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GENERAL ANESTHETICS EXPAND CELL MEMBRANES AT SURGICAL CONCENTRATIONS

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SUMMARY

1. Surgical concentrations of halothane, chloroform, ether and methoxyflurane protected human erythrocytes from osmotic hemolysis.

2. The minimum alveolar concentration values required for human anesthesia are identical to the concentrations which produced 8 % anti-hemolysis ($AH_{8\%}$).

3. The 8 % reduction in hemolysis could be accounted for by an expansion of the erythrocyte membrane by 0.4 % in surface area, which is the same as the value predicted by other workers on the basis of pressure reversal of general anesthesia.

4. If a similar process occurs in excitable membranes, then it can be concluded that general anesthesia is associated with a membrane expansion of 0.4 %, while local anesthesia requires 2 % expansion. It is possible that the anti-hemolysis test to determine the $AH_{8\%}$ may be useful in predicting the minimum alveolar concentration for an individual person.

INTRODUCTION

The erythrocyte membrane appears to be a useful and meaningful model for studying the non-electrical effects of anesthetics on cell membranes because there are many similarities between the action of anesthetics on nerve membranes and their action on erythrocyte membranes. It is known, for example, that all lipid-soluble anesthetics protect erythrocytes from hypotonic hemolysis[1].

Although there are early reports in 1913 (see ref. 2) and in 1921 (ref. 3) of chloroform and ether having anti-hemolytic effects, the concentrations used were extremely high and far exceeded those found in the bloodstream during general anesthesia in humans. OKUMURA *et al.*[4] have found that low concentrations of halothane, methoxyflurane and ether were also anti-hemolytic, with high concentrations of these volatile anesthetics being directly lytic to the erythrocyte; the biphasic pattern was identical to that for the other anesthetics[2].

The present results indicate that halothane, chloroform, ether and methoxyflurane protect human erythrocytes from osmotic hemolysis at concentrations that are found in the blood during surgical anesthesia.

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METHODS

The method for testing the anti-hemolytic effects of the volatile anesthetics was modified from an earlier method[5] such that the cells were pre-equilibrated with the anesthetic before being subjected to hypotonic hemolysis. Fresh venous blood (human) was heparinized (50 I.U./ml blood), washed twice with isotonic medium (0.9 % NaCl in 15 mM Tris–HCl buffer, pH 7), and the erythrocytes finally resuspended in isotonic medium at a hematocrit of about 5.5 %. Aliquots of 0.2 ml of the cell suspension were incubated with 1.0 ml of a solution of the anesthetic dissolved in isotonic medium. The mixture was mixed once using a vortex mixer and then left to pre-incubate in the unstoppered 13 mm × 100 mm pyrex testtube for 5 min at room temperature (24°). An aliquot of either 1.3 or 1.4 ml, containing the anesthetic dissolved in 15 mM Tris–HCl buffer, was then rapidly injected (using a 2 ml syringe with aluminum needle) into the incubation tube while vigorously mixing the cell suspension on a vortex mixer. The tube then remained unstoppered (at 24°) for a further 10 min at which time it was centrifuged at 1500 × *g* at 24° for 90 sec. Between 6 and 12 tubes were usually done in one run, where the first and the last tubes differed by not more than 30 sec of incubation time. The supernatants were immediately decanted into cuvettes (1-cm path-length; each cuvette pre-cleaned with 0.1 M HCl and rinsed with acetone) and the absorbance read at 540 nm in a Zeiss spectrophotometer.

All glassware was pre-cleaned with chromic acid and rinsed at least 6 times with water which had been deionized and glass-distilled. Using Hamilton 1-μl syringes, the anesthetic solutions were prepared by adding aliquots of the pure drug directly into 200 ml of isotonic or of buffer medium inside a 250-ml screw-top bottle. The anesthetics were dissolved using a laboratory shaker. Because there was a 50-ml air pocket in the screw-top bottle, it could be calculated from the data of EGER *et al.*[6] that less than 3 % of each anesthetic was lost in this air pocket, and the concentrations recorded in this paper make allowance for this fact. Reproducible results with halothane were obtained when the halothane solutions were used only once after opening the freshly prepared stock bottles. The halothane used was obtained from Ayerst Labs., Montreal; the methoxyflurane was from Abbott Labs., Montreal; the chloroform and diethyl ether were from Fisher Scientific, Toronto.

RESULTS

Low concentrations of halothane, chloroform, ether, methoxyflurane and ethanol all protected human erythrocytes from osmotic hemolysis. This is shown in Fig. 1 and Table I. The value of 1.0 on the scale of relative hemolysis (ordinate in Fig. 1) indicates 25 % hemoglobin release which occurred in the absence of any drug.

The anesthetic concentration which had an anti-hemolytic effect of 8 % (*i.e.* down to a relative hemolysis of 0.92 in Fig. 1) was denoted by $AH_{8\%}$. These values for $AH_{8\%}$ were obtained from Fig. 1 where the dose-response curves intersect the relative hemolysis value of 0.92. The $AH_{8\%}$ values obtained experimentally at 24° are listed in the first column of Table I.

In order to compare the $AH_{8\%}$ value to the value for the human minimum alveolar concentration required for general anesthesia[7,8], it was necessary to convert the $AH_{8\%}$ at 24° to the value at 37°. Direct measurement of the $AH_{50\%}$ as a function of

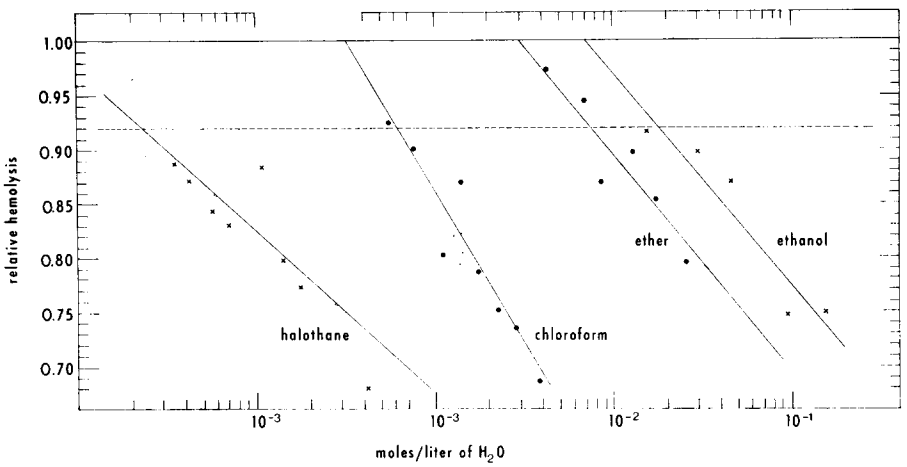

Fig. 1. Volatile anesthetics protect human erythrocytes from hypotonic hemolysis at surgical concentrations. The relative hemolysis value of 1.0 represents 25% hemoglobin release. The $AH_{8\%}$ values are obtained from the intersection of the dose–response line and the interrupted line at 0.92 relative hemolysis.

TABLE I

ERYTHROCYTE PROTECTION BY GENERAL ANESTHETICS

The table shows detailed values used in deriving the molarities for the $AH_{8\%}$ at 37°, and the human minimum alveolar concentration at 37°.

	$AH_{8\%}$ (*at 24°*) *moles/l*	$AH_{8\%}$ (*at 37°*) *moles/l***	*Human minimum alveolar concn. at 37°*			
			% atm[6]	*Molarity in air*	*Water/gas****	*Molarity in water*
Halothane	2.2×10^{-4}	2.9×10^{-4}	0.76	3×10^{-4}	0.85	2.6×10^{-4}
Chloroform	6.1×10^{-4}	8×10^{-4}	0.67*	2.6×10^{-4}	3.8	9.9×10^{-4}
Ether	7.5×10^{-3}	9.8×10^{-3}	1.92	7.5×10^{-4}	13	9.8×10^{-3}
Methoxyflurane	2.7×10^{-4}	3.5×10^{-4}	0.16	6.3×10^{-5}	4.5	2.9×10^{-4}
Ethanol	1.8×10^{-1}	2.4×10^{-1}				3.9×10^{-1}†

* Normalized according to ref. 6; 0.67% = 0.77% · (0.76%/0.87%), where 0.77% is the chloroform minimum alveolar concentration for dogs, 0.76% is the halothane minimum alveolar concentration for humans, and 0.87% is the halothane for dogs[6].

** $AH_{8\%}$ at 37° = 1.3 · $AH_{8\%}$ at 24° since it requires a 30% higher anesthetic concentration to yield the same anti-hemolytic effect at 37° (ref. 9).

*** Ostwald water/gas partition coefficients[6,7].

† Concentration which maintains sleep[25].

temperature[9] showed that the $AH_{50\%}$ increased by an average of 30 %, on elevating the temperature from 24 to 37°. In order to convert the $AH_{8\%}$ at 24° to the value expected at 37°, therefore, the $AH_{8\%}$ values were multiplied by the average factor of 1.3, and the net result is shown in Table I. It is generally observed that more anesthetic is required to exert the same effect at elevated temperature[6,9,10]. The results of EGER *et al.*[6], for example, indicated that the halothane minimum alveolar concentration for dogs was 0.346 mmole/l air (= 0.88 %) at 37°, and 0.126 mmoles/l air (= 0.32 %) at 24°; since the water/air coefficients were 0.85 and 1.58, respectively, the

halothane molarities in the aqueous phase of the alveolar blood were 0.294 and 0.199 mmoles/l water, respectively, a difference of about 33 %. A similar calculation for cyclopropane[6] indicates that the minimum alveolar concentration dropped from 17.4 to 14.9 mmoles/l alveolar water going from 37 to 24°, a difference of about 15 %.

The data in Table I indicate that the values for $AH_{8\%}$ at 37° are very similar or identical to the human minimum alveolar concentration required for general anesthesia (see Fig. 2).

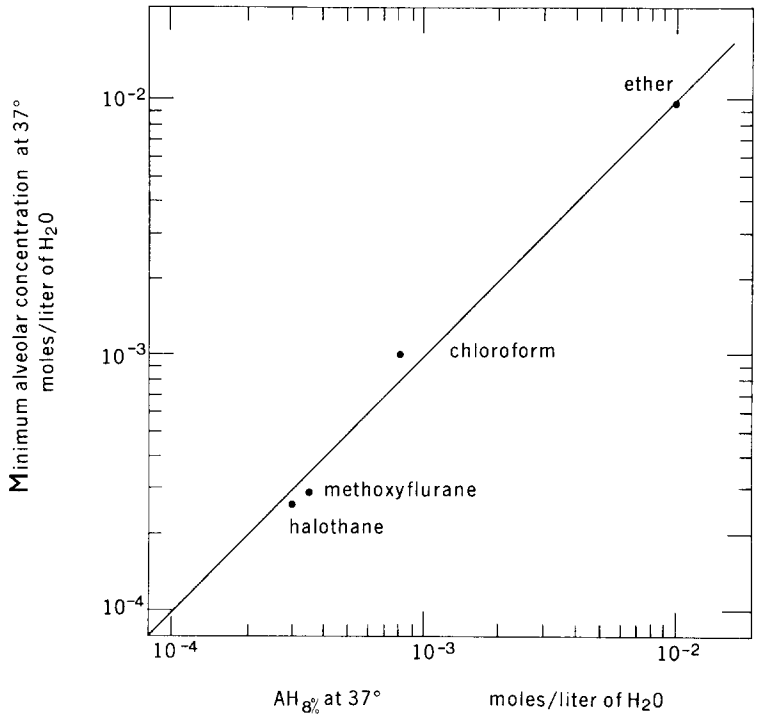

Fig. 2. The concentrations of volatile anesthetics required for human anesthesia (minimum alveolar concentration) are virtually identical to the $AH_{8\%}$ values at 37°. The $AH_{8\%}$ values are for 1 person only, while the minimum alveolar concentration values (taken from ref. 6) were obtained from a population of people.

By extrapolation the anesthetic concentrations which caused 50 % anti-hemolysis ($AH_{50\%}$) were $1.05 \cdot 10^{-2}$ M for chloroform, $2.6 \cdot 10^{-1}$ M for ether, 10^{-1} M for halothane, $1.1 \cdot 10^{-2}$ M for methoxyflurane, and 1.6 M for ethanol. The $AH_{50\%}$ value for ethanol is approximately the same as that recently reported by SANDERSON *et al.*[11].

DISCUSSION

These results confirm earlier observations[2-4] that chloroform and ether inhibit hypotonic hemolysis. The results extend this phenomenon to other volatile anesthetics and ethanol and demonstrate that the anti-hemolytic effects can be detected at very low concentrations of the volatile anesthetic.

There may be important theoretical and practical consequences of the fact that

the volatile anesthetic concentrations for protecting human erythrocytes ($AH_{8\%}$ at 37°) have values which are close to the human minimum alveolar concentrations required for general anesthesia.

From a theoretical point of view it is possible to calculate the amount of biomembrane expansion which would be expected to occur when human beings are under general anesthesia. It is known that lipid-soluble anesthetics expand the membrane area of erythrocytes[12,13] and that this drug-induced membrane expansion can completely account for the anti-hemolytic effect[14]. At 8 % anti-hemolysis, the erythrocyte membrane expands by about 0.4 % in area[13]. There is reason to believe that the same physicochemical process of drug-induced membrane expansion may take place in nerve membranes, because all lipid-soluble anesthetics inhibit hypotonic hemolysis and stabilize nerve membranes at about the same concentrations[1], and because both erythrocyte and synaptosome membranes are fluidized at the same concentrations and to the same extent by anesthetics [15]. Tentatively, it may be concluded, therefore, that general anesthesia is associated with a membrane expansion of about 0.4 % in surface area of nerve cell membranes.

Previous work[13] indicates that a drug concentration which elicits local anesthesia is associated with a membrane expansion of about 2 % in surface area. At clinically useful concentrations, therefore, the general anesthetics exert about one-fifth the membrane effect (0.4 % expansion) compared to local anesthetics (2 % expansion). This is not surprising since general anesthetics are thought to anesthetize small diameter nerve fibers more readily than large fibers[16].

It is known that general anesthetics inhibit the emission of light by luminous bacteria[17,8]. If this is a direct effect of the anesthetics on the luciferase enzyme, then it is possible to estimate the amount of drug-induced expansion of the enzyme. EYRING and his colleagues (see ref. 17 for complete references) have found that the activated state of the enzyme is "more voluminous by 54 cm^3/mole than the initial state, which corresponds to the compact native enzyme". They further found that the inactive or partially unfolded state of the enzyme is more voluminous by 20 cm^3/mole than the activated state. As EYRING[17] states, the addition of hydrophobic anesthetic molecules unfolds the enzyme, assuming that the anesthetic directly acts on the luciferase. Accordingly, the anesthetic suppression of the bacterial luminescence is associated with an expansion of the luciferase by about 20 cm^3/mole enzyme. Bacterial luciferase has a molecular weight of about 80000 (ref. 18), corresponding to an approximate volume of about 60000 cm^3/mole enzyme. The anesthetic-induced expansion, therefore, amounts to 20 cm^3 per 60000 cm^3, or the order of 0.03 % expansion in volume. This value of 0.03 % expansion for the anesthetic-induced expansion of luciferase is based on extremely indirect data, involving pressure and temperature experiments on the luciferase within the intact bacteria. There are no experiments reported describing the effect of anesthetics or hydrophobic drugs on purified luciferase. It is possible that the anti-luminescent effects may be secondary to anesthetic-induced alterations in the cytoplasmic levels of Ca^{2+}, Mg^{2+} or other ions which may act as ligands for this enzyme[19]. It is known, for example, that low concentrations of volatile anesthetics increase the binding of Ca^{2+} to the cell membrane[20]; this might alter the cytoplasmic Ca^{2+} in the bacteria.

In addition to this similarity of drug-induced expansion, it is known that anesthesia is reversed by high atmospheric pressure[21,17]. High pressure also restores the per-

meability of lipid vesicles which are permeable in the presence of anesthetics[22]. LEVER *et al*[21] have calculated that the pressure which reverses anesthesia is associated with a compression of 0.4 %, a value identical to that found in the present study for the general anesthetic-induced expansion of the membrane. These observations lend further strength to the idea that membrane expansion is an important concomitant, if not the essential requirement, of cell membrane anesthesia. The analogy between anesthetic-induced expansion of the membrane and of the luminescent protein is depicted in Fig. 3. This figure also illustrates that re-compression of the membrane returns the excitability of the membrane back to its original state.

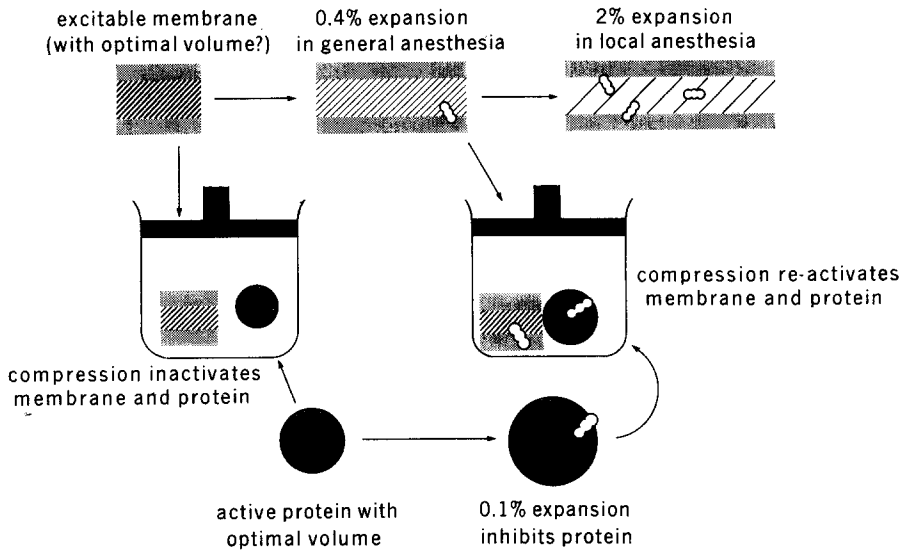

Fig. 3. Analogy between anesthetic-induced expansion of membranes and expansion of luminescent protein. General anesthetics expand both the membranes and the luminescent protein by about 0.1–0.4% in area. Recompression in the presence of the anesthetic re-activates both the luminescence and the animal's excitability[17].

It is known that the mean minimum alveolar concentration value of halothane in chronic alcoholics is about 1.3 % (refs. 23, 24) and that it is considerably higher than the mean halothane for normal people (0.78 %, refs. 6, 24). It has also been observed by DUNDEE[25] that 2 patients who were habitual drinkers of ethanol required a venous blood level of ethanol of between 230 and 280 mg per 100 ml in order to induce sleep; this compared with an average value of about 180 mg per 100 ml for normal people. It would be interesting to test the anti-hemolytic effects of halothane and of ethanol on erythrocytes from these habitual drinkers of ethanol. It may be that the chronic alcoholism brings about a state of "reduced expandibility" of the membrane.

Further refinements in the experimental methods for testing the anti-hemolytic effects of general anesthetics may someday lead to a simple anti-hemolysis test wherein the minimum alveolar concentration for a particular compound may be predicted for a particular patient.

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