Biochimica et Biophysica Acta 819 (1985) 161–169
Elsevier

BBA 72741

The effect of general anesthetics on the proton and potassium permeabilities of liposomes

Gail L. Barchfeld and David W. Deamer

Department of Zoology, University of California, Davis, CA 95616 (U.S.A.)

(Received May 8th, 1985)

Key words: Anesthetic-membrane interaction; Liposome; Ion permeability; Pump-leak hypothesis

The pump-leak hypothesis of general anesthesia proposes that anesthetics act by increasing the functional proton permeability of membranes, particularly those of synaptic vesicles. Since transmembrane proton gradients are required for neurotransmitter accumulation, decay of such gradients by an uncompensated anesthetic-induced leak would result in loss of neurotransmitter from the vesicles, followed by synaptic block and anesthesia. We have tested this hypothesis by determining the effect of four different general anesthetics on the relative permeabilities of liposome membranes to protons and potassium ions. In all cases, physiologically relevant levels of anesthetics caused a 200 to 500 percent increment in ionic permeability. There was no marked preference for protons, suggesting that the anesthetics did not induce a leak specific for this ionic species. Instead the anesthetics appeared to produce a more general defect available to both protons and potassium ions which resulted in a functional increment in proton permeability. These observations were compared with available data on proton transport rates by synaptic vesicle ATPase enzymes. The magnitude of the anesthetic-induced leak could not be compensated by the ATPase, which is only capable of a 40 percent increase in rate when uncoupled. We consider these results to be consistent with the pump-leak hypothesis.

Introduction

General anesthesia has proven refractory to analysis at the molecular level, and several alternative mechanisms are being investigated. Most of these are based on physicochemical parameters of anesthetic-membrane interactions, such as anesthetic effects on lipid bilayer fluidity [1], membrane phase transitions [2], changes in membrane dimensions [3], permeability changes in the lipid bilayer [4,5], and protein interactions [6–8]. Anesthesia is induced by a variety of chemically nonreactive species, suggesting low site specificity, and is reversed by high pressure [9]. In addition, any mechanism for general anesthesia must also be able to account for cold narcosis, or anesthesia without anesthetic [10]. It is observed that anesthetic potency in whole animals, such as mice and amphibian larvae, is proportional to the octanol/water partition coefficient of a given anesthetic. This suggests a nonpolar site of action which could involve membrane lipids, membrane proteins, or both [11].

Although the actual site of anesthesia is still unknown, for induction of both general and local anesthesia action potentials must be interrupted without disturbing the neuronal resting potential. In the stellate ganglion of the cat, 10- to 20-fold lower concentrations of a given anesthetic are required to block synaptic transmission than to block

Abbreviations: Pipes, 1,4-piperazinediethanesulfonic acid; FCCP, *p*-trifluoromethoxy carbonyl cyanide phenylhydrazone.

axonal action potentials [12,13]. Since this corresponds to the approximate difference in anesthetic concentrations needed to induce general and local anesthesia [14], it follows that general anesthetics may function by depressing synaptic transmission of nerve impulses. Synapses vary in their sensitivity to anesthetics. The reticular activating system and interneuronal elements are most sensitive and the cardiovascular and respiratory systems are most resistant to anesthetics. Therefore it is the pattern of synaptic interruptions which produces whole animal anesthesia [15].

Synaptic involvement suggests several possible sites of action for anesthetics. For instance, it is known that synaptic vesicles actively transport protons in order to accumulate neurotransmitters. Acetylcholine is concentrated in electric organ synaptic vesicles of *Torpedo ocellata*, and as a result of the proton transport, the internal pH is about 5.2 [16]. Similar transmembrane proton gradients may permit catecholamines to preferentially distribute into adrenergic vesicles by an unknown mechanism. Bangham et al. have proposed a 'pump-leak' hypothesis in which general anesthetics act by permitting the collapse of this pH gradient [17]. More specifically, the pump-leak hypothesis of anesthesia proposes that general anesthetics increase the ionic permeability of membranes such that membrane pumps are unable to maintain normal ionic concentration gradients across cellular membranes. As anesthetics increase the functional proton permeability of these membranes, the interior pH rises, leading to redistribution of the catecholamines, and presumably to depression of synaptic activity and induction of anesthesia [10]. It was found that general anesthetics did, in fact, increase the proton permeability of liposomes and caused catecholamine release from isolated synaptic vesicles [17].

This concept specifically rests upon the enhancement of functional membrane proton permeability, and one may ask if anesthetics have a general effect on cation permeability, or if the effect is preferentially enhanced for protons. The proton permeability of both biological and model membranes is several orders of magnitude greater than that of other ions, possibly due to Grotthuss conductance through associated water molecules within the hydrophobic portion of the bilayer [18]. Anesthetics increase both the amount of water in the bilayer and the permeability of the bilayer to water [19,20]. This is due to the increased randomness of membrane structure caused by anesthetics, as demonstrated by studies using scanning calorimetry, monolayer penetration, NMR, and spin label techniques [2,21]. Do anesthetics simply increase the total water content of membranes, or could the relative amount of structured water be increased as well? If general anesthetics cause increased amounts of hydrogen-bonded water in the bilayer, then one would expect that the relative permeability to protons would undergo greater increments than potassium permeability as anesthetic concentration is increased.

In the present study, we have approached this question by determining the effect of several general anesthetics on the proton and potassium permeabilities of large unilamellar vesicles. We established pH gradients across the vesicles in the presence of general anesthetics and developed a new method for monitoring the decay rates of small transmembrane pH gradients. Proton flux across the membranes was calculated from the rate of change in intravesicular pH based on the spectral absorbance of encapsulated 6-carboxyfluorescein (6-CF). We did not distinguish between protons and hydroxide ions in these experiments, and protons should be regarded as proton equivalents in the present discussion. Potassium flux was measured by monitoring the decay rates of transmembrane potassium gradients with an ion selective electrode. In this way, we were able to compare the permeability of protons to the permeability of potassium ions in similar liposomes. We tested *n*-butanol, chloroform, diethyl ether, halothane, and, as a control, the nonanesthetic *n*-dodecanol [14] by comparing the effects of these compounds on the relative permeability of liposomes to protons and potassium ions. The validity of using model membranes to study anesthesia has been demonstrated by the correlations between the responses of whole animals, synaptic vesicles, and liposomes to isopotent anesthetics [22]. A significant question is whether the induced increase in proton permeability is large enough to compromise the pH gradient being maintained by the membrane pump. We addressed this question by comparing the induced proton leak to literature values

for the activity of the H^+ translocating ATPase of synaptic vesicles [23].

Methods and Materials

Preparation of liposomes. For proton permeability experiments, egg phosphatidylcholine and egg phosphatidic acid (Avanti Inc., Birmingham, AL), and cholesterol (Nu-Chek Prep) at mole ratios of 65 : 10 : 25, in a total of 30 μmoles, were combined and the organic solvents removed by evaporation under N_2. Large unilamellar vesicles were formed by reverse phase evaporation [24] in the following manner. The lipids were redissolved in 3 ml diethyl ether and 1 ml aqueous phase (0.2 M K_2SO_4/0.19 M Pipes/5 mM EDTA/1 mM 6-carboxyfluorescein, pH 7.0). The aqueous phase was of sufficiently high osmotic strength to minimize any minor osmotic effects. K_2SO_4 provided a counter-ion (K^+) to H^+ flux in the presence of valinomycin [18] and an impermeant anion (SO_4^{2-}) to preclude possible electroneutral movement of protons, for instance, in the form of HCl [25]. The lipids were vortexed and bath sonicated under N_2 to a homogeneous dispersion. The 6-carboxyfluorescein was previously passed over a LH-20 lipophilic column to remove hydrophobic contaminants [26]. The organic phase was removed by rotary evaporation under reduced pressure in a flash evaporator for 15 min, 2 ml aqueous phase was added, and rotary evaporation continued an additional 40 min. the vesicles were passed through 0.2 μm pore polycarbonate filters followed by gel filtration on a 1.5 × 20 cm Sephadex G-50 column equilibrated with 0.33 M K_2SO_4, 5 mM EDTA, and 1 mM Pipes (pH 7.0).

For potassium permeability experiments, a total of 120 μmoles of the lipids in the same mole ratios were prepared as described above, except that the aqueous phase was 0.5 M KCl/10 mM Pipes-Tris/0.5 mM EDTA (pH 7.0). The same evaporation and sizing procedures were followed, and the vesicles were filtered on a 1.5 × 35 mm G-50 Sephadex column equilibrated with 0.5 M choline chloride/10 mM Pipes-Tris/0.5 mM EDTA (pH 7.0). The choline chloride was previously recrystallized from absolute ethanol to remove any amines and ammonium salts present as trace contaminants.

Experiments were performed at ambient temperature, and the vesicles were 0.05 to 0.1 μm in diameter, based on their trapped volume [27].

Proton flux measurements. Filtered liposomes in aliquots of approx. 1 μmole lipid were combined with valinomycin and aliquots of isotonic buffer saturated with anesthetic. To establish a pH gradient, Pipes (pH 6.0) was added for a final concentration of 10 mM in 1 ml. The change in intravesicular pH was monitored by measuring the absorbance of 490 nm (A_{490}) of the trapped 6-carboxyfluorescein with a Perkin-Elmer λ-3-spectrophotometer for 5 min. External pH was independently measured with a glass electrode and pH meter. Vesicular light scattering and A_{465} were measured by scanning the sample from 400 to 550 nm immediately following the kinetic experiment.

Calculation of proton permeability coefficients. The absorbance spectrum of 6-carboxyfluorescein is pH dependent such that the ratio of A_{490}/A_{465} is linear with pH from about pH 6 to pH 7, A_{465} being isospectic [28]. This relationship is described by the equation

$$\mathrm{pH} = 1.14 \cdot (A_{490}/A_{465}) + 4.71$$

determined by a separate experiment. Therefore, from the rate of change in A_{490} one may calculate the rate of change in intravesicular pH. From this, J (proton flux may be calculated by the equation

$$J = (\mathrm{dpH}/\mathrm{d}t) \cdot B \cdot V/A$$

where $\mathrm{dpH}/\mathrm{d}t$, calculated from the initial slope of A_{490} vs. time, is the rate of change in pH; B is the vesicular buffer capacity, determined independently by acid-base titration of the aqueous phase; V is the trapped volume of the vesicles, calculated from the A_{465} of a known volume of unfiltered vesicles and of the sample; and A is the calculated total surface area of the liposomes based on total lipid present, assuming 0.7 nm^2 per lipid molecule [29]. P (permeability coefficient) can then be calculated from the equation

$$P = J/\text{concentration gradient}$$

The actual proton gradient is taken to be the difference between the external pH, which is constant once the gradient is established, and the internal pH as determined by A_{490}.

Potassium flux measurements. Filtered liposomes in aliquots of approx. 2.5 μmoles lipid were combined with isotonic buffer containing saturated anesthetic, pH 7.0, for a total volume of 4 ml. Leakage of trapped potassium was measured with an ion selective electrode and a pH meter. The K^+ electrode was calibrated with K^+ standards at each time point during the 3 days of each experiment.

Calculation of potassium permeability coefficients. For the purposes of calculations, an exponential decay rate was assumed. The data points were entered in a Hewlett-Packard 41C calculator logarithmic curve fitting program. From the resulting equations, the initial slopes, representing the rate of potassium efflux, were obtained by differentiation of the log curves

$$[K^+] = a + b \cdot \ln t$$

to

$$d[K^+]/dt = b/t$$

J (potassium flux) was calculated from the following equation

$$J = (d[K^+]/dt)/A$$

where $d[K^+]/dt$ is the initial slope and A is the surface area of the liposomes. Permeability was calculated in the same manner as for protons, assuming an initial driving potassium concentration gradient of 0.5 M.

Results and Discussion

Proton permeability

Typical results for an anesthetic titration demonstrating the collapse of a pH gradient, are shown in Fig. 1. The decrease in A_{490} through time reflects the influx of protons, which causes a decrease in intravesicular pH. As anesthetic activity increased from 0 to 0.15, so did the rate of proton influx. The activity of the anesthetic is defined as C/C_0, where C equals the concentration of anesthetic in the sample and C_0 equals the concentration of anesthetic in a saturated solution. Anesthetic activity has been shown to be proportional to potency [12]. The clinically relevant anesthetic levels for a variety of general anesthetics

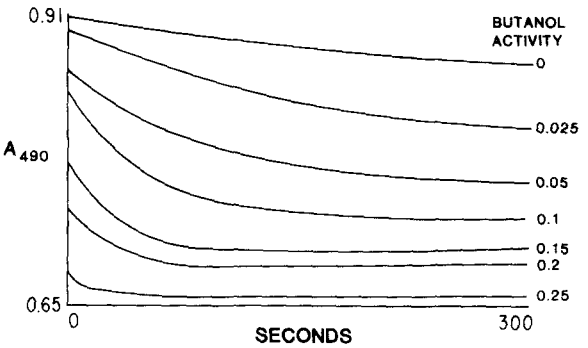

Fig. 1. Example of the response of encapsulated 6-carboxyfluorescein to proton flux induced by increasing anesthetic concentrations. 6-Carboxyfluorescein was entrapped in liposomes as described in Methods and Materials, and pH gradients ranging near half a pH unit were imposed by addition of acid. The pH-dependent absorbance of the dye at 490 nm was monitored, beginning about 3 s after the gradient was established. Valinomycin (135 ng/ml) was present to permit free counter-ion flux of potassium so that proton diffusion potentials did not interfere with intrinsic proton flux rates. In the absence of anesthetic (top curve) a slow decrease in A_{490} occurred, reflecting decay of the pH gradient due to passive proton flux. As the anesthetic concentration was increased, the rate of proton flux also increased. The anesthetic here was *n*-butanol, but four other general anesthetics produced similar increments. Addition of the protonophore FCCP caused an immediate collapse of the gradient (not shown). The liposomes contained 0.2 M K_2SO_4, 0.19 M Pipes, 5 mM EDTA, 1 mM 6-carboxyfluorescein (pH 7.0). External solution was 0.33 M K_2SO_4/5 mM EDTA/10 mM Pipes (pH 6.3).

range from 0.01 to about 0.1 activity [14,17]. The anesthetic concentrations are presented in terms of activity so that the effects of anesthetics with widely differing water/octanol partition coefficients can be compared at equivalent potencies. Fig. 2 shows the resultant permeability coefficients obtained for the various anesthetics at different activities. The proton permeability coefficient increased from an average of $3 \cdot 10^{-4}$ cm/s at 0 activity to $10 \cdot 10^{-4}$ cm/s at 0.15 activity. All the anesthetics had a remarkably similar effect on proton permeability, with an enhancement of 2- to 5-fold in the clinical range. Diethyl ether consistently had a more modest effect than theother anesthetics, in accordance with the lower activity commonly observed by other researchers [12]. Our results are in excellent agreement with earlier studies of Bangham et al. using small unilamellar vesicles [17]. At equivalent anesthetic activities, they observed a 2-fold increase in proton permea-

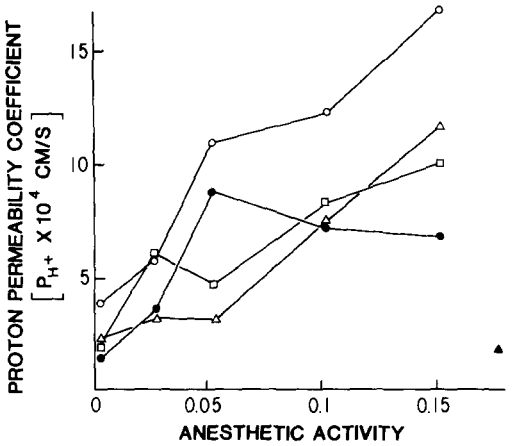

Fig. 2. Effect of anesthetics on absolute proton permeability. Proton permeabilities were calculated from decay of pH gradients under conditions given in Fig. 1, using four general anesthetics. Each point represents a mean value calculated from at least three different experiments. Anesthetics used included *n*-butanol (△), chloroform (□), diethyl ether (●), and halothane (○). The activity parameter on the abscissa represents the decimal fraction of a saturated solution of anesthetic in buffer and is a convenient way to take into account the different partition coefficients of general anesthetics which govern their actual concentrations in the membrane phase. Saturated concentrations are: *n*-butanol, 1.0 M; diethyl ether, 816 mM; chloroform, 62 mM; halothane, 18 mM. The proton permeability of liposomes prepared with a lipid:dodecanol mole ratio of 40:1 as described in Methods and Materials is shown at the lower right (▲).

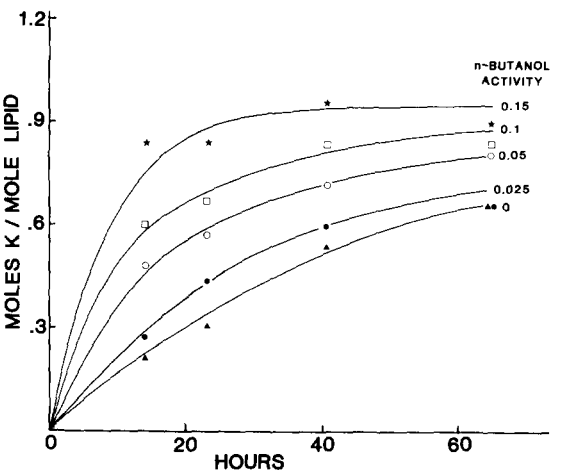

Fig. 3. Example of anesthetic effect on potassium ion flux. As the concentration of anesthetic (*n*-butanol) increased, there was a corresponding increment in potassium efflux measured by a potassium selective electrode. Other anesthetics caused similar increments. Liposomes contained 0.5 M KCl, 10 mM Pipes, 0.5 mM EDTA, pH 7.0. External solution was 0.5 M choline chloride/10 mM Pipes/0.5 mM EDTA (pH 7.0).

bility, and we have extended this work to the comparative investigation reported here.

Potassium permeability

Anesthetic titrations done with potassium loaded vesicles resulted in enhanced potassium efflux, as shown in Fig. 3. The average potassium permeability coefficients for the various anesthetics increased from $7 \cdot 10^{-11}$ cm/s at 0 activity to $18 \cdot 10^{-11}$ cm/s at 0.15 activity (see Fig. 4). These permeability coefficients are 2 orders of magnitude greater than typical reported values [4], but the difference is likely due to real permeability differences between large unilamellar vesicles and small unilamellar vesicles, possibly caused by the effects of differing radii of curvature. Overall, we observed a 3-fold enhancement in potassium permeability in the clinical range, which is in qualitative agreement with earlier work using multilamellar vesicles [30] and small unilamellar vesicles [4,5].

n-Dodecanol controls

We chose *n*-dodecanol as a control because it is homologous to *n*-butanol, and *n*-alkanols with more than 11 carbons are not anesthetic [7,9]. On titrating a saturated *n*-dodecanol solution into our liposomes, we observed no effect on permeability (data not shown). It has been suggested that the reason large alkanols are not anesthetic is due to their extremely low solubility in water, making it difficult to deliver sufficient quantities to the anesthetic site [7]. To circumvent this difficulty, we then incorporated *n*-dodecanol directly into the liposomes during preparation. At a lipid to dodecanol mole ratio of 40:1, P_{H^+} and P_{K^+} fell within the range of control values (0 activity of anesthetic) obtained in the other experiments (see Figs. 2 and 4). The mole ratios of lipid to anesthetic at 0.05 activity are given in Table I. Based on these ratios and the decrease in concentration required for nerve block as one goes up the *n*-alkanol series (butanol 10^{-2} mol/l H_2O, nonanol 10^{-5} mol/l H_2O, Ref. 14), a mole ratio of 40:1 is clearly sufficient to elicit an effect on membrane permeability if such an effect exists at clinically relevant levels. We suggest that the lack of anesthetic capacity is due to a nonperturbing intercalation of *n*-dodecanol within membranes.

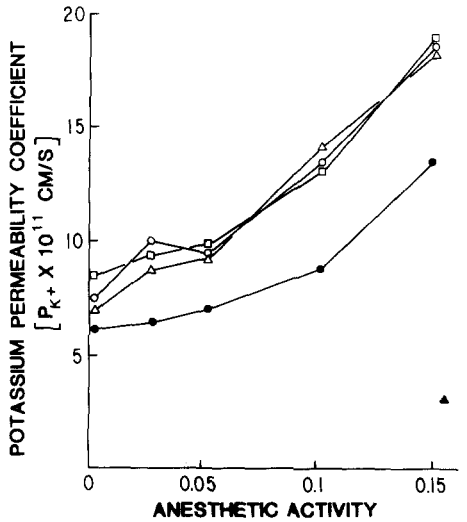

Fig. 4. Effect of anesthetics on absolute potassium permeability. Potassium permeability was calculated from the efflux rates measured under conditions given in Fig. 3, using four different general anesthetics. Each point represents a mean value calculated from the results of three different experiments. Anesthetics used are given in Fig. 2. The potassium permeability of liposomes prepared with a lipid : dodecanol mole ratio of 40 : 1 is shown at the lower right (▲).

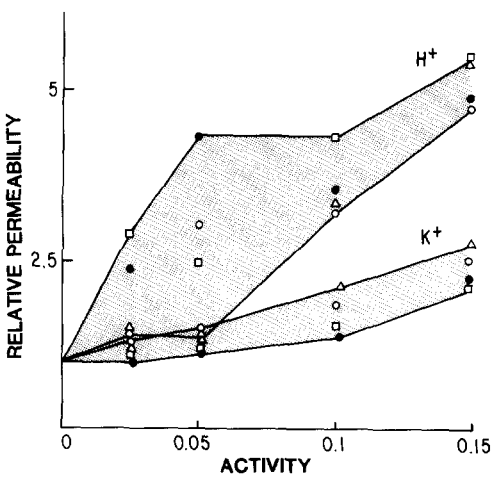

Fig. 5. Relative permeability increments induced by anesthetics were calculated for protons and potassium ions by taking the permeability coefficient in the absence of anesthetic as 1.0. Each point represents the mean of at least three experiments taken from the data shown in Figs. 2 and 4, and the shaded areas represent the range of all values for the four anesthetics studied. It is clear that anesthetics produce significant increments in the permeability of liposomes to both cations, with the effect on proton permeability being somewhat greater.

TABLE I

CONCENTRATIONS AND LIPID/ANESTHETIC RATIOS AT 0.05 ANESTHETIC ACTIVITY

Anesthetic	0.05 anesthetic activity	
	Lipid/anesthetic (mole ratio)	Aqueous concn. (mM)
n-Butanol	20 : 1	50
Chloroform	25 : 1	10
Diethyl ether	30 : 1	163
Halothane	130 : 1	3.6

Anesthetic effects on the relative permeabilities of H^+ and K^+

As an aid in the comparison of anesthetic effects on proton and potassium permeabilities, the permeability coefficients were converted to relative permeabilities (RP). RP equals P/P_0 where P is an experimental permeability coefficient and P_0 is the control value of P in a given experiment. Fig. 5 illustrates that the anesthetics appear to affect both H^+ and K^+ permeability in a similar way and to a similar extent; RP_{H^+} increases 2- to 5-fold and RP_{K^+} increases 3-fold, with RP_{H^+} consistently higher than RP_{K^+}. Comparison of Fig. 2 and Fig. 4 reveals a discontinuity in the permeability increments at the low end of the activity scale which is similar for both proton and potassium experiments, a further indication that the permeability increments result from a common cause. We propose that anesthetics create a defect in the bilayer which can be used for the passive transport of both protons and potassium ions. Alkanol anesthetics have previously been shown to increase the permeability of liposomes to water [20], and since proton permeability is related to water permeability [31], it follows that proton permeability should also be increased by anesthetics. Although proton RP increases more than potassium RP, it does not appear that anesthetics preferentially enhance a 'proton-wire' type of conductance under these conditions. Flux, a measure of ionic conductance, for protons and potassium ions was remarkably close under our conditions. Even though proton concentrations and potassium ion concentrations differed by six orders of magnitude, measured fluxes of both species were in the range of 10^{-14} mol · cm^{-2} · s^{-1}. This reveals an ap-

proximate equivalence in the conductivities of these two ionic species which is paralleled in biological membranes [32]. Since previous work has shown that general anesthetics decrease the cooperativity between phospholipid molecules in dipalmitoylphosphatidylcholine multilamellar vesicles [2,21], it is possible that the increase in ionic permeability results from a similar decreased intermolecular cooperativity both in liposomes and biological membranes.

Is anesthetic enhancement of lipid bilayer permeability physiologically significant?

The present study confirms and extends previous observations that anesthetics increase lipid bilayer permeability to cations. We have demonstrated that anesthetics do not produce a marked enhancement of specific proton permeability, but do increase the number of defects in the bilayer which are used for passive flux of both protons and potassium ions. We may now ask whether the observed increments in P_{H^+} are sufficient to deplete the catecholamine stores of synaptic vesicles. Bangham et al. have shown that clinical levels of general anesthetics will cause pH gradients to decay in isolated synaptic vesicles [17], followed by loss of catecholamine content. Since these vesicles were not supplied with exogenous ATP to permit active pumping, presumably only the leak component of the pump-leak mechanism was operational, and it is not clear whether the vesicles would lose their catecholamine content in vivo. This question may be approached by considering the H^+ translocating ATPase of electric organ synaptic vesicles. Anderson et al. [23] have determined that these synaptic vesicles maintain a proton gradient by means of a H^+ translocating ATPase, and that accumulation of acetylcholine occurs by a H^+/acetylcholine antiporter. The active transport of protons by the ATPase is balanced by a passive outward leak of the same magnitude. When the outward leak is markedly increased, for instance, in the presence of the protonophore FCCP, the ATPase activity only increases by about 40%. Under the same conditions, active uptake of acetylcholine by a putative H^+/acetylcholine antiporter in the vesicles is completely inhibited, but stored acetylcholine is not released [16]. These results indicate that the ATPase is normally operating at close to maximal activity and is not able to compensate for the effects of the protonophore, resulting in uncoupling of the proton pump and acetylcholine uptake. In the liposome results reported here, the intrinsic proton permeability increased 200% to 500% in the presence of anesthetics. It follows that the ATPase may not be able to compensate for even modest increases in membrane permeability. These observations are consistent with the hypothesis that general anesthetics exert their primary effect by causing decay of proton gradients in synaptic vesicles, followed by loss of catecholamines.

The pump-leak hypothesis of anesthesia is attractive for a number of reasons. First, it accounts for whole animal responses to temperature changes in the presence of anesthetics. Anesthetic potencies decrease with increasing temperature in dogs and goldfish [7,33], whereas anesthetic potencies increase as temperatures drop below normal body temperature [9]. According to the pump-leak hypothesis, as temperature rises so does membrane permeability, but the membrane pump, also having a positive temperature coefficient, can compensate for the enhanced permeability. Conversely, as temperature decreases, both permeability and pump activity also decreases, but presumably the pump and metabolism slow down to such an extent that they are unable to keep up with the leak and cold narcosis ensues [10,33]. Second, the hypothesis explains why lipid vesicles respond to temperature increments and to anesthetics in a similar way, but whole animals do not [33]. Mechanisms of anesthesia based on changes in membrane fluidity, phase transitions, and simple permeability effects all predict an increase in potency with an increase in temperature [7], and for model systems such as small unilamellar vesicles, anesthetic effects in fact increase with rising temperatures [4]. Third, since anesthetics exert relatively similar effects on cation permeability in general, the pump-leak hypothesis can be invoked not only for proton gradients but also for other energetically maintained ionic gradients.

For instance, the calcium gradient across the presynaptic membranes could also be affected by general anesthetics. Synapses of giant squid neurons have a large Ca^{2+} current, indicating the presence of many Ca^{2+} channels [34]. A calcium

ion influx is the primary signal for neurotransmitter release [35], and proper levels of intrasynaptic calcium must be maintained for normal functioning [35]. It is interesting to note that the synaptic delay of the frog neuromuscular junction, which is 0.5 ms at 20°C, increases to 7 ms at 2°C [26]. Since there is no increase in the time for neurotransmitter to diffuse across the synaptic cleft, we suggest that the increase in synaptic delay is due to a diminished efficiency of the Ca^{2+} pump. This is exactly the effect one would predict for cold narcosis based on the pump-leak hypothesis of anesthesia. In addition, clinical levels of general anesthetics have been demonstrated to affect various membrane functions involving calcium. For instance, anesthetics cause a release of Ca^{2+} from the sarcoplasmic reticulum and a resultant muscle contraction [14,37,38]; an increase in the rate of miniature endplate potentials at the neuromuscular junction [14]; an increase in membrane permeability to Ca^{2+} [14]; and in malignant hyperpyrexia induced by general anesthetics, the sarcoplasmic reticulum is apparently unable to resequester Ca^{2+} [38]. There are two likely ways for anesthetics to affect Ca^{2+} distribution at the synaptic level. First, they may simply cause an increase in plasma membrane Ca^{2+} permeability which results in elevated intracellular Ca^{2+} levels and an inappropriate miniature endplate potential pattern, causing hyperactivity and then depressed synaptic transmission as neurotransmitter stores become depleted. Low doses of anesthetics have been commonly observed to cause hyperactivity [15]. Second, anesthetics may alter the molecular environment of the Na^{+}-Ca^{2+} exchange protein, the Ca^{2+}-ATPase pump, or the Ca^{2+} channel itself such that the normal calcium gradient is compromised sufficiently to interfere with the function of the Ca^{2+} channel and resultant neurotransmitter release. These two hypotheses are not mutually exclusive and validation of either awaits experimental verification.

In conclusion, given the nonspecificity of general anesthetic action, anesthetics may act by a cumulative effect on various membrane functions involving ion gradients coupled to nerve cell function. Confirmation of anesthetic effects on synaptic vesicles does not rule out the possibility of other physiological effects, and in future studies it will be necessary to establish which of these effects play primary roles in producing anesthesia.

Acknowledgements

This study was supported in part by the Office of Naval Research contract N00014-85-K-0242.

References

1 Metcalfe, J.C., Seeman, P. and Burgen, A.S. (1968) Mol. Pharmacol. 4, 87–95
2 Mountcastle, D.B., Biltonen, R.L. and Halsey, M.J. (1978) Proc. Natl. Acad. Sci. USA 75, 4906–4910
3 Miller, K.W., Paton, W.D., Smith, R.A. and Smith, E.B. (1973) Mol. Pharmacol. 9, 131–143
4 Johnson, S.M. and Bangham, A.D. (1969) Biochim. Biophys. Acta 193, 92–104
5 Johnson, S.M., Miller, K.W. and Bangham, A.D. (1973) Biochim. Biophys. Acta 307, 42–57
6 Veda, I. and Kamaya, H. (1973) Anesthesiology 38, 425–436
7 Franks, N.P. and Lieb, W.R. (1982) Nature 300, 487–493
8 Hille, B. (1980) in Progress in Anesthesiology, Vol. 2 (Fink, R., ed.), pp. 1–68, Raven Press, New York
9 Smith, E.B. (1974) in Molecular Mechanisms in General Anesthesia (Halsey, M.J., Milar, R.A. and Sutton, J.A., eds.), pp. 112–131, Churchill Livingstone, New York
10 Bangham, A.D. (1983) in Liposome Letters (Bangham, A.D., ed.), pp. 261–268, Academic Press, London
11 Franks, N.P. and Lieb, W.R. (1978) Nature 274, 330–342
12 Brink, F. and Posternak, J.M. (1948) J. Cell Comp. Physiol. 32, 211–233
13 Richards, C.D. (1974) in Molecular Mechanisms in General Anesthesia (Halsey, M.J., Milar, R.A. and Sutton, J.A., eds.), pp. 90–111, Churchill Livingstone, New York
14 Seeman, P. (1972) Pharmacol. Rev. 24, 583–655
15 Schaefer, H. (1963) in Mechanisms of Anesthesia (Cullen, S.C. and Featherstone, R.M., eds.), Vol. 1(4), pp. 977–979, Little, Brown, Boston
16 Angel, I. and Michaelson, D.M. (1981) Life Sci. 29, 411–416
17 Bangham, A.D., Hill, M.W. and Mason, W.T. (1980) Progr. Anesthesiol. 2, 69–77
18 Nichols, J.W. and Deamer, D.W. (1980) Proc. Natl. Acad. Sci. USA 77, 2038–2042
19 Seeman, P., Sha'afi, R.I., Galey, W.R. and Solomon, A.K. (1970) Biochim. Biophys. Acta 211, 365–368
20 Hochster, H.S. and Prestegard, J.H. (1977) J. Membrane Biol. 35, 303–307
21 O'Leary, J.T., Ross, P.D. and Levin, I.W. (1984) Biochemistry 23, 4636–4641
22 Hill, M.W. (1978) Ann. N.Y. Acad. Sci. 308, 101–110
23 Anderson, D.C., King, S.C. and Parsons, S.M. (1982) Biochemistry 21, 3037–3044
24 Szoka, F., Jr. and Papahadjopoulos, D. (1978) Proc. Natl. Acad. Sci. USA 75, 4194–4198
25 Gutknecht, J. and Walter, A. (1981) Biochim. Biophys. Acta 641, 183–188

26 Klavsner, R.D., Kumar, N., Weinstein, J.N., Blumenthal, R. and Flavin, M. (1981) J. Biol. Chem. 256, 5879–5885
27 Deamer, D.W. and Uster, P.S. (1983) in Liposomes (Ostro, M.J., ed.), pp. 27–52, Marcel Dekker, New York
28 Thomas, J.A., Kolbeck, P.C. and Langworthy, T.A. (1982) in Intracellular pH: Its Measurement, Regulation, and Utilization in Cellular Functions (Nuccitelli, R. and Deamer, D., eds.), pp. 105–124, Alan R. Liss, New York
29 Luzzati, V. (1968) in Biological Membranes: Physical Fact and Function (Chapman, D., ed.), pp. 71–123, Academic Press, New York
30 Bangham, A.D., Standish, M.M. and Miller, N. (1965) Nature 208, 1295–1297
31 Elamrani, K. and Blume, A. (1983) Biochim. Biophys. Acta 727, 22–30
32 Deamer, D.W. (1982) in Intracellular pH: Its Measurement, Regulation and Utilization in Cellular Functions (Nuccitelli, R. and Deamer, D., eds.), pp. 173–187, Alan R. Liss, New York
33 Hill, M.W., Hoyland, J. and Bangham, A.D. (1980) J. Comp. Physiol. 153, 327–332
34 Reuter, H. (1983) Nature 301, 569–574
35 Gill, D.L., Cheuh, S. and Whitlow, C.L. (1984) J. Biol. Chem. 259, 10807–10813
36 Kuffler, S.W. and Nicholls, J.G. (1976) in From Neuron to Brain, pp. 177–191, Sinauer Associates, Sunderland, MA
37 Fiehn, W. and Hasselbach, W. (1969) Eur. J. Biochem. 9, 574–578
38 Strobol, G.E. and Bianchi, C.P. (1971) Anesthesiology 35, 465–473