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PHYSICAL MECHANISMS OF ANESTHESIA

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INTRODUCTION

It still remains unclear how anesthetics exert their pharmacological effects on excitable tissues and produce the physiological phenomenon known as anesthesia.

The study of the mechanism of anesthetics is as old as anesthesia itself. The literature on this subject has developed for over a century. One of the major problems in developing a theory of the mechanism for these agents is the failure to define a structural requirement for activity; the other is the complexity of the system affected, the central nervous system (CNS). At one end of the spectrum, there is the complexity of the central nervous system, primarily due to the histological heterogeneity of the different cell types that comprise the CNS (1). At the other end is the complexity of the structure and function of the basic cellular membrane, which may also vary with cell types (1).

Anesthesia can be produced by a wide variety of chemical agents, ranging from inert rare gases to steroid molecules (2, 3). The diversity of chemical structure of anesthetics suggests a lack of a specific receptor site, but the excellent correlation of lipid solubility with potency implies an interaction with hydrophobic regions in the membrane. Since physical properties such as lipid solubility are governed by intermolecular forces (4, 5), these properties, compared to anesthetic potency, have been the primary focus of research into the physical mechanisms of anesthesia and have led to the basis for a unitary theory of anesthesia which suggests that all anesthetics act via

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the same physical mechanism. A unitary theory of mechanism for all anesthetics cannot explain the diversified activities of various chemical agents often loosely termed as anesthetics. The method of comparing physical properties with anesthetic potency has a major limitation of providing correlations or "rules" and offering only a whisper of direction toward mechanism. Recent studies of the perturbation of membrane properties with various "probes" have provided considerable information about the mechanism of action. It would appear that "physical" mechanisms of anesthetics may soon be explained at the molecular level.

There have been many excellent reviews published on this subject within the past few years (3, 6–14); therefore no attempt has been made to review all of the literature.

Anesthetic Agent

Since a wide variety of chemical agents are capable of producing an anesthetic state, an *anesthetic* can generally be defined as a drug or any agent capable of reversibly blocking neural conduction without any significant effect on resting membrane potential (3). This definition would include many agents which are usually not classified pharmacologically as anesthetics, such as tranquilizers, anticonvulsants, detergents (2, 3). In this review, no distinction is made between local and general anesthetics, although the actions at the molecular level may be different (15, 16).

Anesthetic State

Anesthesia is a reversible drug-induced pertubation of neuronal behavior. It is difficult to define in the absence of a clear concept or definition of consciousness (17). Anesthesia has been regarded as a result of a functional disorganization or even excitation at various levels of neuronal activity of the nervous system (18, 19). Neurophysiological studies of anesthesia have provided ample evidence to support the concept that a single state of anesthesia does not exist (18, 20, 21), but rather a continuum or multidirectional progression of anesthetic states, represented by excitation and depression of the CNS (21, 22). This progressive inactivation of facilitatory and/or inhibitory processes in the CNS yields the "anesthetic state" (23). Anesthesia, often associated with a loss of responsiveness and amnesia, should not be synonymous only with depression of neuronal behavior (18). Since molecular structure of anesthetics can influence the neurophysiological effects observed in man (24) and EEG patterns have been associated with individual anesthetics (18), it is difficult to imply a similar spectrum of activity for all anesthetics (20, 25, 26).

SITE OF ACTION

Axonal and Synaptic Pathways

Many anesthetic agents have been shown to block synaptic transmission at lower concentrations than required for blocking axonal conduction (27–29); therefore it is generally accepted that general anesthetics are acting on some aspect(s) of synaptic physiology. Alternatively, gaseous and volatile anesthetics are capable of blocking conduction along peripheral nerves (30–33). The concentrations for blocking peripheral axonal conduction are approximately 10–20 fold greater (3, 30) than for general anesthesia. One explanation for this difference has been attributed to the increased sensitivity of chemical synapses in the CNS (13, 27, 34). Moreover, it is conceivable that different synapses may have varying degrees of stability and susceptibility to anesthetics (13), and variations may be evident in axons of varying diameter (28, 35, 36).

It has also been suggested that general anesthesia is a form of local anesthesia (3), the blockade of conduction in fine unmyelinated nerve terminal axons resulting in apparent synaptic inhibition.

Whether the mechanisms of local and general anesthesia are similar remains to be determined. The "physical" mechanisms described in this review may not apply to local anesthetics. The physical mechanisms specific to local anesthetics have been reviewed recently by Strichartz (16), and also discussed at length by Hille (37, 38).

There is evidence to suggest that the alteration of excitatory postsynaptic potentials (EPSP) by anesthetic agents occurs at more than one site (39, 40). Experiments have demonstrated that anesthetic agents decrease the amount of excitatory transmitter release (41) and also increase the release of inhibitory transmitter (42). Furthermore, it has been postulated that the postsynaptic membrane properties are altered, by either a decrease in chemosensitivity of the neurotransmitter receptor (3) or a stabilization of the postsynaptic membrane, thus inhibiting action potential generation (34, 43). Sato, Austin & Yai (44) have suggested that anesthetic activity is a result of the alteration of the cable properties of the dendrites of the postsynaptic neuron, i.e. an increase in the postsynaptic membrane potential and conductance. Barker (45) postulates that the primary effect of anesthetic agents is a selective depression of postsynaptic excitation.

The results obtained in experiments on peripheral synapses suggest a double action of anesthetic activity, that is, a decrease in transmitter release, and a depression of sensitivity of postsynaptic membrane to transmitter substance (46, 47). The lack of knowledge of transmitter substance(s) in the brain presents difficulties in interpretation of data; thus, only indirect or

suggestive evidence of a double action in the central nervous system has been provided (48–50). Although most of the data is conflicting there appears to be agreement that all the anesthetics studied so far cause a decrease in EPSP amplitude and reduce the lifetime of open postsynaptic ionic channels (51).

Cellular Membrane

The primary effects of anesthetics occur at the level of the cell membrane (3, 52, 53). The cellular membrane is generally regarded as a fluid mosaic structure (54). It is composed as a matrix of a phospholipid bilayer embedded with functional proteins. The membrane phospholipids are amphiphatic, i.e. having distinct polar and apolar regions, and position into a bilayer configuration when in an aqueous environment. The long apolar hydrocarbon chains are directed toward the center of the bilayer, and the polar head groups remain in contact with the aqueous phase on the surface. The membrane proteins are often viewed as globular entities that are bound to the exterior and interior surfaces of the bilayer; some penetrate the membrane partially, others completely. The proteins are also amphiphatic. Intracellular structures such as microtubules and microfilaments form a complex network or cytoskeleton which probably aids in the organization of the membrane components (53).

Cellular membranes are asymmetrical in structure and function (55). Although some of the proteins may be immobile, there is lateral diffusion of various membrane constituents (56). The membrane may be regarded as a two-dimensional fluid (57), and lateral movement of cell components may be involved in the function of the cell and its response to the external environment (58). The membrane, therefore, must be considered a dynamic structure. Although an understanding of the composition and function of membranes has advanced greatly over the years (53), the lipid bilayer region is relatively better understood and often emphasized in the description of anesthetic-membrane interactions (10). Research in the area of lipid-protein interactions is relatively new, and the status of these studies has been recently reviewed (57).

Intramembrane Location

The precise location or regions within the cellular membrane which can be regarded as the specific site of action for anesthetics remain unknown. There have been numerous suggestions over the years, accounting for all of the major components and/or phases within the membrane, for example, lipid [for references see (3)], protein (59–61), lipoprotein (62), and cellular water (63, 64). Hydrophobic or nonaqueous sites have been given preference (65); however, the possibility exists that more than one region is involved (66,

67). The many attempts to define the molecular site of action of anesthetics form the basis of many of the early physical theories of anesthesia. The diversity of chemical structure of the many compounds that can produce anesthesia has biased many investigators to suggest that the interaction cannot occur at a specific receptor, but rather occurs via a nonspecific or physical mechanism, mainly hydrophobic. This has evolved into a "hydrophobic site theory." Considering the large number of hydrophobic sites within the membrane, the membrane could be regarded as a receptor.

Selectivity of the Site of Action

A large number of chemical agents or drugs can interact with specific receptors on different cells. These drugs are regarded by pharmacologists as specific
that can be correlated with biological activity. A comparative analysis of the structure with activity is termed structure-activity relationship (SAR). It is apparent that many cells, or cellular membranes, can distinguish or recognize a large variety of structures and exhibit a corresponding biological response in relation to that structure. This emphasizes the molecular diversity of the cellular membrane (8). The complete lack of a common chemical structure among anesthetics tends to suggest that the membrane or site of action is not capable of recognition or selection of a specific configuration of chemical structure and is affected by most compounds that are capable of hydrophobic interaction.

Selectivity of the anesthetic site, however, does exist. Although perhaps subtle when compared to specific drug-receptor
observations by many investigators of anesthetic-dependent excitant phenomena (19, 68) and variation in patterned activity (69) suggest that the site is indeed capable of responding to slight structural differences. Selectivity may exist at a single site of action comparable to the classic drug-receptor interaction, or at multiple sites (11). These sites may possess similar physical characteristics that would provide an explanation for the correlation of potency and lipid solubility (14, 70, 71). Multiple sites may also explain the differences in sensitivity of various cell types (72), and also the selectivity exhibited at the level of the synapse (73). A structure-activity relationship does exist for certain depressants, for example, the barbiturates (74) and the steroid anesthetics (75; see also 11). Although the structure and organization of lipid bilayers are not as complex as neuronal membranes, recent experiments have shown that liposomes are capable of a high degree of structural discrimination (76–79). Anesthetics may interact with a critical site, and via structural perturbations, either ordered or disordered, produce a depression of the essential functional components (80), or selectively perturb different membrane lipids (81, 82).

Although there appears to be a common modality among most anesthetics, it would be an oversimplification
a common mechanism at the cellular level. The precise mechanism of sequential events following the simple interaction with hydrophobic moieties may differ.

MECHANISMS OF ACTION

Many of the earlier theories of anesthesia resulted from correlations of anesthetic potency with various physical properties of these agents. A common relationship (physicochemical property) may only explain the differences in relative potencies and the ability of the various compounds to reach the active site within the hydrophobic milieu of the cellular membrane. This approach to the study of mechanisms has been questioned (83), for these properties relate only to the intermolecular forces of the anesthetic molecules, and provide little information about the interactions of anesthetic molecules and membrane constituents (11). This method provides some information as to the site of action, but very little toward mechanism. Many of these studies employ a homologous series that obviously follows regular relationships (6, 84). The majority of anesthetics follow a relatively simple relationship between physical property and anesthetic potency, except for the fully fluorinated compounds that have weak intermolecular forces (6). The simple relationship of the majority of anesthetics neither explains the various patterns of activity observed at the cellular level nor elucidates the precise molecular pharmacology of these agents. Thus, the many theories proposed for anesthesia have so far only provided rules of anesthesia rather than mechanisms of action (3, 85).

Hydrophobic (Lipid) Theories

Over 100 years ago a strong correlation between anesthetic potency and oil solubility was found for a wide variety of compounds [for references see (3)]. This observation led to the classical Meyer-Overton theory, or rather rule, of anesthesia. K. H. Meyer (see 3) modernized the original theory of H. H. Meyer and Overton and proposed, "Narcosis (anesthesia) commences when any chemically indifferent substance has attained a certain molar concentration in the lipids of the cell. This concentration depends on the nature of the animal or cell but is independent of the narcotic." The range of concentrations of anesthetic in the oil phase was found to be rather narrow, from 0.03 to 0.06 mol of drug per liter of oil (see also 3, 85–88).

The relationship between concentration in the oil phase and the aqueous phase is the partition coefficient. The partitioning of various anesthetics has been studied in a variety of solvent/aqueous systems (see 3, 6) in which the

physicochemical properties of the solvents are well characterized. The most suitable property is the solubility parameter δ which provides a measure of the cohesive energy density or strength of the intermolecular forces within the solvent. For the majority of anesthetics a relatively good correlation exists between δ and anesthetic potency; however, the fluorinated compounds deviate considerably (4). The minimal deviation for all anesthetics examined occurs when a solvent such as benzene is used (6). Benzene has a solubility parameter of 9.2, which suggests that the site of action of anesthetics has a δ of approximately 9 $(J\ cm^{-3})^{1/2}$. Mullins has suggested that the site for synaptic blockade may have a δ equal to 10. Both values are close to values for liquid hydrocarbons (see 10).

An approach to establishing the existence of a definite concentration in the oil (membrane) phase was that of Ferguson (89) who pointed out that thermodynamic activity could be calculated from the isonarcotic concentration at equilibrium defined relative to the pure liquid state. Thermodynamic activities for a variety of anesthetics were calculated to be between 10^{-2} and 10^{-1} mol fraction at equianesthetic concentrations. This was supported by Brink & Posternak (90). The validity and usefulness of this approach has been questioned (3, 6, 7). A limitation of this principle is that the activity is based on the pure liquid state, excluding gaseous anesthetics. It does not provide any information on classification or identification of the anesthetic site.

Aqueous Theories

The two classical theories of mechanism proposing the site of action to be the aqueous phase are those of Miller (63) and Pauling (64). Miller postulated that anesthetics were capable of ordering the water molecules in "icebergs." These icebergs would stabilize the excitable tissue membrane and thus produce anesthesia. Pauling proposed the formation of clathrates or gas hydrates. The correlation of hydrate formation with anesthetic potency is much weaker than the correlation of lipid solubility with potency. Many anesthetics cannot form hydrates and the stability of hydrates at 37°C body temperature is not well supported (see 10).

Extensions of the Hydrophobic Theories

Mullins (7) proposed an extension to the Meyer-Overton rule of anesthesia, suggesting that the potency of an anesthetic is related not only to the concentration in the membrane, but also to the volume occupied by the anesthetic. He predicted that anesthesia occurs when a critical volume fraction of anesthetic exists within the membrane phase. In dilute solutions, the volume fraction of an anesthetic is equal to the mole fraction times the partial molar volume (6). The partial molar volume is usually approximated

by the molar volume (6). It was suggested that upon reaching a critical volume of anesthetic within the membrane, permeability of ions would be depressed, thus resulting in a loss of excitability.

Many experiments have tested the hydrophobic theory by determining the concentration of anesthetic in the membrane phase (see 3, 85, 86) and characterizing the anesthetic-membrane interaction (see 3). These studies provided support that the membrane concentrations are on the order of 0.05 molal at anesthetic concentrations. To test Mullins' hypothesis, the membrane concentration was multiplied by the molar volume; these calculations support the hypothesis that equinarcotic effects occur at equal volume occupations of membrane phase (see 3, 6). Since the molar volumes are all within a factor of 2, correction is relatively minor. In addition, there may be a dependence on molecular shape (see 91).

Critical Volume Hypothesis

Mullins' theory, however, could be extended to suggest that a certain critical volume as a result of adsorption of an anesthetic would *expand* the membrane. Expansion of membrane dimensions would provide a basis for a mechanism of action. The phenomena of pressure reversal (see below) provided support for this modification

The critical volume hypothesis states that "anesthesia occurs when the volume of a hydrophobic region is caused to expand beyond a certain critical amount by the adsorption of molecules of an (inert) substance." This theory is similar in concept with other proposed mechanisms (see 6). As Miller & Smith (6) state, the expansion created by the dissolved anesthetic can be readily calculated (especially for gaseous anesthetics) from the partial molal volume. The critical concentration of anesthetic in the hydrophobic phase is estimated to be approximately 0.05 molal (at general anesthetic concentrations), and the corresponding volume increase is of the order of 0.5%. Utilizing an estimated compressibility of the site of action, expansion of 0.5% can be antagonized by a pressure of $\sim$ 100 atm—precisely the order required experimentally to reverse the effects of anesthesia in the intact organism (92).

Membrane Expansion

The critical volume hypothesis suggests that adsorption of an anesthetic into a hydrophobic region would create an increase in the volume of the site of action. This hypothesis, therefore, predicts membrane expansion as the physical mechanism of action of anesthetics. Consistent with this theory of mechanism of action are the many observations that monolayers, bilayers, bulk phase solvents and oils, and even rubber are expanded by anesthetics (reviewed in 3). The swelling or expansion of most of these phases is approximately equal to the volume of occupation of the anesthetic.

Perhaps the most convincing data in support of membrane expansion by anesthetics are the surface area measurements of erythrocytes (3, 93–95). The erythrocyte membrane increases in surface about 2–3% at anesthetic concentrations, and is reported to be as high as 10–12% (95). The expansion by volatile anesthetics at clinical anesthetic concentrations was estimated to be approximately 0.4% (93). Recently it has been found by direct measurements that lecithin monomolecular films are expanded by 0.5% (96). Based on the pressure required for reversal of anesthesia, and compressibilities of a phase resembling a membrane, it was calculated that general anesthesia can be associated with an expansion of about 0.4% (92). The volume of occupation by the anesthetic molecules can only account for less than 10% of the total expansion measured (3). The excess in expansion has been suggested to be a result of extensive conformation change in membrane proteins (61). This excess expansion or dilatation is seen only when the system contains water (97).

It is assumed that the increase in surface area is an indication of a uniform swelling in the volume of the membrane, that is, an increase in all directions (98). Stated to be the first direct evidence, a study by Seeman (60) demonstrated that ethanol expanded the specific volume of erythrocyte and synaptosomal membranes. In this same study liposome membranes were only expanded by 0.01% compared to a membrane expansion of 0.5%. This suggests that membrane protein conformation change may be the major effect. Recently it was reported that benzyl alcohol produces a large expansion in lecithin bilayers (99, 100), and *n*-alkanes create a thickening of bilayers (101) as estimated from observed anesthetic-induced decreases in capacitance.

Trudell has recently questioned the "expansion" data of erythrocytes, and suggests that the membrane volume expansions are of the order of 0.026% for general anesthetics and 0.13% for local anesthetics at effective concentrations (102). In this same article he provides references to theoretical and experimental studies which demonstrate that a phospholipid bilayer membrane decreases in thickness with associated increase in surface area. A decrease in thickness of membrane upon interaction with anesthetic agents has been suggested previously (5, 10).

Recent experiments (103) have examined the changes in cellular geometry and shape of intact erythrocytes in the presence of chlorpromazine. The results of these studies confirm earlier reports of increased membrane surface area (3), and cell shape changes (104) induced by chlorpromazine. In addition, a time-dependent reversal of shape occurred, and a model was predicted to correlated shape change with area expansion. Inside-outside ratios were calculated for the cell membrane, related to shape, and compared to area increase. It was concluded that in order for the drug-exposed cell to have an area-ratio at equilibrium similar to that of the control cell,

the membrane thickness must decrease from 100 Å to 82 Å. This represents an 18% thinning of the cell membrane and more than accounts for the observed 9% expansion of membrane area (S. H. Roth and A. W. L. Jay, in preparation).

The question remains whether membrane expansion does provide a physical mechanism of anesthesia. It would appear that an increase in surface area combined with a decrease in membrane thickness can accommodate some of the most recent findings,
ment is still lacking.

Pressure Reversal of Anesthesia

The phenomenon of pressure reversal or antagonism has been regarded as strong evidence to support the critical volume hypothesis (105) and membrane expansion theory (3, 93) of anesthesia. The original observations of pressure antagonizing the effects of anesthetics were by Johnson and coworkers (106, 107) on bacterial luminescence. Later it was reported that hydrostatic pressure could restore the spontaneous swimming motion of anesthetized tadpoles (108, 109). Subsequent studies have demonstrated this phenomenon on newts (92, 105), mice (110), isolated neural preparations (111–115), and model membranes (116–118). It has been well documented that pressure can reverse the effects of most anesthetics in the intact animal (119). It was originally suggested that pressure simply opposes the expansion and increased fluidity of the membrane in the presence of an anesthetic (92). Recent studies have shown that pressure can restore the ordering of membrane components (120) and may even induce molecular configuration changes of the hydrocarbon chains of phospholipids or anesthetics (121). Conflicting results of the ability of pressure to reverse anesthesia have been reported (112). Pressure does not antagonize all aspects of anesthesia (122) and has been shown to enhance the anesthetic effect (123). Pressure alone can produce CNS stimulation, tremors, and convulsions in animals (124), and a physiological phenomenon known as high pressure neurological syndrome (HPNS) in man (125, 126). Pressure can inhibit synaptic transmission (115), possibly in increasing synaptic failure (127), and alter current parameters in nervous tissue (127, 128). The slopes of the dose-response curves of anesthetics are different at high pressure (129), which suggests the antagonism is not direct. Pressure reversal may be a multineuronal phenomenon acting indirectly as a functional antagonist (130) that is as complicated in mechanism as anesthesia itself.

Fluidization

Fluidity is described as a measure of resistance to lateral diffusion of molecules in the membrane phase. Fluidity has become one of the most fre-

quently described properties, and recent studies of this parameter are perhaps the most exciting in the field of molecular (membrane) pharmacology.

When the hydrocarbon chains of the membrane bilayer exist in an all *trans* geometry or conformation they are perpendicular to the plane of the bilayer (131), and packed into an ordered hexagonal formation (53). This ordered state is referred to as the gel or solid phase and exists below the critical temperature (T_c). In contrast, the fluid or liquid crystalline phase is disordered and the hydrocarbons maintain an "average" orientation perpendicular to the bilayer plane, but are disordered by rapid transformations of conformation (*trans-gauche*) (102). The transition from one phase to the other occurs at the critical temperature (T_c), which is related to membrane composition and solution characteristics (53). It is well established that various agents can perturb membrane components and create transition changes, thus affecting the fluidity of the membrane. A perturbation yielding a transition change from solid to liquid crystalline may be regarded as "loosening" (3) and is often referred to as fluidization or disordering. The mechanism of anesthetic-induced membrane expansion has not been established yet (98), but experiments measuring fluidity changes of biological and model membranes in the presence of various drugs have yielded some very exciting results and predicted modes of action (132). Alteration of fluidity can be monitored as a change in mobility of the drug per se, or of mobility of a labeled probe by fluorescence spectrophotometry, or as resonance changes of spin labels measured by electron spin resonance (ESR) and nuclear magnetic resonance (NMR) (see 11).

In general, anesthetics increase the fluidity of the membrane phase; that is, they induce a transition from gel to crystalline liquid in the same manner as an increase in temperature (102, 133). The anesthetic-induced shift in transition temperature (134) suggests that the pool size of melted lipids has increased. This melting phenomenon is concentration dependent; the degree of response is linearly related to dose (118). It was demonstrated (135) that halothane produced a biphasic response on both model and brain synaptosomal membranes. The authors attributed their findings to the filling of spaces within the membrane by halothane, which would relate to the critical volume model. There has been some limited degree of disagreement as to whether fluidity changes occur at clinical concentrations. Boggs, Yoong & Hsia (136) have reported no detectable change in bilayer order at clinical doses, yet the controversy appears to be resolved on the basis of defining "clinical concentration." Kaufman (11) has pointed out this discrepancy and it would appear that fluidization does occur at clinical levels.

Utilizing spin probes that localize the spin label at different levels within the membrane phase, it was reported that the fluidization appears to be

generalized rather than localized in one region (137); however, variations in perturbation have also been reported (138, 139).

The ability of various molecules to fluidize membranes appears to correlate with their biological activity. Lawrence & Gill (76, 77) have demonstrated that biologically active cannabinoids, steroid anesthetics, and alcohols were able to fluidize lecithin-cholesterol bilayers, and the inactive compounds produced much less disordering. The effectiveness of the active compounds correlated very well with molar volume occupation, which supports the critical volume theory. The steroid anesthetics provided additional information, in that structure or molecular orientation was also important in the ability to fluidize. The comparative difference in activity or orientation of the steroid isomers was suggested to be a result of hydrogen bonding. The fluidizing effects of barbiturates (140), local anesthetics, and alcohols (141) have also been demonstrated. Localization or selectivity of anesthetic-induced fluidization has been suggested to be related to the annular lipid layer surrounding the sodium channel (141), resulting in relaxation or alteration of the channel. However, there has been some evidence to suggest that a poor correlation exists between fluidity and permeability (142). Recently, Trudell has postulated that anesthetics may alter or eliminate lateral phase separations in the bilayer, that is, the boundaries between the gel and fluid phases necessary for solvated membrane protein function (132).

The importance of fluidity to fusion of vesicles in presynaptic terminals, transmitter release, and postsynaptic responses to chemical stimulation all are implicated (see 11); thus alteration in fluidity may affect any one or all of these processes at the synaptic junction. It would appear that the sensitivity of various membranes may differ in their response to drug-induced fluidity changes, which may account for the variation in biological responses observed for different anesthetics. Fluidity is dependent on the composition of the membrane; different membranes exhibit different degrees of fluidity (143), and selectivity of anesthetic action is also dependent on membrane composition (81).

Fluidity changes may explain the phenomenon of membrane area expansion, and thus provide a mechanism of anesthesia. Consistent with a theory that fluidization may provide a mechanism is the observation that pressure can reverse anesthetic-induced transition changes in membrane bilayers (118); however, this is not yet definitive.

Anesthetic-Protein Interactions

Since many functions of the living cell are dependent on membrane proteins (57), it is essential that the effects of anesthetics on membrane proteins be established. The majority of studies have used pure proteins or polypep-

tides, since isolation of membrane proteins is difficult and requires solvents that can be classified as anesthetics. The proteins susceptible to anesthetic interaction include enzymes, receptors, membrane transport proteins, and structurally organized proteins (microfilaments and microtubules). A limited account of this topic is presented here in view of recent reviews (3, 6, 11).

Anesthetics are capable of binding to most proteins (3), which contain hydrophobic sites. The conformation changes that occur as a result of this interaction are relatively small (10). Techniques such as X-ray diffraction and optical rotatory dispersion have been frequently used to study conformation changes (10, 11).

Eyring and others (61) have proposed a theory of anesthesia based on conformational changes in proteins, referred to as protein conformational change (PCC) or unfolding theory. These theories have resulted primarily from studies of anesthetic effects on chemiluminescence of luciferase, a water-soluble enzyme from firefly tail extract [see (11) for references]. This theory could also relate to lipoproteins that are involved in transmitter release and postsynaptic membrane conductance.

Trudell has recently proposed a theory of anesthesia which suggests that anesthetics alter lateral phase separations in membrane lipids, which induces inhibition of conformational changes in proteins associated with neural function (132). This theory is compatible with the coupling theory of Lee (141).

The effects of anesthetics on enzyme systems have been reviewed (see 11); this interaction is usually not regarded as a primary action for anesthesia (3, 6). The roles of microtubules and microfilaments are not completely understood. Studies related to these structures have been described elsewhere (10, 11). Generally the concentrations of anesthetics effective on these structures are relatively high, which suggests that the effects are secondary to anesthesia.

CONCLUSIONS

Physical mechanisms of anesthesia emphasize the hydrophobic interaction. Many of the studies of mechanism of action have stressed the lipid bilayer, and little information has been provided on the interaction of anesthetics with membrane proteins or lipoproteins.

Lipid solubility is apparently essential for activity, yet does not always predict anesthetic effectiveness. A well-known observation, still unexplained completely, is the "cut-off" phenomenon, where beyond a certain member of a homologous series of anesthetics, further increases in hydrocarbon length will result in a decrease in pharmacological activity. Fergu-

son (89) referred to this phenomenon as the "cut-off" point. He attributed this "cut-off" to low aqueous solubility, such that insufficient quantities of the drug were able to enter the site of action. Mullins (8) explained the phenomenon as a result of large molecules being able to "fit" into the site of action without perturbing the membrane. The biological activity of a homologous series increases with increasing lipophobicity, reaches a maximum, then begins to decrease with further increase of hydrophobicity. A dramatic "cut-off" point does not exist (144, 145). Activity is related to hydrophobic character in a bilinear relationship (146) and is demonstrated experimentally as a symmetrical parabola. Although many explanations have been suggested for the "cut-off" phenomenon (see 3), perhaps the best explanation is that the active or hydrophobic binding sites in the membrane

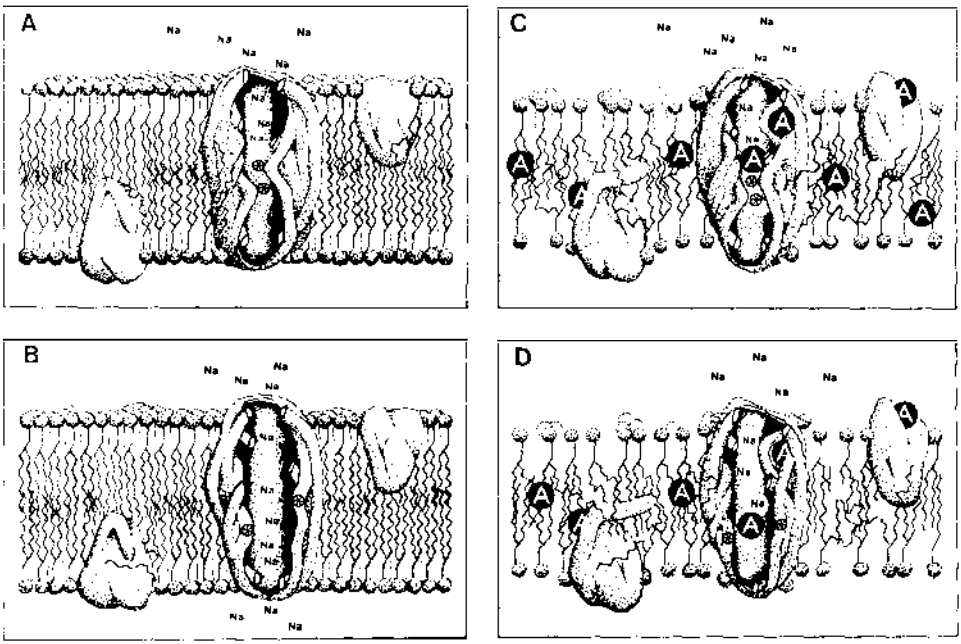

Figure 1 Anesthetic-membrane interactions: possible mechanisms of action. *A.* Membrane at rest. The membrane is composed of a phospholipid bilayer embedded with function protein (ionophore channel). The channel is "gated" by charged (⊛) or bulky constituents. *B.* A voltage-dependent conformational change of ionophore displaces the "gates," and creates an active (open) channel for ions (current) to flow. *C.* Anesthetic molecules bind to hydrophobic (and perhaps charged) sites within the membrane. Fluidization of bilayer and alteration of conformation of functional proteins result in an increase of membrane surface area (membrane expansion) with a decrease in membrane thickness. *D.* A sufficient voltage-dependent conformational change of ionophore to form an open channel cannot occur because of anesthetic-induced perturbation of bilayer and functional proteins. Anesthetic may also effectively block the channel.

exhibit a size discrimination effect (59), providing a method of selectivity at the site of action. This selectivity may provide an explanation for partial anesthetics (20, 76, 77).

In Figure 1, a schematic diagram is presented to demonstrate the effects of anesthetics on membranes. Anesthetics may localize in various hydrophobic regions of the membrane. Localization may be dependent on lipid solubility as well as size, shape, binding characteristics, etc of the anesthetic molecule. Upon interaction with one or more sites, drug-induced perturbations would result both in a change in membrane dimensions, i.e. increase in surface area and decrease in thickness, and in a change in the conformation of functional proteins within the membrane. Some anesthetics may affect certain sites more effectively than others; sites may be associated with the lipid bilayer and/or the proteins.

It is conceivable that the interaction of anesthetics with the lipid phase results in the generalized nonspecific effects of anesthesia, while the interaction with membrane proteins provides the selectivity and specificity of action.

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