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Alcohol effects on lipid bilayer permeability to protons and potassium: relation to the action of general anesthetics

G.L. Barchfeld and D.W. Deamer

Departments of Animal Physiology and Zoology, University of California, Davis, CA (U.S.A.)

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Past work has shown that general anesthetics perturb the membranes of isolated synaptic vesicles, thereby increasing permeability to protons and inhibiting the ability of the vesicles to take up catecholamines. It has been proposed that such effects may produce anesthesia through inhibition of synaptic transmission. The mechanisms of perturbation is unknown. Two possible explanations include alterations of dielectric constant or production of defects as anesthetics partition into the bilayer phase. In order to choose between these alternatives, we measured the effect of nine alcohols and two alkanes on liposome permeability to protons and potassium. Ionic permeability was increased by alcohols and alkanes to similar degrees, thereby ruling out direct effects on the membrane dielectric constant caused by partitioning of anesthetics into the bilayer. Other experiments confirmed earlier reports that the enhanced permeability caused by anesthetics is not specific for protons. We conclude that these membrane perturbants act by increasing the number of transient, ion-conducting defects normally present in the bilayer structure.

Introduction

A remarkable variety of small, relatively nonpolar molecules as diverse as chloroform, diethyl ether and *n*-butanol are able to partition into neuronal membranes and in some manner inhibit excitability and synaptic transmission, thereby producing the phenomenon of general anesthesia. Because the action of anesthetics involves complex cellular and even tissue level function, little progress has been made toward understanding the mechanism at the molecular level. Two alternative approaches to this question are now being considered. The first assumes that the primary action of anesthetic molecules involves the lipid bilayer, either by altering certain physical properties directly or by increasing permeability to ions [1–8]. The second proposal is that anesthetic molecules act on membrane proteins involved in nervous system function [9,10] with only minor effects on the lipid moiety (see Refs. 11–14 for review).

Using the assumption that the primary site is the lipid bilayer, Bangham and Mason [15] suggested that anesthetics may increase the functional proton permeability of synaptic vesicle membranes beyond the ability of the proton pump to maintain the pH gradient required for accumulation of neurotransmitter (See Ref. 16 for review). Anesthesia ensues when synaptic transmission is inhibited in CNS tissues that produce arousal and response to stimulation. This concept, the pump-leak hypothesis, has been tested in previous stud-

Correspondence: D.W. Deamer, Department of Zoology, University of California at Davis, Davis, CA 95616, U.S.A.

ies. First, it was shown that general anesthetics did in fact increase proton permeability of liposomes, and that the ability of synaptic vesicles to accumulate and maintain dopamine gradients was inhibited by similar levels of anesthetics [15]. The effect on liposome proton permeability was confirmed by our laboratory [17] and extended to comparisons of proton and potassium permeabilities in liposomes.

In the present study, we have further tested the pump-leak hypothesis by investigating bilayer permeability to potassium ions and protons. We were particularly interested in relating the permeability effects to partition coefficients of the anesthetics and the dielectric constant of the hydrophobic lipid phase. Therefore, the effects of nine alcohols and two alkanes on membrane permeability were systematically assessed in liposomes.

Methods

Preparation of liposomes

Egg phosphatidylcholine and egg phosphatidic acid (Avanti Inc., Birmingham, AL) at mole ratios of 9:1 were combined and the organic solvents removed by evaporation under nitrogen gas, followed by evacuation for 30 min. Large unilamellar vesicles were then prepared by reverse phase evaporation [18] by dissolving lipids in 6 ml anhydrous diethyl ether and 2 ml aqueous phase, followed by vortexing and bath sonication to produce a homogeneous dispersion. The organic phase was removed by rotary evaporation under reduced pressure in a flash evaporator for 15 min; 4 ml aqueous phase was added, and rotary evaporation continued an additional 40 min. The resulting vesicles were then sized by filtering ten times through 0.1 μm pore polycarbonate filters [19] using approximately 200 psi nitrogen pressure.

In a typical run, 4-ml aliquots containing 20 mM lipid were prepared as described above, then passed through Sephadex G-50 columns to a final lipid concentration of 10 mM. Internal solutions contained 0.2 M potassium sulfate and different buffer concentrations depending on the ion to be measured. When potassium ion flux was monitored, the external solution contained choline chloride, which was previously recrystallized from absolute ethanol to remove any trace amines or ammonium salts. The ethanol was removed by vacuum filtration, and the choline chloride crystals were stored under vacuum in a dessicator. In some experiments the alcohols were passed through Dowex anion and cation exchange resins to remove trace ionic contaminants (none was detected). Experiments were performed at ambient temperature (near 23° C).

Proton flux measurements

Proton flux was measured in two ways to be certain that results were not a function of the experimental method being used. In the first, liposomes were prepared with 50 mM buffer inside and 1 mM buffer outside (see legends for details) and external pH was monitored by a Fisher Accumet pH meter attached to a chart recorder [20]. External pH was allowed to equilibrate with the internal pH, and after equilibrium was reached, a small pH gradient was produced by the addition of dilute sulfuric acid. Potassium sulfate (0.2 M) was present to reduce any osmotic or ionic strength effects to negligible levels, and valinomycin was added to allow counterion movement to compensate for proton flux. This procedure was repeated several times for each sample, with each measurement taking 10 to 20 min. Calibration was done by the addition of known amounts of acid.

The second method was modified from Kano and Fendler [21]. Pyranine (0.5 mM) was encapsulated together with buffer and potassium sulfate, as described above, and known pH gradients ranging from 0.3 to 1.3 units were established by adding 0.05 M sulfuric acid. The initial rate of decay of the gradient was measured by decreasing fluorescence of the dye as the interior pH became acidic (excitation 430 nm, emission 515 nm). At the end of an experiment, the liposomes were made permeable to ions by gramicidin A addition, and the fluorescence change was calibrated by back-titration with 0.1 M sodium hydroxide.

J (net proton flux) was calculated from the equation

$$J = \frac{C}{A} \cdot \frac{\mathrm{dpH}}{\mathrm{d}t}$$

where net proton flux is defined as moles proton equivalents (hydrogen and hydroxide ions) per

unit area per second; dpH/dt, calculated from the initial slope of pH vs. time, is the rate of change in pH; C is the calibration relating change in pH to moles of proton equivalents; and A is the calculated total surface area of the liposomes based on total lipid present, assuming 0.7 nm^2 per lipid molecule [22].

Potassium flux measurements

Leakage of trapped potassium from the filtered potassium loaded liposomes, in aliquots of approx. 10 μmoles lipid, was measured with an ion selective electrode and Fisher Accumet pH meter.

J (net potassium flux) was calculated from the equation

$$J = \frac{C}{A} \cdot \frac{dK^+}{dt}$$

where dK^+/dt is the initial slope of the moles of potassium ion per second appearing in the external choline chloride medium, monitored with the calibrated potassium electrode; C is the conversion factor relating electrode output in millivolts to potassium concentration, and A is liposome surface area. Because protons are many orders of magnitude more permeable than potassium ions [20] they are able to act as counter ions so that diffusion potentials do not limit potassium flux.

Additions of normal alcohols and diols

The *n*-alcohols (ethanol, propanol and butanol) were added directly to the liposome preparations in the desired concentrations. To minimize exposure of the liposomes to high concentrations of alcohols, the alcohols were rapidly mixed (less than one second) with the liposomes. The diols and glycerol (up to 5 M) were prepared ahead in appropriate buffers and mixed with the desired concentrations of lipid in the form of liposomes. Franks and Lieb [10] noted that normal alcohols, particularly those with longer chains, do not immediately equilibrate with the membrane phase. Therefore liposomes were incubated with the various alcohol concentrations for at least 15 min before making proton flux measurements following a pH shift. (This was not possible with potassium flux measurements. These were estimated from initial rates of efflux following dilution of potassium containing liposomes into choline chloride solutions with the desired concentrations of alcohols present.)

In some experiments, the effects of pentane and hexane on proton permeability were similarly tested. Because these compounds are relatively insoluble in aqueous phases, their concentrations were adjusted as 'activities' rather than molarities [23] where an activity of 1.0 represents a saturated solution of the alkane in buffer.

Results

Fig. 1 shows typical data produced by the two methods for monitoring proton flux. Absolute values for proton flux measured by the pyranine method and the pH meter agreed within a factor of 2. The differences in absolute flux values obtained by the two methods probably arise from the fact that calibration of the pyranine method requires internal volume to be estimated from the amount of trapped pyranine dye. The pH meter does not require this estimate, and simply measures flux of proton equivalents across a known area of lipid bilayer in the form of liposomes. Both methods provided very similar data for relative changes in flux produced by the alcohols, and this increased our confidence in the results reported here. It should be noted that valinomycin was added in mole ratios of 1 : 500 with the phospholipid to prevent proton diffusion potentials from interfering with proton flux measurements. Flux was estimated from initial rates that were approximately linear and developed within 5–10 s after the pH gradient was established.

Examples of potassium flux measurements are illustrated in Fig. 2, which shows the effect of 1,2- and 1,4-butanediol on this parameter. Flux was calculated from initial rates, using a calibration equation for the electrode which relates the logarithm of potassium concentration to electrode output in millivolts. Note that higher concentrations of the diols increased flux rates several-fold. The addition of gramicidin A released the total potassium trapped in the liposomes, thereby providing an estimate of encapsulation efficiency.

Fig. 3 shows a comparison of proton flux measured by the pyranine method as affected by increasing concentrations of *n*-butanol, 1,2-

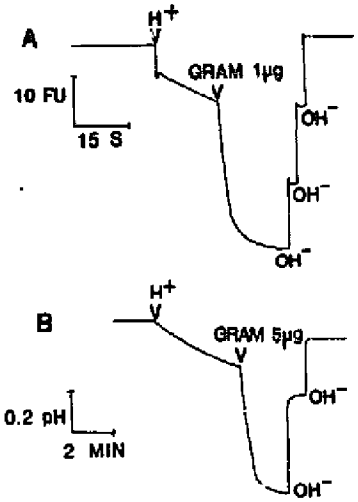

Fig. 1. Comparison of methods for monitoring proton flux. (A) The fluorescence of encapsulated pyranine (0.5 mM) was monitored at 430 nm excitation and 515 nm emission. In this experiment, liposomes containing 0.2 M potassium sulfate and 5 mM buffer (pH 7.4, see below) were suspended in 2 ml of the same medium. An acid pulse (H^+) lowered the external pH to 6.6, producing a pH gradient of 0.8 units. Valinomycin was present at a mole ratio of 1:500 with the phospholipid (1.0 mM egg phosphatidylcholine:phosphatidic acid, 9:1 mole ratio). After an initial rate was obtained, the gradient was released by addition of gramicidin A (GRAM) followed by calibration of the trapped dye with known additions of potassium hydroxide (OH) in increments of $0.88 \cdot 10^{-6}$ mol. Net proton-hydroxide flux was calculated from the initial rate of change in fluorescence, using the calibration and volume ratio as described in Methods. (B) The trace is from a recording pH meter, and the conditions were the same as in (A) except that phospholipid concentration was 5 mM, and the external buffer concentration 1.0 mM to permit measurement of pH changes associated with proton flux. To provide a substantial source/sink of protons, the internal buffer was 0.2 M, with external potassium sulfate concentrations adjusted appropriately to provide osmotic balance. The buffer was an equimolar mixture of Aces, Mes, Tes and Tricine (Good buffers, Sigma, Inc. St. Louis, MO) which gives essentially linear buffer capacity between pH 6 and 8. Concentrations are expressed as total buffer ions present.

butanediol and 1,4-butanediol. The marked effect of butanol on proton permeation has been reported in earlier studies [17] and related to anesthetic mechanisms. The 1,2- and 1,4-diols were much less effective, as might be expected from their lower partition coefficients, and aqueous concentrations in the molar range were required to increase proton flux by 2–3-fold. The two diols were approximately equivalent in their effect on potassium flux (Fig. 4).

Because the 1,4-diol has been reported to have a significantly higher membrane partition coefficient than the 1,2-diol (0.268 vs. 0.06, calculated in Ref. 24) it was surprising that the 1,4-diol had less effect on proton permeability. Therefore we determined the octanol-water partition coefficients for the two diols by permitting 204 mg of each to distribute between 1.00 ml of water and *n*-octanol. Following 1.0 min vortexing and 1 h incubation at 25°C, 200 µl aliquots of the clear supernatant were removed, and the content of the diols was

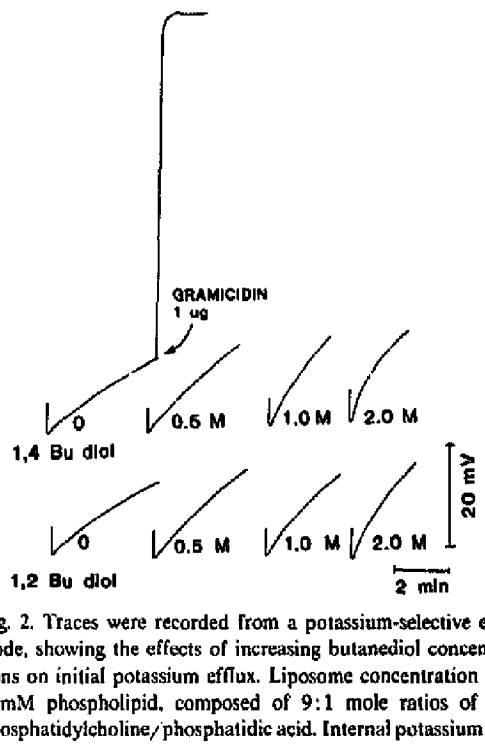

Fig. 2. Traces were recorded from a potassium-selective electrode, showing the effects of increasing butanediol concentrations on initial potassium efflux. Liposome concentration was 5 mM phospholipid, composed of 9:1 mole ratios of egg phosphatidylcholine/phosphatidic acid. Internal potassium was 0.4 M (0.2 M K_2SO_4/5 mM buffer, pH 8.0) and the liposomes were filtered through Sephadex G-50 into 0.3 M choline chloride/5 mM buffer just before beginning flux measurements. Butanediol was added directly to the cuvette to produce the desired final concentrations, followed by choline chloride/buffer to 0.6 ml. Aliquots of the liposome suspension (0.6 ml, final volume 1.2 ml) were then added to initiate flux. Potassium flux was calculated from the calibration of millivolt response to known potassium chloride additions as described in Methods. Note the different initial rates for the traces in the presence and absence of 1,2- and 1,4-butanediol.

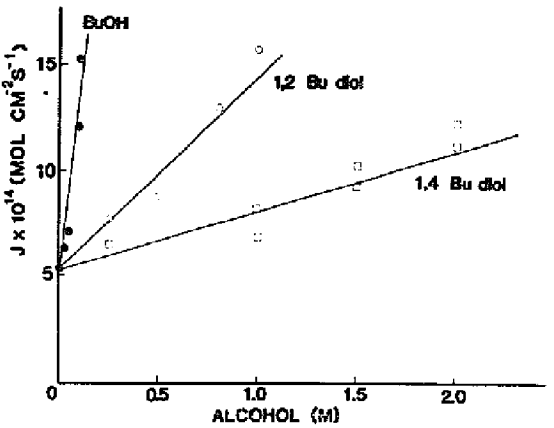

Fig. 3. Effect of *n*-butanol and butanediols on proton flux, measured by the pyranine method (see Fig. 1.) *n*-Butanol markedly increased the flux of protons at relatively low concentrations, while similar increments could only be produced by butanediols at molar concentrations. Note that the 1,2-diol had a significantly greater effect than the 1,4-diol.

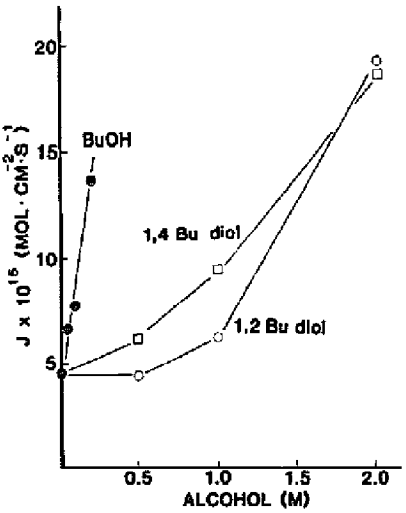

Fig. 4. Effect of *n*-butanol and butanediols on potassium flux. Butanol (0.05 M) increased potassium flux about 2-fold, similar to its effect on proton flux (see Fig. 3). However, there was little difference between 1,2- and 1,4-butanediol, in contrast to their markedly different effects on proton flux.

determined by gas chromatography-mass spectrometry. Standards were also run for each sample by known additions of diols to *n*-octanol which had been similarly treated. The partition coefficients for the 1,2- and 1,4-butanediol were 0.172 and 0.050, respectively, and these values were used

TABLE I

CONCENTRATIONS AND MEMBRANE PARTITION COEFFICIENTS OF *n*-ALCOHOLS AND ALKANES AFFECTING PROTON/POTASSIUM PERMEABILITY IN LIPOSOMES.

C is the aqueous concentration (molar) required to increase proton or potassium flux 2-fold under the experimental conditions described in Methods. These values were estimated from data similar to that illustrated in Figs. 3 and 4. $P_{m/b}$ is the membrane partition coefficient taken from Ref. 24, and C_m is the membrane concentration of each solute calculated from C and $P_{m/b}$. In the case of pentane and hexane, proton flux was doubled at 0.1 activity of the alkane (one-tenth saturated solution) and *C* was calculated from their solubility in water [34].

	C (M)		$P_{m/b}$	C_m	
	H^+	K^+		H^+	K^+
Alcohols					
Ethanol	1.1	–	0.096	0.10	–
Propanol	0.175	0.2	0.438	0.077	0.087
Butanol	0.05	0.1	1.52	0.076	0.15
Ethyleneglycol (4 M)	(No effect)				
1,2-Propanediol	0.5	0.2	0.019	0.01	0.004
1,3-Propanediol	0.5	0.25	0.03	0.015	0.075
Glycerol (7.5 M)	(No effect)				
1,2-Butanediol	0.6	1.0	0.034 [a]	0.02	0.034
1,4-Butanediol	1.5	1.2	0.01 [a]	0.015	0.012
Alkanes					
Pentane	$4.2 \cdot 10^{-4}$	–	63	0.026	–
Hexane	$1.1 \cdot 10^{-5}$	–	200	0.0022	–

[a] Calculated from partition coefficients measured by GC-MS, see Results.

for the calculations summarized in Table I. The value for the 1,4-diol was confirmed by the two calculated values in Leo, Hansch and Elkins [25] which were 0.04 and 0.06.

Although the aqueous concentrations of solutes which affect ion permeability vary widely, the underlying mechanism presumably involves a physical effect produced by solutes as they partition into the bilayer. One would therefore expect the actual increments in permeability to reflect the membrane concentration of the solute. This can be tested by calculating membrane concentration according to Roth and Seemen (Ref. 26, taken to be 0.2 P, where P is the octanol partition coefficient) and determining the membrane concentration of solute required to increase ion flux by a factor of 2 [27]. The results for nine alcohols and two alkanes are shown in Table I. The three normal alcohols all required similar membrane concentrations to cause a doubling of proton and potassium flux, ranging from 0.08 to 0.15. The propanediols and butanediols were more effective in this regard (0.004 to 0.075 calculated membrane concentrations) but required much higher aqueous concentrations, in the molar range. Ethylene diol and glycerol had no effect on proton or potassium permeability, even at concentrations near 7 M, while the two alkanes tested, pentane and hexane, caused permeability increments similar to that of the *n*-alcohols.

The results with the butanediols led to a prediction regarding their anesthetic effects, which was tested as follows. If anesthetics work by the pump-leak mechanism, it would be expected that anesthetic potency of different compounds would be related to their effect on proton permeability. It follows that even compounds such as the butanediols should produce anesthesia at appropriate concentrations. We therefore tested *n*-butanol, 1,2-butanediol and 1,4-butanediol for their relative anesthetic action on Medaka, a freshwater killifish maintained in the laboratory. Thirty fish of approximately the same size were divided into three groups of ten each and exposed to *n*-butanol and the butanediols. The righting reflex was used as an indicator of anesthesia. The concentrations of 0.05 M (*n*-butanol), 0.6 M (1,2-butanediol) and 1.5 M (1.4-butanediol) were chosen because each produced approximately 2-fold increments in proton permeability in liposomes. Control groups of fish were run in which the above concentrations were halved.

The experiment was carried out twice, with similar results. Butanol was a highly effective anesthetic: all of the fish lost their righting reflex in 1–2 min (67 ± 42 s) and all survived this level of exposure, recovering within 10 min. The fish exposed to the butanediols required 1–2 h to lose the righting reflex (5960 ± 720 seconds). Approximately the same amount of time was required for recovery in the 1,2-butanediol group, but the 1.5 M 1,4-butanediol proved to be a toxic concentration – the fish recovered swimming ability, but none survived 24 h later. This was not a direct toxicity, since during the same period, none of the fish in the control groups (0.3 M 1,2-butanediol and 0.75 M 1,4-butanediol) lost the righting reflex, and all survived.

Discussion

This investigation has established that alcohols containing up to four carbons generally increase the permeability of lipid bilayers to protons and potassium ions when membrane concentrations reach 10 to 100 mM. However, ethylene glycol and glycerol had no measurable effect on permeability (Table I). The lack of a glycerol effect on proton permeability was unexpected. In earlier studies, Gutknecht [28] reported that high glycerol concentrations (6 M) markedly reduced the permeability of planar lipid membranes to protons, by nearly 10-fold. In preliminary studies with liposomes, we obtained evidence that appeared to confirm this observation [29]. However, discrepancies began to appear when we compared results obtained with the pyranine and pH meter methods in the present investigation. We therefore re-examined experimental protocols and found that the relatively high viscosity of higher glycerol concentrations did not permit adequate mixing with magnetic stirring alone. When magnetic stirring was accompanied by mixing with nitrogen bubble agitation, no effect of glycerol or ethylene glycol on proton permeability could be measured.

Mechanisms of alcohol effects on ion permeability

All other alcohols increased proton and potassium permeabilities of liposome membranes. We

have examined two alternative explanations for this effect. The first is that the partitioning of alcohols into the membrane increases the membrane dielectric constant, and thereby reduces the Born energy barrier to ion permeation. For instance, Dilger et al. [30] produced a small increment in membrane dielectric by exchanging chlorodecane ($\varepsilon = 4$) for decane ($\varepsilon = 2$) as the solvent in planar lipid membranes, and found that the permeability to anions (perchlorate and thiocyanate) increased by three orders of magnitude. Similarly, Perkins and Cafiso [31] observed in a liposome system that 50 mol% decane mixed with phospholipid reduced proton flux 10-fold, while the same amount of chlorodecane caused a 100-fold increment.

Could dielectric effects caused by alcohols partitioning into the membrane account for the increments in cation permeation measured here? At first, this might seem unlikely in view of the small concentrations of alcohol required to cause measurable increments in membrane permeability. However, the dielectric constant of butanol is 17, and the membrane concentration required to double bilayer permeability to potassium is approx. 76 mM (Table I). Assuming a membrane dielectric of 2.00, and that the resulting dielectric constant is simply the sum of the fractions of hydrocarbon and alcohol, 76 mM butanol partitioning into the bilayer would increase the dielectric constant from 2.00 to 2.05. (Exact values are not necessary in this approximation, since the result will be a ratio of relative effects.)

When this increment in dielectric is used to calculate the Born energy of a hydrated potassium ion to move from the aqueous phase to the hydrocarbon interior of the bilayer, about 168 kJ · mol^{-1} are required in the absence of butanol, and 164 kJ · mol^{-1} in the presence of butanol

$$W(\text{joules/mol}) = k(1/\varepsilon_{hc} - 1/\varepsilon_{w})$$

where W is the Born energy, k is approx. $3.48 \cdot 10^5$ J/mol [32] and ε_{hc} and ε_{w} are the dielectric constants of the hydrocarbon phase and the aqueous phase, respectively. The permeability of the bilayer to ionic flux is proportional to $\exp(-W/kT)$ [30] and the relative permeabilities can therefore be estimated from the calculated Born energies. When this is carried out, we find that 76 mM butanol in the bilayer should increase the ionic permeability by over 5-fold. As noted above, this ratio is relatively parameter insensitive with respect to the absolute values of Born energies. However, the absolute permeability changes are extremely sensitive to the membrane dielectric constant. For instance, increasing this parameter from 2.00 to 2.25 increases permeability by over three orders of magnitude when calculated in the same way.

It was possible to test this concept by measuring the effect of *n*-alkanes on proton permeability. Since the dielectric constant of alkanes would closely match that of the hydrocarbon interior of a bilayer, there would be no significant increase in membrane dielectric caused by the anesthetic itself. When such measurements were carried out with pentane and hexane at activities in the same range as those of the *n*-alkanols, marked increments in proton permeability were readily measured (Table I). We conclude that the effects of anesthetics on membrane permeability to cations are not necessarily related to increased dielectric constant of the bilayer hydrophobic phase caused by the anesthetic itself.

The alternative possibility arises from the fact that no bilayer is a perfect barrier to ion flux. Instead, even in the absence of perturbants there is a measurable permeability to both cations and anions, which results from transient defects appearing in the membrane [32,33]. It follows that the presence of solute molecules in the membrane hydrocarbon phase may simply increase the number of such defects, and that these in turn permit increased ion flux to occur. The evidence reported here supports this alternative. However, there was considerable variability in the effects of different alcohols on membrane permeability. For instance, the 1,2-diols appeared to be more active in this regard than the corresponding normal alcohols and 1,3- and 1,4-diols. This suggests that the nature of the defect may have some relationship to the chemical structure of the perturbants, rather than depending simply on the amount of perturbant which has partitioned into the bilayer.

Relation to anesthetic effects of alcohols

Finally, it was of interest to compare the effect

of alcohols on proton permeability with their activity as general anesthetics. The pump-leak hypothesis suggests that any substance capable of partitioning into membranes and discharging pH gradients should be an anesthetic, assuming, of course, that toxic effects do not override anesthesia. It also predicts that their capacity to discharge pH gradients should be related to their effectiveness as anesthetics. We were able to test this conjecture with a comparison of 1,2- and 1,4-butanediol. When partition coefficients are compared, the 1,2-butanediol is about 3-fold higher. In fact, the aqueous concentration required to double both proton and potassium ion flux was about a third that of the 1,4-diol, and when we tested relative anesthetic effects on fish, the 1,2-diol had apparent anesthetic activity (which required 1 to 2 h to develop) at approximately a third the aqueous concentration required for the 1,4-diol to affect the fish. When the effective concentrations of the butanediols were halved, neither caused loss of the righting reflex or toxic symptoms following a 2 h exposure.

It is not clear, of course, whether it is appropriate to consider both 1-butanol and the butanediols to be 'anesthetics'. However, both required similar membrane concentrations to double proton permeability in liposomes, and both produced insensibility in fish, the only difference being that the diols required 100-times longer exposure to produce an effect. The longer exposure times may simply reflect lower uptake and transport rates of the diols in the fish vascular and nervous system. It is interesting that McGreery and Hunt [24] observed that the 1,2-diol produced 'intoxication' in rats, while the 1,4-diol did not. Intoxication is a different phenomenon than narcosis/anesthesia, but presumably arises from related effects of alcohols on nervous tissue.

In summary, the results reported here are generally consistent with the pump-leak model of general anesthesia, in that effects of alcohols on proton and potassium permeability of lipid bilayers are qualitatively related to their anesthetic activity. However, the results cannot be taken uncritically as support for the pump-leak hypothesis. First, the membrane concentrations required to produce significant increments (2-fold) of proton and potassium flux in liposomes are usually 2–5-times that required to cause anesthesia in vivo, and the liposome model system fails in this regard. A second reservation concerns kinetics of pH gradient decay and release of neurotransmitters from synaptic vesicles. Anesthesia can ensue in less than a minute when an organism is exposed to a general anesthetic, and yet decay of pH gradients across liposome membranes requires many minutes, even at high anesthetic concentrations. Future investigations of the pump-leak hypothesis will address these questions both in liposomes and in relevant biological membranes, including synaptic vesicles and chromaffin granules.

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References

1 Johnson, S.M. and Bangham, A.D. (1969) Biochim. Biophys. Acta 193, 92–104.
2 Trudell, J.R., Hubbell, W.L. and Cohen, E.N. (1972) Fed. Proc. 31, 549.
3 Trudell, J. (1977) Anesthesiology 46, 5–9.
4 Johnson, S.M., Miller, K.W. and Bangham, A.D. (1973) Biochim. Biophys. Acta 307, 42–57.
5 Lee, A.G. (1976) Nature 262, 545–548.
6 Miller, K.W. and Pang, K.-Y.Y. (1976) Nature (London) 263, 253–255.
7 Hill, M.W. (1974) in Molecular Mechanisms of Anesthesia (Halsey, M.J., Millar, R.A. and Sutton, J.A., eds.), pp. 132–144, Churchill Livingstone London.
8 Reyes, J. and Latorre, R. (1979) Biophys. J. 28, 259–280.
9 Seeman, P. (1972) Pharmacol. Rev. 24, 583–655.
10 Franks, N.P. and Lieb, W.R. (1985) Nature 316, 349–351.
11 Mullins, L.J. (1975) in Molecular Mechanisms of Anesthesia (Fink, B.R., ed.), Vol. 1, pp. 237–242, Raven Press, New York.
12 Franks, N.P. and Lieb, W.R. (1982) Nature 300, 487–493.
13 Franks, N.P. and Lieb, W.R. (1984) Nature 310, 599–601.
14 Miller, K.W. (1985) Int. Rev. Neurobiol. 27, 1–61.
15 Bangham, A.D. and Mason, W.T. (1980) Anesthesiology 53, 135–141.
16 Bangham, A.D., Hill, M.W. and Mason, W.T. (1980) Progr. Anesthesiol. 2, 69–77.
17 Barchfeld, G.L. and Deamer, D.W. (1985) Biochim. Biophys. Acta 819, 161–169.
18 Szoka, F. and Papahadjopoulos, D. (1978) Proc. Natl. Acad. Sci. USA 75, 4194–4198.

19 Hope, M.J., Bally, M.B. Webb, G. and Cullis, P.R. (1985) Biochim. Biophys. Acta 812, 55–65.
20 Nichols, J.W. and Deamer, D.W. (1980) Proc. Natl. Acad. Sci. USA 77, 2038–2042.
21 Kano, K. and Fendler, J.H. (1978) Biochim. Biophys. Acta 509, 289–299.
22 Luzzati, V. (1968) in Biological Membranes: Physical Fact and Function (Chapman, D., ed.), p. 105, Academic Press, New York.
23 Brink, F. and Posternak, J.M. (1948) J. Cell. Comp. Physiol. 32, 211–233.
24 McGreery, M.J. and Hunt, W.A. (1978) Neuropharmacology 17, 451–461.
25 Leo, A., Hansch, C. and Elkins, D. (1971) Chem. Rev. 71, 525–616.
26 Roth, S. and Seeman, P. (1972) Biochim. Biophys. Acta 255, 207–219.
27 Verkman, A.S. (1987) J. Bioenerg. Biomembr. 19, 481–493.
28 Gutknecht, J. (1984) J. Membr. Biol. 82, 105–112.
29 Barchfeld, G.L. and Deamer, D.W. (1986) Biophys. J. 49, 517a.
30 Dilger, J.P. McLaughlin, S.C.P., McIntosh, T.S. and Simon, S. (1979) Science 206, 1196–1198.
31 Perkins, W.R. and Cafiso, D. (1986) Biochemistry 25, 2270–2276.
32 Hauser, H. Oldani, D. and Phillips, M.C. (1973) Biochemistry 12, 4507–4517.
33 Deamer, D.W. and Bramhall, J. (1986) Chem. Phys. Lipids 40, 167–188.
34 Bell, R. (1973) Chem. Phys. Lipids 10, 1–10.