol [4,7,20] whilst the effects of nitrous oxide on the transition are more similar to those of hydrophilic substances such as the shorter-chain alcohols. Lee [20] has argued that hydrophobic molecules (*n*-octanol compared with shorter-chain alcohols) preferentially affect the lower end of the transition by partitioning into vacancies or dislocations in the gel state. It appears that *n*-propane and cyclopropane have the same property, and like methoxyflurane at low dosage show the further complication of a biphasic transition (Fig. 2). Even at a very high dosage (T_m = 21°C) nitrous oxide does not affect transition width.

Generally, the results show that despite their small size, inert gases, including helium, affect the phase transition and perturb bilayer structure in much the same way as the larger and more conveniently studied membrane probes.

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References

1 Miller, K.W., Paton, W.D.M., Smith, R.A. and Smith, E.B. (1973) Mol. Pharmacol. 9, 131—143
2 Macdonald, A.G. (1975) J. Cell Physiol. 85, 511—528
3 Macdonald, A.G. and Wann, K.T. (1978) Physiological Aspects of Anaesthetics and Inert Gases, Academic Press, London
4 Lee, A.G. (1975) Prog. Biophys. Mol. Biol. 29, 5—56
5 Jain, M.K. and Wu, N.M. (1977) J. Membrane Biol. 34, 157—201
6 Trudell, J.R., Payan, D.G., Chin, J.H. and Cohen, E.N. (1974) Biochim. Biophys. Acta 373, 436—443
7 Macdonald, A.G. (1978) Biochim. Biophys. Acta 507, 26—37
8 Johnson, S.M., Miller, K.W. and Bangham, A.D. (1973) Biochim. Biophys. Acta 307, 42—57
9 Plachy, W.Z. (1976) Biophys. J. 16, 138a
10 Kamaya, H., Ueda, I., Moore, P.S. and Eyring, H. (1979) Biochim. Biophys. Acta 550, 131—137
11 Mountcastle, D.B., Biltonen, R.L. and Halsey, M.J. (1978) Proc. Natl. Acad. Sci. U.S.A. 75, 4906—4910
12 Chin, J.H., Trudell, J.R. and Cohen, E.N. (1976) Life Sci. 18, 489—498
13 Finch, E.D. and Kiesow, L.A. (1979) Undersea Biomed. Res. 6, 41—45
14 Smith, E.B. (1974) in Molecular Mechanisms in General Anaesthesia (Halsey, M.J., Miller, R.A. and Sutton, J.A., eds.), pp. 112—131, Churchill Livingstone, Edinburgh
15 Hill, M. (1974) Biochim. Biophys. Acta 356, 117—124
16 Battino, R., Evans, F.D. and Danforth, W.F. (1968) J. Am. Oil Chem. Soc. 45, 830—833
17 Allott, P.R., Steward, A., Flook, V. and Mapleson, W.W. (1973) Br. J. Anaesth. 45, 294—300
18 Miller, K.W., Hammond, L. and Porter, E.G. (1977) Chem. Phys. Lipids 20, 229—241
19 Smith, E.B. (1969) in The Physiology and Medicine of Diving and Compressed Air Work (Bennett, P.B. and Elliott, D.H., eds.), pp. 183—192, Baillière Tindall and Cassell, London
20 Lee, A.G. (1977) Biochim. Biophys. Acta 472, 285—344