J. Physiol. (1985), **362**, *pp.* 233–244
With 4 text-figures
Printed in Great Britain

THE PHYSIOLOGICAL EFFECTS OF HYDROSTATIC PRESSURE ARE NOT EQUIVALENT TO THOSE OF HELIUM PRESSURE ON *RANA PIPIENS*

BY BARBARA A. DODSON, ZYGMUND W. FURMANIUK, JR AND KEITH W. MILLER

From the Harvard Medical School, Departments of Anaesthesia and Pharmacology, Massachusetts General Hospital, Boston, MA 02114, U.S.A.

(*Received 22 May 1984*)

SUMMARY

1. The effects of helium pressure and hydrostatic pressure on *Rana pipiens* were compared.

2. Both agents caused paralysis at pressures greater than 135 atmospheres (1 atm = 101·325 kPa), but the median pressure for hydrostatic-pressure-induced paralysis was 35 atm less than that for helium pressure.

3. When the ability of both pressurizing agents to reverse urethane-induced anaesthesia was compared, it was found that hydrostatic pressure raised the median dose for anaesthesia 2·2-fold more per atmosphere than did helium pressure.

4. Animals that were lightly anaesthetized by urethane at 110 atm hydrostatic pressure became more deeply anaesthetized when helium was admitted isobarically into the pressure chamber. This difference in depth of anaesthesia between hydrostatic pressure and helium pressure is consistent with helium possessing an inherent anaesthetic effect.

5. The abilities of other gases to pressure-reverse urethane anaesthesia were also determined. The degree of attenuation of the full pressure reversal effect observed with hydrostatic pressure was proportional to the lipid solubility of the gases, increasing in the order helium, neon, hydrogen, nitrogen and argon.

6. Our data on the difference between hydrostatic and helium pressure are consistent with the critical volume hypothesis.

INTRODUCTION

Much research has been directed at the effects of pressure on biological systems. These studies must, however, be interpreted with respect to the pressurizing agent used. It has long been known that mechanical compression leads to excitation in aquatic animals (Regnard, 1891). This pressure-induced excitability has also been observed in mechanically compressed flurocarbon-breathing mammals as well as in both fish and mammals compressed with helium and is referred to as the high pressure neurological syndrome (for reviews see Brauer, Hogan, Hugon, MacDonald & Miller, 1982; and Halsey, 1982). Another pressure-induced physiological phenomenon is the reversal of anaesthesia. This pressure-induced reversal of anaesthesia has been

reported for all classes of general anaesthetics using a variety of animal models (Lever, Miller, Paton & Smith, 1971; Halsey & Wardley-Smith, 1975; Miller & Wilson, 1978). Like pressure-induced excitation, pressure reversal of anaesthesia can be induced by either helium or hydrostatic pressure. Although theoretically the effects of helium and hydrostatic pressure are not equivalent (Miller, Paton, Smith & Smith, 1973), no direct quantitative comparison of the ability of hydrostatic and helium pressure to reverse anaesthesia has been made. Consequently, it is not firmly established whether helium possesses any pharmacological properties of its own which are normally masked by pressure effects.

This study directly addresses the question of whether helium has any such properties or simply acts as a pressure-transducing medium in the same way as a compressed fluid. We used amphibia to allow a comparison of hydrostatic and gaseous pressure under relatively physiological conditions. As it is much easier in these animals to quantitate the behavioural end-point of general anaesthesia than that of pressure *per se*, our study mainly uses the pressure reversal of a non-gaseous anaesthetic to probe the physiological effects of the various pressurizing media. Some consideration is also given to effects of pressure *per se*.

METHODS

Experiments were performed at 23 ± 1 °C on pre-limb bud tadpoles approximately 2·0 cm in length (*Rana pipiens*. Conn. Valley Biological Supply Co., Southampton, MA, U.S.A.). Groups of five animals were placed in neutral, oxygenated, distilled water, containing anaesthetic when appropriate, and were transferred into a 0·3 l stainless-steel high pressure chamber fitted with a Plexiglas viewing port. In the mechanical compression studies, the chamber was completely liquid filled, whilst in studies with gases, a 100 ml gas space was left which was flushed with O_2. Pressure was increased either mechanically by a hand-driven booster pump or by compressed gas from cylinders (Yankee Oxygen, Boston, MA, U.S.A. All were greater than 99·99% pure).

Hydrostatic pressure was measured by a 7000 psi Heise gauge (Model 800, Newton, CT, U.S.A.) (accuracy $\pm 0{\cdot}1$% of full scale) cross-calibrated with a Master Test gauge (Type 200, Marsh Instrument Co, Skokie, IL, U.S.A.). Gas was dissolved by rotating the chamber and equilibration was checked for by decompressing 1 ml samples via a six-way high pressure liquid chromatography sampling valve and collecting them over 2 M-sucrose in an inverted graduated tube. The volume of gas leaving solution in 1 min was compared to the literature solubility values (Wilhelm & Battino, 1973).

Helium was added under isobaric conditions to a mechanically compressed chamber via a concentric dual inlet pipe. The inner pipe was connected via a valve to a helium supply, and the outer pipe to the hydraulic booster pump and a micrometer-controlled bleed valve. After mechanical compression of the water-filled system, the helium pressure in the supply line was equalized with that of the chamber. Helium was then admitted to the chamber by opening the valve on the central inlet and allowing water to be displaced slowly through the bleed valve on the outer pipe. Approximately 100 ml of solution were vented under these isobaric conditions.

The level of anaesthesia or paralysis was defined by loss of rolling response. The animals were tipped by manually rotating the chamber, and their ability to right themselves (rolling response) was determined as previously described (Lever *et al.* 1971). Each group of animals was exposed to one anaesthetic concentration and their responses were determined at several pressures.

Dose–response curves for urethane at fixed levels of the pressurizing agents were generated. Pressure–response curves were also generated for the pressurizing agents themselves. The curves and their respective median effective doses (ED_{50}s) and slopes were analysed using a program based on the method of Waud (1972) for quantal responses. Statistical analysis on the results was performed using Student's t test, Tukey ω test, or analysis of variance as appropriate (Snedecor & Cochran, 1973).

RESULTS

Pressure–anaesthetic interactions

Anaesthesia was induced with 24 mM-urethane, a dose just sufficient to induce 100 % mean loss of rolling response at ambient pressure. When these animals were compressed mechanically to 110 atm, anaesthesia was reversed (Fig. 1 *A*) and the mean loss of rolling response fell to 20 %. Helium was then admitted to the chamber under isobaric conditions (see Methods). Approximately 1 h was required for the

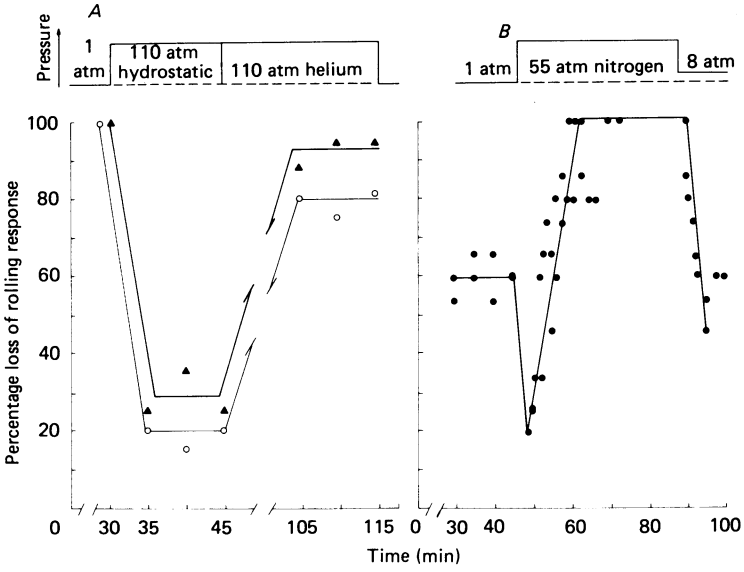

Fig. 1. The effect of pressure on a fixed anaesthetic concentration. *A*, the mean loss of rolling response in animals exposed to 0·14 mM-octanol (▲) or 24 mM-urethane (○) drops from 100 to 25 % with the application of 110 atm of hydrostatic pressure. The isobaric switching of hydrostatic to helium pressure increased mean loss of rolling response to 85 %. *B*, the mean loss of rolling response in animals exposed to 14 mM-urethane (●) first decreased from 60 to 20 % with the application of 55 atm of nitrogen. With the dissolution of nitrogen into solution, this mean loss of rolling response increased to 100 %. The response of animals partially anaesthetized with urethane at 110 atm hydrostatic pressure was independent of time over 3 h. In both Fig. 1 and 2, each point represents the mean response of five animals.

helium to reach equilibrium with the aqueous phase. During this period, anaesthesia deepened progressively until a plateau was reached with 80 % mean loss of rolling response. This helium-induced increase in loss of rolling response is consistent with helium possessing a weak anaesthetic effect. Identical results were obtained using equivalent concentrations of *n*-octanol (Fig. 1 *A*) ruling out a urethane-specific effect.

Because of the slow rate of dissolution of inert gases in water, it was possible to separate the effects of pressure *per se* from those of the compressing gas even if the compressing gas was itself an anaesthetic. Tadpoles were equilibrated with an approximately ED_{50} dose of urethane (Fig. 1 *B*) and were then rapidly compressed to 55 atm with nitrogen, a partial pressure which itself is sufficient to induce anaesthesia in greater than 50 % of the animals (Table 2). The mean loss of rolling

response fell from 60 to 20%, a degree of reversal comparable to 55 atm of mechanical compression. During the next 20 min, the dissolution of nitrogen reversed this effect and complete loss of rolling response was obtained. Subsequent decompression to 8 atm caused a transient return to approximately control level of anaesthesia followed shortly by the onset of decompression sickness which terminated the experiment.

TABLE 1. The variation of the ED_{50} for urethane anaesthesia caused by the pressurizing agents

Pressurizing agent	Total pressure (atm)	ED_{50} (mM)	Slope of dose–response curve	Number of animals
Hydrostatic	1	13·7 ± 1·3*	5·5 ± 2·0*	30
	27·5	17·5 ± 1·6	6·3 ± 2·7	25
	55	21·1 ± 1·5	6·2 ± 1·7	45
	82·5	25·5 ± 1·8	6·3 ± 2·4	30
	110	28·4 ± 1·4	8·9 ± 2·6	45
Helium	1	13·6 ± 0·7	6·8 ± 1·6	75
	55	16·0 ± 0·8	6·6 ± 1·6	65
	110	19·8 ± 0·8	7·2 ± 1·6	75
	150	22·7 ± 1·3	9·8 ± 4·4	20
Neon	1	13·8 ± 1·3	5·6 ± 2·4	35
	55	14·1 ± 1·0	6·7 ± 2·5	35
	110	14·6 ± 0·8	8·8 ± 2·9	35
Hydrogen	1	14·5 ± 1·0	5·5 ± 2·6	20
	55	13·1 ± 1·0	6·5 ± 3·0	20
	110	11·9 ± 0·8	6·5 ± 3·2	20
Nitrogen	1	14·4 ± 1·0	6·4 ± 2·3	30
	10	14·6 ± 1·0	6·6 ± 2·3	30
	20	12·4 ± 0·4	4·5 ± 1·3	40
	30	10·7 ± 1·0	3·9 ± 1·4	35
Argon	1	14·1 ± 0·9	8·5 ± 3·3	35
	10	11·9 ± 1·0	5·2 ± 1·7	35
	15	10·7 ± 1·2	4·2 ± 1·9	25
	20	8·9 ± 1·1	3·7 ± 1·7	25

* Values are means ± standard deviations.

Typical urethane dose–response curves are shown in Fig. 2. Hydrostatic pressure caused a greater rightward shift in the urethane dose–response curve than did helium pressure, whilst nitrogen caused a leftward shift. The results of analysing all such data are shown in Table 1. The slopes of the dose–response curves for urethane for the different pressurizing agents were not significantly different from each other for pressures up to and including 110 atm ($P = 0{\cdot}01$). This lack of difference in the slopes allows us to use the ED_{50} in comparisons of the relative potency of urethane under the various pressurizing conditions. Hydrostatic pressure produced the greatest reversal of anaesthesia with an 107% increase in the ED_{50} of urethane at 110 atm. Helium at the same pressure increased the ED_{50} of urethane by only 46%. At pressures up to and including 110 atm, neon failed to produce a significant change ($P = 0{\cdot}05$) from the 1 atm base-line ED_{50} of urethane. At 110 atm, the hydrogen-induced change in ED_{50} of urethane was minimal (significant at $P = 0{\cdot}05$ but not $P = 0{\cdot}01$). Nitrogen and argon both acted additively with urethane decreasing the

ED_{50} of urethane 26 and 37 % at 30 and 20 atm, respectively. The pressures of nitrogen and argon that could be studied were limited by their own anaesthetic effects. In the absence of any other anaesthetic, they caused loss of rolling response at median pressures (EP_{50}) of 42 and 24 atm respectively (Table 2). These values are somewhat higher than those reported for newts (Miller *et al.* 1973; Smith, 1974) but comparable to those reported for mice (Miller, Wilson & Smith, 1978; Smith, Smith, Eger, Halsey & Winter, 1979).

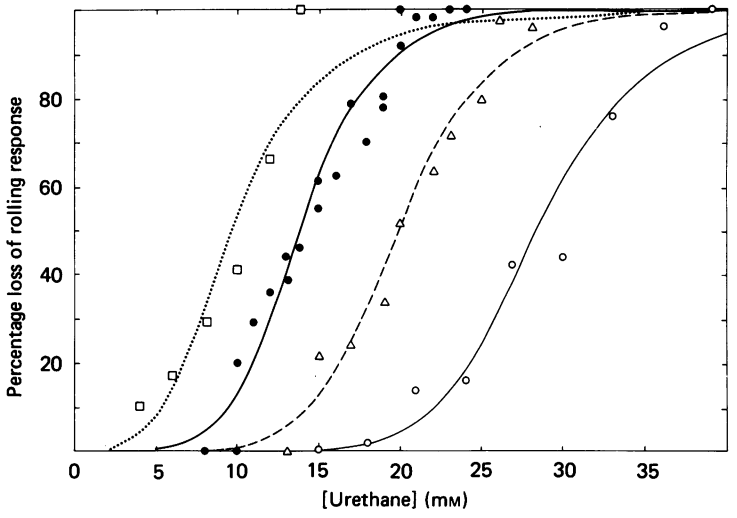

Fig. 2. Accumulative dose–response curves for urethane-induced loss of rolling response. The data were obtained under control conditions of 1 atm of pressure (●), 110 atm of hydrostatic pressure (○), 110 atm of helium (△) and 30 atm of nitrogen (□). The greater degree of rightward shift by hydrostatic pressure as compared to helium is consistent with helium possessing an intrinsic anaesthetic potency. The lines were drawn by eye.

Pressure–inert gas interactions

We observed a loss of rolling response with all the pressurizing agents, except for neon, where the maximum working pressure was limited to 120 atm (Table 2). Hydrostatic pressure caused complete loss of rolling response by 160 atm, with an EP_{50} of 137 atm. This is in the range observed with newts (Lever *et al.* 1971) and can be unambiguously assigned as a pressure effect. As with other species, nitrogen and argon both cause anaesthesia in tadpoles with the slopes of the dose–response curves of both nitrogen and argon not significantly different from that of the anaesthetic urethane ($P = 0{\cdot}05$). Both gases also reduced the ED_{50} of urethane (Table 1).

The effect of helium alone is to cause loss of rolling response with an EP_{50} of 172 atm, 35 atm higher than that for hydrostatic pressure, and in the same pressure range as the helium-induced paralysis in newts (Lever *et al.* 1971). The slope of the dose–response curve of helium is greater than that for the anaesthetic gases ($P < 0{\cdot}001$) and similar to that for hydrostatic pressure (Table 2).

We noted a mean loss of rolling response of 62 % at the maximum pressure of 220 atm of hydrogen and calculated an EP_{50} of 198 atm. On the basis of the slope of this dose–response curve and the slight additivity with urethane-induced

TABLE 2. Ability of pressurizing agents to cause loss of rolling response

Pressuring agents	EP_{50} (atm)	Slope of dose–response curve	Number of animals
Hydrostatic	137 ± 2·2*	28 ± 8·6*	50
Helium	172 ± 3·1	20 ± 5·5	50
Neon	Not determined	(No effect at 120 atm)	
Hydrogen	198 ± 12·5	6·9 ± 3·6	30
Nitrogen	41·5 ± 3·4	4·5 ± 1·1	55
Argon	24·4 ± 2·6	4·0 ± 1·2	50

* Values are means ± standard deviations.

anaesthesia, hydrogen appears to have an anaesthetic effect. As with nitrogen and argon, we found the anaesthetic potency of hydrogen closer to that reported in mice than that for newts. Hydrogen succeeded in postponing the paralysing effect of pressure to well beyond 200 atm.

Theoretical interpretation

Our current understanding of the central nervous system does not provide an adequate framework for a detailed understanding of the physiological mechanisms underlying the actions of anaesthetics or pressure. None the less, certain theoretical models have proved reasonably successful in predicting the combination of mechanical pressure, inert gas partial pressure and anaesthetic concentration which will produce the same physiologic end-point. One such model that has been successfully used to describe this interaction is the critical volume hypothesis in which anaesthesia is said to occur when the absorption of an inert substance causes hydrophobic regions of an excitable membrane to expand beyond a certain critical volume. Pressure counteracts this expansion, thus reversing anaesthesia (Miller *et al.* 1973). Thus, when an anaesthetic agent, such as urethane is in the surrounding aqueous solution, the relative expansion, $E_{50}^{P_a}$, of the hydrophobic region at the median anaesthetic dose at any pressure, P_a can be expressed as

$$E_{50}^{P_a} = \frac{\bar{V}_2 K_2 x_{50}^{P_a}}{V_m} - \beta P_a + \frac{\bar{V}_3 x_3 P_a}{V_m}, \quad (1)$$

where $\bar{V}_2$ is the partial molar volume of urethane, $x_{50}^{P_a}$ is the mole fraction concentration of urethane in the aqueous phase necessary for the above end-point and K_2 is the membrane/H_2O partition coefficient of urethane in mole fraction units. V_m is the molar volume of the site of action and β its isothermal compressibility with units of (pressure)$^{-1}$. $\bar{V}_3$, x_3 and P_a are the partial molar volume, mole fraction solubility at 1 atm partial pressure and the partial pressure respectively of the inert gas used as pressurizing agent. Because the animals are much smaller than the volume of the surrounding aqueous phase, the concentration at the site(s) of action can be assumed to be in equilibrium with the aqueous phase. The pressure cylinder was flushed with one atmosphere of oxygen (O_2) so that the total pressure, $P_T = P_a + P_{O_2}$. For simplicity of calculations, the assumption is made that this additional gas is inconsequential and that $P_T = P_a$.

The hypothesis assumes the critical relative expansion, V_c, under those conditions causing anaesthesia in half the animals, should be a constant independent of pressure in the range studied, with $V_c = E_{50}^{P_1} = E_{50}^{P_a}$, where P_1 indicates a total pressure of 1 atm. For $P_a = 1$ atm, β is inconsequential and V_c becomes the first term of eqn. 1. Therefore, assuming K_2, x_3 and β to be independent of pressure (see Miller *et al.* 1973)

$$\frac{x_{50}^{P_a} - x_{50}^{P_1}}{x_{50}^{P_1}} = \frac{1}{V_c}\left(\beta - \frac{\overline{V}_3 x_3}{V_m}\right)(P_a - P_1). \quad (2)$$

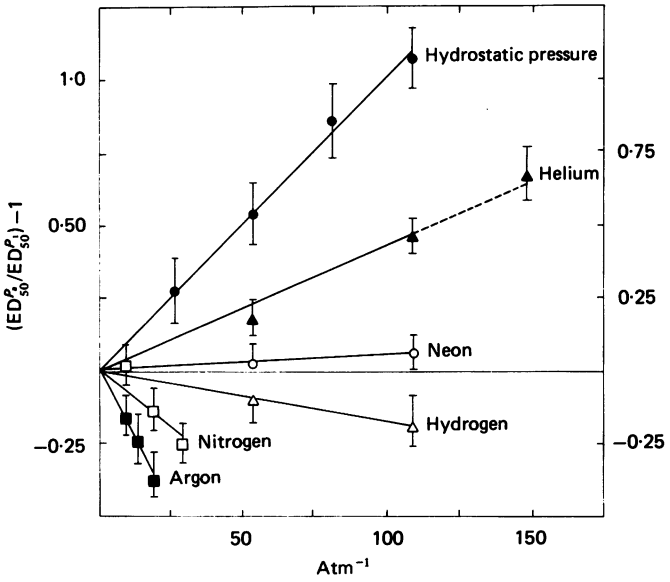

Fig. 3. The ability of mechanical compression and the gases to change the ED_{50} of urethane. The ED_{50}s of urethane for each pressurizing agent (Table 1) are expressed as a function of increasing pressure by the ratio, (ED_{50} at pressure/ED_{50} at 1 atm) − 1. The slopes for each agent were fitted by linear regression through the origin and they allowed a quantitative comparison of the ability of the agents to pressure-reverse urethane (Table 3). The difference in the slope generated for a gas from the slope for hydrostatic pressure is the experimentally determined value of Δ_3 (Table 3) where Δ_3 is formally defined in eqn. (3). Therefore, the larger the value of Δ_3, the less the ability of a gas to pressure-reverse anaesthesia and the greater its own intrinsic anaesthetic potency.

This prediction is tested in the experiments summarized in Fig. 3 where, for the pressure range studied, the dependence on pressure was essentially linear in each case (Table 3). In no case could an improvement in fit be obtained by adding a quadratic term ($P = 0{\cdot}005$). Similar linear relationships have been seen with newts under helium pressure with various gaseous and volatile anaesthetics (Miller *et al.* 1973; Smith, 1974). In mammals, however, where hyperbaric helium imposes unavoidable respiratory and thermal stresses, the linear dependence of ED_{50} on pressure remains controversial even when discussion is restricted to gaseous anaesthetics to avoid the possible pharmacokinetic problems of intravenous agents (Winter, Smith, Smith & Eger, 1976; Halsey, Wardley-Smith & Green, 1978). The linearity observed with aquatic animals is thus noteworthy.

The critical volume hypothesis further predicts the rate of change of the ED_{50} of

urethane with gaseous compression to be less than that under mechanical compression by a factor related to the volume fraction of gas dissolved at the site of action. This difference in rate of change is defined as Δ_3, the difference between the slopes of eqn. (1) for gaseous and mechanical compression. That is,

$$\Delta_3 = \frac{\overline{V}_3 x_3}{\overline{V}_2 K_2 x_{50}^P}. \tag{3}$$

TABLE 3. Results of analysis in Fig. 3 and experimental parameters used in Fig. 4

Pressurizing agent	Slope* ± S.D. ($\times 10^{-3}$)	Δ_3† ($\times 10^{-3}$)	$\overline{V}_3$†‡	x_3† ($\times 10^{-4}$) at 25 °C	
				Benzene§	Olive oil‖
Hydrostatic	9·95 ± 1·31	—	—	—	—
Helium	4·15 ± 0·75	5·81	36	0·77	7·1
Neon	0·55 ± 1·07	9·40	33	1·18	8·3
Hydrogen	−1·63 ± 0·86	11·58	35	2·58	22·8
Nitrogen	−9·57 ± 3·27	19·52	53	4·46	30·6
Argon	−18·92 ± 5·36	28·87	44·6	8·82	59·2

* From Fig. 3.
† Δ_3, $\overline{V}_3$ and x_3 as defined in eqn. (3).
‡ From Miller *et al.* (1973).
§ From Wilhelm & Battino (1973).
‖ From Miller & Smith (1973).

We have tabulated in Table 3 our experimental values of Δ_3 for the five gases studied.

In practice the hydrophobic site central to the critical volume hypothesis has often been modelled by bulk solvents such as olive oil, octanol or benzene. Using benzene to provide values for $\overline{V}_3$ and x_3, Fig. 4 illustrates the predicted linear relationship between x_3 and attenuation of pressure reversal ($r = 0{\cdot}988$). Similarly olive oil gave a comparable fit ($r = 0{\cdot}969$). When Δ_3 is plotted against ($\overline{V}_3 x_3$) to test eqn. (3), an even better fit was obtained with $r = 0{\cdot}993$ for benzene and $r = 0{\cdot}990$ for olive oil.

By reversing anaesthesia with hydrostatic pressure it is no longer necessary to assume additivity between the effects of helium and the other anaesthetic. Thus the slope of the hydrostatic pressure line in Fig. 3 defines β/V_c to be approximately 10^{-2} (see Table 3) and, since for most fluid systems β lies in the range 10^{-5}–10^{-4}, we can estimate V_c to be between 10^{-3} and 10^{-2} (0·1–1·0%), the range suggested by bulk solvent calculations. Furthermore, the partition coefficient for urethane between phosphatidylcholine:cholesterol (2:1) bilayers and buffer is 1·1 (Pang, Braswell, Chang, Sommer & Miller, 1980). Its molar volume in the solid state is 81 ml/mol and the mean molar volume of the lipids can be estimated to be 640 ml, yielding an estimated expansion, V_c, of 0·2% at an aqueous concentration of 14 mM. This expansion is well within the range estimated above and with that obtained by excess volume dilatometry for volatile anaesthetics in this bilayer (Kita, Bennett & Miller, 1981). This estimate of V_c yields a compressibility, β, of 2×10^{-5} per atmosphere, which is close to that calculated for newts using olive oil as a model solvent (Miller *et al.* 1973). Unfortunately, no experimental determination of the compressibility of this lipid bilayer has been reported.

Finally, the critical volume hypothesis has been extended to include effects of pressure. If one assumes paralysis occurs when some site is fractionally compressed

beyond a critical amount, then by similar arguments to those above, one can deduce that the compressibility of the paralysis site is higher than that for the anaesthetic site. This suggests that the site at which pressure induces paralysis is distinct from that at which it reverses anaesthesia, as previously shown in mammals (Miller *et al.* 1978; Smith, Dodson & Miller, 1984).

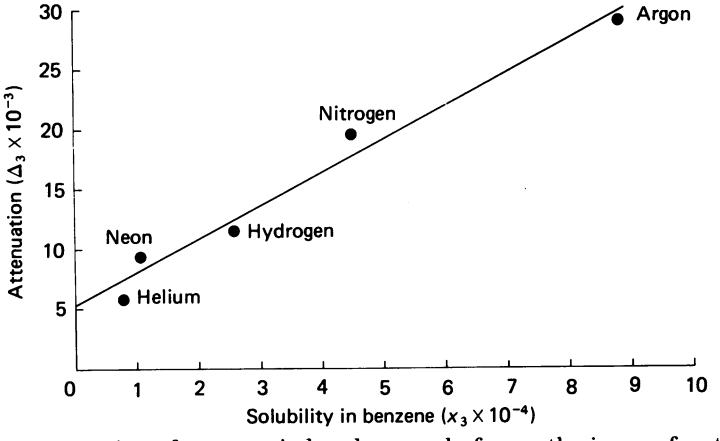

Fig. 4. The attenuation of pressure-induced reversal of anaesthesia as a function of the lipid solubility of the pressurizing gas. The lipid solubility of a gas is expressed as x_3, the mole fraction solubility in benzene (Table 3). Attenuation is expressed by the experimentally determined value of Δ_3 (Table 3). Linear regression shows a good correlation ($r = 0{\cdot}987$) between the lipid solubility of a gas and its ability to pressure-reverse anaesthesia.

DISCUSSION

Our data support the prediction of the critical volume hypothesis that helium pressure is not equivalent to hydrostatic pressure in its ability either to induce high pressure paralysis or to pressure-reverse anaesthesia. In both cases the presence of helium attenuated the effects of hydrostatic pressure. This difference is most dramatically revealed in the isobaric switching experiments where helium was admitted to a mechanically compressed chamber (Fig. 1). This manoeuvre clearly exposed the underlying anaesthetic effect of helium. More systematic studies with other gases (Figs. 2 and 3) suggest that the magnitude by which they differ from hydrostatic pressure in their ability to pressure-reverse anaesthesia is related to their lipid solubility (Fig. 4), as suggested by the critical volume hypothesis.

Whether helium and hydrostatic pressure cause equivalent effects has been recently reviewed (Brauer *et al.* 1982). The proportion of newts paralysed at 200 atm decreased in the order of hydrostatic pressure, helium pressure, neon pressure and hydrogen pressure (Lever *et al.* 1971; Miller *et al.* 1973). Barthélémy, Belaud & Saliou (1981) found that anaesthetics and helium both increased the survival time of trout mechanically compressed to 151 atm and speculated that this might be caused by the 'narcotic' properties of helium. On the other hand, in a preliminary report no difference was seen in the somatosensory-evoked potentials of helium and liquid breathing dogs compressed to pressures of up to 101 atm (Harris, Coggin, Roby, Turner & Bennett, 1982).

In principle, hydrostatic pressure and helium pressure might appear to be

equivalent under two conditions. First, some mechanism might be at work that is insensitive to the presence of inert gases (Brauer *et al.* 1982). Secondly, equivalence might occur even if an expansion mechanism similar to the critical volume hypothesis is postulated. This would require the site of action of pressure to have a high compressibility, and the solubility for helium in it to be low. In this case the difference in helium and hydrostatic pressure might be small enough to escape detection in the tolerable pressure range (Smith *et al.* 1984).

We have suggested that the decreased ability of helium to reverse anaesthesia, when compared to hydrostatic pressure, stems from its solubility in non-polar regions. Similarly, the balance between the ability of a gas to expand such regions and the compression caused by the mechanical pressure will then define whether a given gas will cause net compression (pressure-like action) or net expansion (anaesthetic-like action). For gases which cause little net effect, such as neon and hydrogen (Fig. 3), a small net volume change would result from the difference between a large expansion and a large compression term. Therefore, the dividing line between expanding and compressing gases defined by fan-shaped diagrams of the type in Fig. 3 provides a sensitive tool both for categorizing events and for defining mechanisms. This fan-shaped pattern has also been reported in the pressure reversal of nitrous oxide anaesthesia in mice by different gases (Kent, Halsey & Eger, 1976) suggesting a remarkable conservation in the site of action of anaesthesia in these two species.

The physical nature of the non-polar physiological phase in which the gases are supposed to act is not specified by the critical volume hypothesis. Data on proteins are somewhat limited and it is not yet possible to test a protein model. However, several lipid models have exhibited fan-like patterns similar to that seen in Fig. 3 (Bennett, Papahadjopoulos & Bangham, 1967; Chin, Trudell & Cohen, 1976).

Alternative models

Although the critical volume hypothesis predicted results consistent with our data, its success might have arisen by chance, and other models might also explain the interactions between pressure and anaesthetics. The first of these is the hypothesis of linear additivity of the effects of gaseous anaesthetics and of pressure (Brauer *et al.* 1982). This model proposes no mechanism but formally resembles the critical volume hypothesis and therefore provides a good description of our data.

The second model is the multi-site expansion hypothesis in which several sites of limited occupancy are allowed for anaesthetics. Physical properties, such as compressibility, of the sites are assumed to vary with both the anaesthetic employed and pressure (Halsey *et al.* 1978). The hypothesis explains the non-linearity and variability of pressure reversal observed with some intravenous anaesthetics in mammals, although pharmacodynamic explanations have not entirely been ruled out. Several of the structurally diverse anaesthetics appear to act by specific allosteric mechanisms in addition to their effects based on lipid solubility (Olsen, 1982; Miller, Sauter & Braswell, 1982; Braswell, Dodson & Miller, 1984). However, with the simple agents used in the present work, we found highly linear pressure reversal (Fig. 3), and it is sufficient to assume that all agents act at a common site by a non-specific mechanism to produce anaesthesia.

A third model assumes that pressure reverses anaesthesia by displacing gaseous

anaesthetics from their site(s) of action on a protein (Franks & Lieb, 1982). The original formulation of this model implicitly assumed that helium did not interact with the anaesthetic site. Our demonstration of the anaesthetic effects of helium is at odds with this assumption. While it is possible to modify the displacement model *post hoc* to account for our new observation, it is significant to note that the hydrophobic phase expansion (or critical volume) model actually predicted the experimental results (Miller *et al.* 1973).

Conclusion

We have shown that helium pressure differs quantitatively from hydrostatic pressure when the abilities of these agents to cause paralysis and to reverse anaesthesia are compared. Helium acts as though it possesses a weak anaesthetic potency and this property can be directly revealed by adding helium at constant pressure to partially anaesthetized animals. The behaviour under hydrostatic pressure is modified by helium and the other gases examined to a degree which is proportional to their solubility in non-polar solvents. These findings are consistent with, but do not prove, the critical volume hypothesis and reconfirm the usefulness of this hypothesis as a simple model for predicting the interactions between pressure and gases.

Supported in part by Grants GM-15904 & GM-07592 from the National Institute of General Medical Sciences.

REFERENCES

BARTHÉLÉMY, L., BELAUD, A. & SALIOU, A. (1981). A study of the specific action of 'per se' hydrostatic pressure on fish considered as a physical model. In *Underwater Physiology VII, Physiology VII*, ed. BACHARACH, A. J. & MATZEN, M. M., pp. 641–649. Bethesda: Undersea Medical Society.

BENNETT, P. B., PAPAHADJOPOULOS, D. & BANGHAM, A. D. (1967). The effect of raised pressure of inert gases on phospholipid membranes. *Life Science* **6**, 2527–2533.

BRASWELL, L. M., DODSON, B. A. & MILLER, K. W. (1984). Allosteric and nonspecific interactions of barbiturates with the acetylcholine receptor from *Torpedo*. *British Journal of Pharmacology* **81**, 30*P*.

BRAUER, R. W., HOGAN, P. M., HUGON, M., MACDONALD, A. G. & MILLER, K. W. (1982). Patterns of interaction of effects of light metabolically inert gases with those of hydrostatic pressure as such – a review. *Undersea Biomedical Research* **9**, 353–396.

CHIN, J. H., TRUDELL, J. R. & COHEN, E. N. (1976). The compressing ordering and solubility disordering effects of high pressure gases in phospholipid bilayers. *Life Science* **18**, 489–498.

FRANKS, N. P. & LIEB, W. R. (1982). Molecular mechanisms of general anaesthesia. *Nature* **300**, 487–493.

HALSEY, M. J. (1982). Effects of high pressure on the central nervous system. *Physiological Reviews* **62**, 1341–1377.

HALSEY, M. J. & WARDLEY-SMITH, B. (1975). Pressure reversal of narcosis produced by anaesthetics, narcotics and tranquillisers. *Nature* **257**, 811–813.

HALSEY, M. J., WARDLEY-SMITH, B. & GREEN, C. J. (1978). Pressure reversal of general anaesthesia – a multi-site expansion hypothesis. *British Journal of Anaesthesiology* **50**, 1091–1096.

HARRIS, D. J., COGGIN, R., ROBY, J., TURNER, G. & BENNETT, P. B. (1982). Slowing of S.E.P. late waves in gas breathing dogs compressed up to 101 bars. *Undersea Biomedical Research* **9**, suppl., 7.

KENT, D. W., HALSEY, M. J. & EGER, E. I. III. (1976). Pharmacological effects of helium, neon, hydrogen and nitrous oxide. In *Underwater Physiology V*, ed. LAMBERTSON, C. J., pp. 581–586. Bethesda: Federation of American Societies for Experimental Biology.

KITA, Y., BENNETT, L. & MILLER, K. W. (1981). The partial molar volumes of anesthetics in lipid bilayers. *Biochimica et biophysica acta* **647**, 131–139.

LEVER, M. J., MILLER, K. W., PATON, W. D. M. & SMITH, E. B. (1971). Pressure reversal of anaesthesia. *Nature* **231**, 368–371.

MILLER, K. W., PATON, W. D. M., SMITH, R. A. & SMITH, E. B. (1973). The pressure reversal of general anesthesia and the critical volume hypothesis. *Molecular Pharmacology* **9**, 131–143.

MILLER, K. W., SAUTER, J. F. & BRASWELL, L. M. (1982). A stereoselective pentobarbital binding site in cholinergic membranes from *Torpedo californica*. *Biochemical and Biophysical Research Communication* **105**, 659–666.

MILLER, K. W. & SMITH, E. B. (1973). Intermolecular forces and the pharmacology of simple molecules. In *A Guide to Molecular Pharmacology-Toxicology*, vol. 1, ed. FEATHERSTONE, R. M., pp. 427–475. New York: Dekker.

MILLER, K. W. & WILSON, M. W. (1978). The pressure reversal of a variety of anesthetic agents in mice. *Anesthesiology* **48**, 104–110.

MILLER, K. W., WILSON, M. W. & SMITH, R. A. (1978). Pressure resolves two sites of action of inert gases. *Molecular Pharmacology* **14**, 950–959.

OLSEN, R. W. (1982). Drug interactions at the GABA receptor-ionophore complex. *Annual Review of Pharmacology and Toxicology* **22**, 245–277.

PANG, K.-Y. Y., BRASWELL, L. M., CHANG, L., SOMMER, T. J. & MILLER, K. W. (1980). The perturbation of lipid bilayers by general anesthetics: a quantitative test of the disordered lipid hypothesis. *Molecular Pharmacology* **18**, 84–90.

REGNARD, P. (1891). Influence de la pression sur la vie aquatique. In *La Vie dans les Eaux*, ed. REGNARD, P., pp. 158–187. Paris: Masson.

SMITH, R. A. (1974). Investigations into the mechanism of anaesthesia: the relationships between anaesthesia and pressure, pp. 28–60. Doctor of Philosophy Thesis, Oxford.

SMITH, R. A., DODSON, B. A. & MILLER, K. W. (1984). The interactions between pressure and anaesthetics. *Philosophical Transactions of the Royal Society B* **304**, 69–84B.

SMITH, R. A., SMITH, M., EGER, E. I. III, HALSEY, M. J. & WINTER, P. M. (1979). Nonlinear antagonism of anesthesia in mice by pressure. *Anesthesia and Analgesia* **58**, 19–22.

SNEDECOR, G. W. & COCHRAN, W. G. (1973). *Statistical Methods*, 6th edn. Ames, Iowa: Iowa State University Press.

WAUD, D. R. (1972). On biological assays involving quantal responses. *Journal of Pharmacology and Experimental Therapeutics* **183**, 577–607.

WILHELM, E. & BATTINO, R. (1973). Thermodynamic functions of the solubilities of gases in liquids at 25 °C. *Chemical Reviews* **73**, 1–9.

WINTER, P. M., SMITH, R. A., SMITH, M. & EGER, E. I. III. (1976). Pressure antagonism of barbiturate anesthesia. *Anesthesiology* **44**, 416–419.