

## DIFFERENTIAL EFFECTS OF GASEOUS AND VOLATILE ANAESTHETICS ON SODIUM AND POTASSIUM CHANNELS

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After 150 years of clinical anaesthesia, the molecular events that lead to anaesthesia have not been determined. This is not because of a lack of ideas [16-19, 56, 57]. However, most striking to the observer is the paucity of long-term and systematic studies. The present review describes results from such a study. The long-term goals were: first, to understand how anaesthetics affect membrane protein function and second, to determine if clinical and molecular actions of anaesthetics may be correlated.

### *Definition of anaesthesia and anaesthetic*

Before the mechanisms of anaesthetics are discussed, the term "anaesthesia" should be defined in practical terms [64]: "Anaesthesia describes the condition of a patient corresponding to the one produced by diethyl-ether which permits surgery to be performed without the patient moving, reacting to pain, or remembering the surgical intervention after recovery from anaesthesia."

Strictly speaking, only a substance that has all these qualities may be called a general anaesthetic. However, clinical practice is such that almost all general anaesthetics are given in combination with other agents at concentrations where, by themselves, they may not produce anaesthesia. Therefore, the term general anaesthetic is used for substances that, at sufficiently large concentrations, could produce anaesthesia, although for a variety of reasons these large concentrations are not used in common clinical practice. While this definition extends across the range of general anaesthetics, basic scientists have broadened the definition further: they include all substances that in animal experiments produce "anaesthesia-like" states, as determined by the absence of certain reflexes (e.g. in response to pain, in response to posture changes, etc), and in addition, anaesthetic-like compounds are included also. These substances may not have been tested in whole animal experiments, but may act similar to general

anaesthetics when used in *in vitro* preparations, at the network, cellular, subcellular or molecular level.

### *Integrative approach towards understanding anaesthetic action*

The action of an anaesthetic at a particular molecular site will depend on how this molecular site is integrated into the function of the CNS. The anaesthetic effect on a particular membrane protein (e.g. a sodium channel) is the result of several and simultaneous actions at presumably distinct sites of the sodium channel macromolecule. The total anaesthetic response of a neurone again results from the summation of the responses of its different ion channels and ion pumps to the anaesthetic and the various inputs it receives from neurones of surrounding networks (fig. 1).

The example in figure 1 illustrates the complexity of possible anaesthetic modified responses of a neurone, although the example is oversimplified, since a typical neurone within the cortex receives thousands of synaptic inputs [38]. Most neurones, even when exposed to an anaesthetic, will not respond to an incoming signal on a single input (fig. 1A and 1E), unless some temporal (fig. 1B) or spatial (fig. 1C) integration has taken place that has not been offset by a simultaneous inhibitory input (fig. 1D). Anaesthetic modifications of the incoming signal (which need not be blocked completely) that prevent a neurone from firing may involve alterations of firing rates (fig. 1F) or temporal shifts (fig. 1G) in the incoming signals. Alternatively, a temporal shift involving inhibitory inputs may lead to removal of inhibition (fig. 1H). A network of neurones responds with integration of the anaesthetic responses from individual member neurones. Partial inhibition may lead to complete inhibition at the next higher level of integration, alternatively, it may result in removal of inhibition (fig. 1). It is not possible to predict the effects of anaesthetic actions at the molecular level without knowing the neuronal network topology. Conversely, knowledge of the network topology will not allow predictions about anaesthetic effects on the CNS, without understanding of anaesthetic actions at the molecular level.

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(Br. J. Anaesth. 1993; 71: 25-38)

### **KEY WORDS**

*Action potential. General anaesthetic. K<sup>+</sup> channel. Lipophilicity. Mechanism of action. Membrane excitability. Meyer-Overton correlation. Na<sup>+</sup> channel.*

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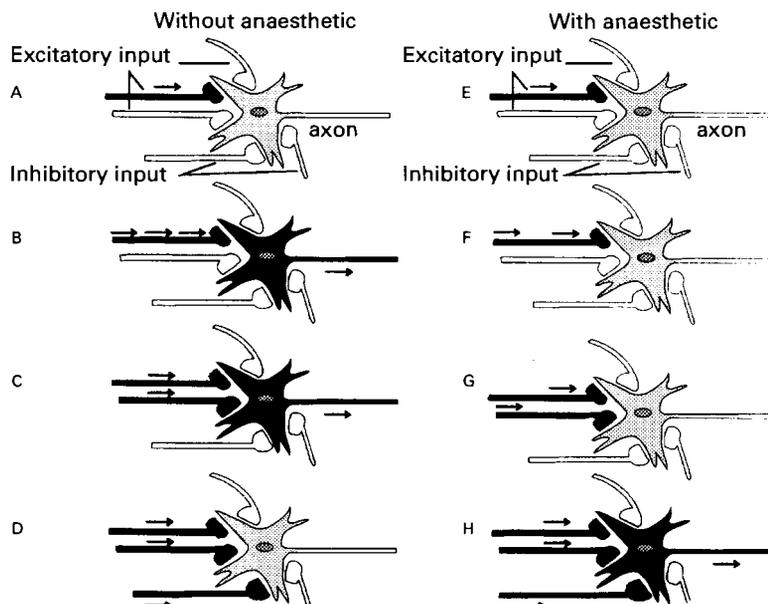


FIG. 1. Spatial and temporal integration of excitation and inhibition within a model synapse and its potential modification by anaesthetics. Resting fibres are drawn white, grey indicates a non-propagating excitation, black symbolizes a propagating excitation. The number of arrows indicates the frequency of incoming signals, their relative shift a temporal shift in incoming signals (see text for explanation).

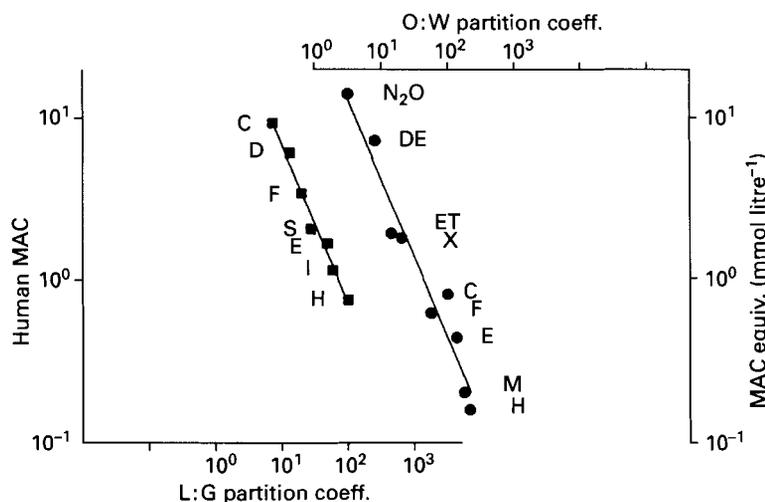


FIG. 2. Left: Meyer-Overton correlation of the logarithms of human MAC values for cyclopropane (C), desflurane (D), fluroxene (F), sevoflurane (S), enflurane (E), isoflurane (I) and halothane (H) vs the logarithms of the lecithin:gas (L:G) partition coefficient (slope  $-0.987$ ). Data from Taheri and colleagues [61], who could not find a statistically significant difference between the correlations of anaesthetic potency with either lecithin:gas or octanol:gas partition coefficients. Since there are more partition coefficient data available for the octanol:water system and to facilitate later comparisons with i.v. anaesthetics, water:octanol coefficients may be used equally as well. Right: Human MAC data converted into equivalent aqueous concentrations [66] vs octanol:water (O:W) partition coefficients [56] (slope  $-0.991$ ). DE = Diethyl ether; ET = ethylene; X = xenon; M = methoxyflurane.

### Identifying key anaesthetic interactions

A vast range of physiological changes and responses are observed in an organism under anaesthesia and various stages of anaesthesia are distinguished clinically. Separate molecular mechanisms may account for the many different manifestations of anaesthesia, in much the same way as separate mechanisms have been found responsible for the different forms of sleep and memory [38]. The large variety of anaesthetic responses could result from a combination of several types of anaesthetic interactions with different proteins and

membranes. It is thus important to identify and understand the nature of these key anaesthetic interactions, and appropriate model systems have to be used in which anaesthetic interactions may be studied systematically.

### Meyer-Overton correlation

The Meyer-Overton correlation (fig. 2) shows that anaesthetic potency and lipid partition coefficients are related linearly over many concentration decades [45]. This finding pointed to membranes as an important site of action of anaesthetics [54]. This simple relationship indicated that the underlying

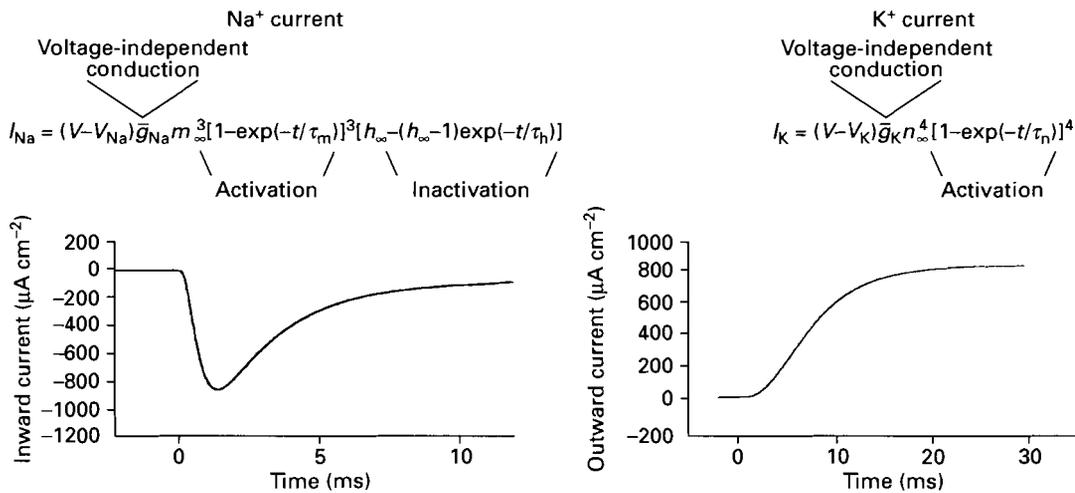


FIG. 3. According to Hodgkin-Huxley formalism [36], sodium currents are the product of three different terms which describe membrane potential-independent conductance ( $\bar{g}_{Na}$ ), activation and inactivation.  $V$  = membrane potential,  $V_{Na}$  and  $V_K$  = sodium and potassium equilibrium potentials, respectively. The activation term contains a steady state variable ( $m_\infty$ ), the cube of which describes the fraction of channels activated in the steady state and an exponential expression including a time constant ( $\tau_m$ ). Potassium currents are a combination of a membrane potential-independent conductance ( $\bar{g}_K$ ) and an activation term containing a steady state variable ( $n_\infty$ ) the fourth power of which describes the fraction of channels activated, and an exponential expression including a time constant ( $\tau_n$ ). All variables, except for  $\bar{g}_{Na}$  and  $\bar{g}_K$ , are functions of membrane potential, that is they assume different values at different membrane potentials (see fig. 5).

mechanism was simple also. Yet, while sharing this common characteristic of lipophilicity, anaesthetic molecules were found to vary greatly in their chemical and physical properties [48, 59]. Thus this suggested a non-specific mode of action, comparable to the action of an antifreeze in a car radiator where depression of the freezing point results from disruption of the ice bonding structure, and depends only on the number of molecules of antifreeze and not on its particular chemical structure. It was thought that anaesthetics dissolve in membranes and thereby disrupt the structure of the membrane. However, an important question remained: how could changes in these physical variables impair the electrical excitability of a membrane?

#### Membrane proteins and ion channels

Lipid bilayer membranes, consisting of a bimolecular leaflet of lipid molecules, constitute the backbone of a biological membrane. Lipid bilayers are not electrically excitable as they are perfect insulators, permitting no ion flow. However, biological membranes also contain many different membrane proteins which are essential for mediating many physiological functions. Direct or indirect interference of anaesthetics with membrane proteins is likely to be of great importance. Opinions have been divided on whether or not anaesthetics interfere with membrane protein function by binding directly to proteins [20, 54] or if the main modes of action occur indirectly through changing the physico-chemical properties of the lipid membrane into which the anaesthetics readily dissolved [15, 46]. In order to differentiate to what extent anaesthetics interfere with the interaction between lipids and membrane proteins, while possibly also interacting with membrane proteins directly, it is important to select appropriate membrane proteins for these studies and to monitor the effects of systematically

changing the structure of membranes, proteins and anaesthetics alike.

Ion channels constitute a class of membrane proteins that render membranes electrically excitable [35] but also susceptible to the actions of anaesthetics [57]. The study of ion channels is therefore of both immediate relevance, and wider interest within the context of characterizing the interactions between anaesthetics, lipids and integral membrane proteins. Because in-depth studies of ion channels are time-consuming and difficult [15, 47, 65], it is not practical to abandon one and begin a new study every time fashions and ideas change as to what ion channel is the most important for anaesthesia [49]. In order to characterize the anaesthetic interactions that different ion channels have in common, a good starting point is a thorough investigation of typical and well characterized ion channels that may serve as model channels.

#### Sodium channel as a model system for ion channels

Voltage-gated sodium channels are obvious and strong candidates to represent a model system. With the possible exception of acetylcholine receptors, sodium channels are unparalleled in the extent to which their structural and functional properties have been characterized [5, 35, 39]. They are ubiquitous in the central and peripheral nervous system, mediating fast propagated action potentials, which are vital to nerve impulse transmission and cell communication [38]. Sodium channels play an important role in signal integration; action potentials are initiated in the axon hillock region where the density of sodium channels is large [38].

Sodium channel function, using voltage-clamp, patch-clamp, lipid bilayer and ion flux techniques, has been described by detailed mathematical models and formalisms (fig. 3) [3, 36, 44, 52]. Their structural properties have been, and are being,

elucidated in numerous studies using biochemistry, spectroscopy and molecular biology techniques [1, 6, 8, 42, 51]. Ion channels may be grouped into families of evolutionary and structurally related gene products; for example, 55% sequence homology exists between sodium and calcium channels [8, 62]. Because sodium channels have served as a model for the description of almost all other voltage-dependent ion channels (e.g. fig. 3) and with suitable modifications even for chemically activated channels [35], they provide a good starting point for the study of anaesthetic interactions with ion channels. The actions of hundreds of agents, including anaesthetics, have been evaluated in pharmacological studies [50, 55, 59]. The suppression of sodium channels by gaseous and volatile anaesthetics follows the Meyer-Overton correlation [63].

#### *Mechanistic basis for action potentials*

The action potential in most nerves and skeletal muscle represents a transient reversal of the resting membrane potential [35], caused by time- and voltage-dependent changes in membrane conductance for sodium and potassium ions as first described in the squid giant axon [36]. Hodgkin and Huxley successfully applied the voltage-clamp technique to study these conductances; rather than eliciting action potentials, they held (clamped) the membrane potential fixed and measured the resulting macroscopic currents (fig. 3), so-called because they are generated by many thousands, if not millions, of ion channels. Inward sodium currents were characterized by a rising (activating) phase, followed by a current decline (inactivation), while outward potassium currents showed no such decline (fig. 3). The shapes, activation and inactivation of these current traces changed with membrane potential. Four distinct functions have been described for the sodium channel: a transmembrane ion pathway, a selectivity filter, an activation and an inactivation mechanism. In the squid giant axon, the potassium channel functions similarly, except that it does not normally inactivate. The activation (inactivation) mechanisms have been formally described as gates that open (close) on membrane depolarization and close (open) on membrane repolarization.

The Hodgkin-Huxley formalism [36] is a detailed mathematical description of sodium and potassium currents in the squid giant axon which fits the experimental macroscopic current data well and the resulting variables are capable of reproducing action potentials mathematically. With slight modifications, the Hodgkin-Huxley formalism has also been applied in the description of ionic currents in other preparations [35]. A description that is based on the observation of macroscopic currents may provide only limited information about events at the molecular level, thus there exists considerable controversy concerning an accurate description of sodium channel function at the molecular or single channel level [52]. Therefore, the Hodgkin-Huxley formalism is used here simply to differentiate between separate actions of anaesthetics on macroscopic currents.

#### *Grouping anaesthetics according to their physico-chemical properties*

Among the inhalation anaesthetics, there are mainly two different classes: alkanes and their derivatives and ether and ether derivatives. The alkanes include cyclopropane (a cyclic alkane), chloroform (derived from the normal or n-alkane methane) and halothane (a n-ethane derivative). The ethers include diethyl ether, the methylethyl ether derivatives methoxyflurane, enflurane and isoflurane. Analysing an anaesthetic in terms of its chemical groups and parent compound gives important information as to its possible mode of action. For example, diethyl ether ( $\text{CH}_3\text{—CH}_2\text{—O—CH}_2\text{—CH}_3$ ) consists of different chemical groups, having either purely lipophilic character ( $\text{—CH}_3$ ,  $\text{—CH}_2\text{—}$ ) or being polar (the ether linkage  $\text{—O—}$ ). Purely lipophilic compounds reside primarily in the membrane interior (e.g. cyclopropane), non-ionic polar compounds reside predominantly at interfaces (e.g. alcohols) and inhalation anaesthetics have properties somewhere in between. By comparison with purely lipophilic molecules (alkanes) and strongly polar uncharged molecules (n-alcohols), the action of diethyl ether may be rationalized as falling somewhere in between, in turn providing the basis for the preliminary characterization of the even more complex halogenated ethers, such as isoflurane and enflurane. In terms of mechanisms of action, it has proved helpful to group gaseous and volatile anaesthetics according to their physico-chemical properties and distinguish between three different groups: (i) purely lipophilic, (ii) uncharged strongly polar, and (iii) inhalation anaesthetic. Haydon and his subsequent co-workers later subdivided the uncharged polar group by including carboxylic esters and ketones and added the group of ionic and ionizable compounds (for a review see [15]).

#### ANAESTHETIC ACTIONS ON SODIUM AND POTASSIUM CHANNELS

##### *Anaesthetic effects on voltage-gated sodium channels*

*Distinct spectra of anaesthetic action.* All anaesthetics that have been investigated so far have depressant effects on sodium channels [7, 15, 55, 59]. Figure 4 shows the effects of typical representatives of the three groups of gaseous and volatile anaesthetics discussed above. The anaesthetic concentrations are chosen such that the peak sodium currents are approximately half-suppressed.

When the actions of the anaesthetics are dissected in terms of the Hodgkin-Huxley formalism (fig. 4) it is clear that, in general, and depending on the concentration, anaesthetics affect all five Hodgkin-Huxley variables of the sodium current. The currents in figure 4 are reconstructed mathematically in such a way that they show the influence of the anaesthetic changes on a single Hodgkin-Huxley variable of the sodium current. Clearly there are instances where the anaesthetic effect on a single variable leads to current increase rather than current suppression. Therefore, the total inhibitory

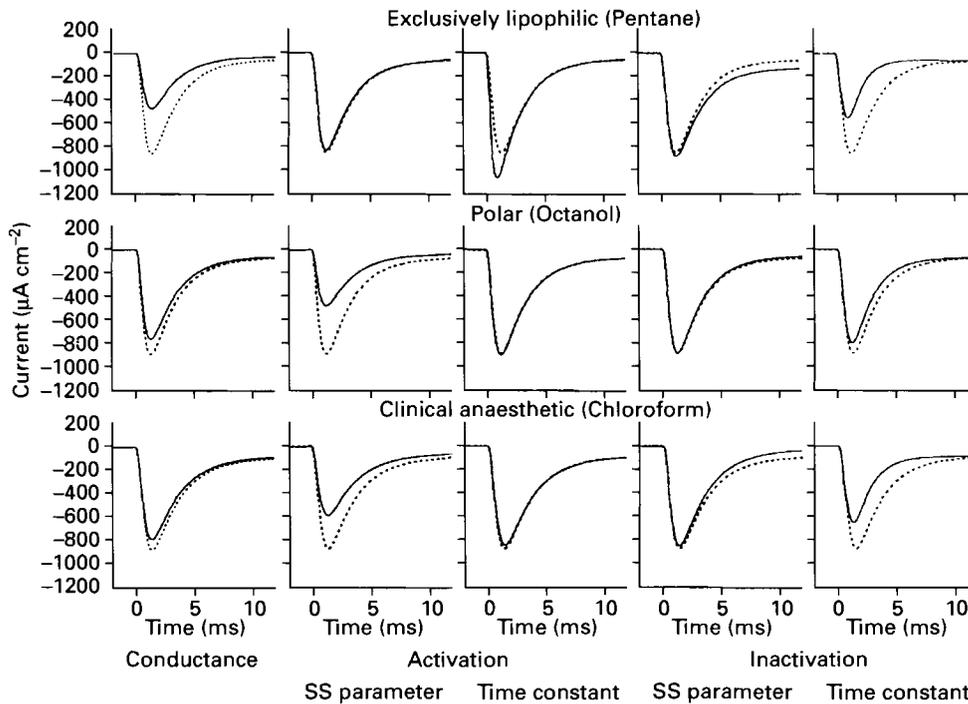


FIG. 4. Based on Hodgkin-Huxley formalism, the effects of three groups of anaesthetics on sodium currents are dissected. A set of five Hodgkin-Huxley variables each were obtained for the control (dashed line) and drug-suppressed currents by fitting the Hodgkin-Huxley equations to the experimental data [29–31]. The Hodgkin-Huxley variables of the control currents are then replaced, only one in each panel, as indicated on the abscissa, with the corresponding variable of the drug-suppressed current. The remaining four variables in each panel are those describing the control current. The resulting theoretical current (continuous line) is shown. SS = Steady state.

anaesthetic responses of sodium currents (fig. 4) results from the integration of separate inhibitory and excitatory anaesthetic actions on distinct sodium channel functions. Any one anaesthetic class suppresses sodium currents by more than one mechanism, while different classes of anaesthetics have distinct spectra of anaesthetic actions.

*Purely lipophilic volatile substances.* Cyclopropane [10] is an example of a purely lipophilic substance. This hydrocarbon was characterized in voltage-clamp experiments together with other cyclic alkanes, n-alkanes and carbon tetrachloride [29]. The molar concentrations of these hydrocarbons required to produce current suppression of 50% varied widely, but correlated well with lipid solubility [29], suggesting that the sites of action are essentially lipophilic.

Because of their lipophilic nature, hydrocarbons are expected to adsorb predominantly into the centre of the chain region of the bilayer, causing the membrane to thicken. This has been shown to be the case in lipid bilayer experiments and attempts have been made to demonstrate a similar effect in the squid giant axon [29]. Because of its size and simple geometry, the squid giant axon membrane, of all the biological membranes studied, is probably the most amenable to this analysis using electrical capacity measurements. Yet, even in this simple preparation, conclusions could be reached, not by direct proof, but only by consistency arguments, since the exact composition of the squid giant axon was unknown and geometrical correction factors caused by other membranes lying in front of the axolemma membrane were uncertain [30]. In so far as the nerve

membrane capacities at 100 kHz were determined by the thickness of the bilayer regions of the membrane, the results are consistent with thickening of the membrane by the hydrocarbons. A direct correlation between structural changes (evaluated via simultaneous electrical capacity measurements) and functional changes on membrane currents by the hydrocarbons was observed [26, 27, 29].

A thickening of the axolemma membrane by hydrocarbons could be expected to affect sodium channel function in several ways. The electric field ( $E$ ) within the membrane may be altered as a result of the thickness ( $d$ ) increase, since the electric field  $E = V/d$ , where  $V$  is membrane potential. Because the electric field is the physical quantity which operates on the gates of the sodium channel when the membrane potential is changed, as a consequence, the time courses and equilibrium values of the electric field-dependent variables of the Hodgkin-Huxley equations may also change [26]. In addition, and together with simultaneous membrane tension increase, as described in lipid bilayers [34], the thickness increase may subject the channels to stresses which could reduce the maximum conductance  $\bar{g}_{Na}$ . Inspection of the anaesthetic effects on the Hodgkin-Huxley variables follows.

All the hydrocarbons caused reduction in the voltage-independent conductance,  $\bar{g}_{Na}$ . This conductance is the product of the number of channels in the membrane, their availability and their single channel conductance. Haydon and co-workers, in proposing the thickness-tension hypothesis [24, 25] argued that, if a channel is normally in equilibrium with the surrounding membrane, a thickness change

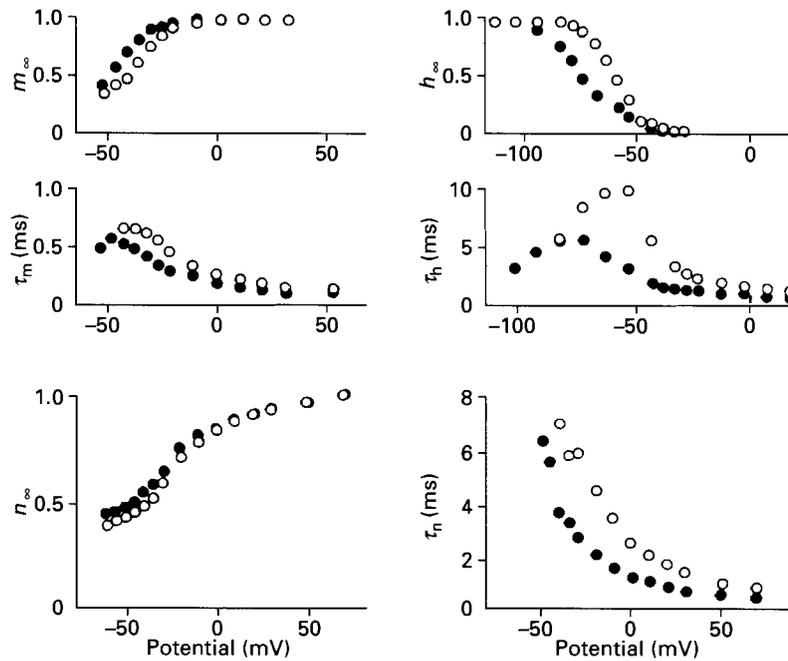


FIG. 5. Influence of *n*-pentane  $275 \mu\text{mol litre}^{-1}$  on variables of the Hodgkin-Huxley formalism (fig. 3) for the sodium and potassium currents ( $\bullet$ ).  $\circ$  = Control values. All parameters are functions of membrane potential.

in the lipid is almost certain to stress the channel, destabilize it, and make it less available for conduction. A direct example of this mechanism was provided by the experiments with gramicidin in lipid bilayer membranes [34]. The antibiotic gramicidin behaves as a cationic ion channel that is not gated by membrane potential. It has been studied extensively in artificial membrane systems using the planar lipid bilayer technique. When lipid bilayers were formed from the same lipid, but using different chain length alkanes, similar additions of gramicidin to the system produced widely different levels of conductance. A mechanism was proposed and a theory developed in terms of mechanical stresses that are set up in the membrane that pull the gramicidin dimer apart in the middle. Essentially quantitative agreement between theory and experiment was found [34], constituting possibly the first and only quantitative example of a specific molecular mechanism by which anaesthetics reduce electrical excitability that has been verified by experiment.

If the hydrocarbon is assumed to thicken the membrane, as the electrical capacity results suggest, then the increase in thickness of the non-polar part of the membrane would change the electric field experienced by electric charges or dipoles in this region (responsible for operating the channel gates), even though the actual membrane potential remained constant. This should lead to both a shift and slope change in the voltage dependence of steady state activation and inactivation variables, as has indeed been observed (fig. 5).

However, while the correlation between electrical capacity (relating to thickness) changes and observed shifts in steady state inactivation was excellent, the slope of the curve was larger than expected [29]. This led to speculation that the average lipid composition of the membrane (as would be reflected in the electrical capacity measurement) differed from

the composition of the lipids in the immediate vicinity of the sodium channel. The shifts in the steady state activation curves were consistently less negative than in the inactivation curves. This suggested that, although the activation process was influenced by the same mechanism (e.g. membrane thickening) as the inactivation process, there was an additional effect superimposed which tended to move the activation curve in the opposite direction. When considering the physical origin of this effect for the hydrocarbons alone, there remained at least two possibilities to explain the additional mechanism; either a change in protein confirmation or an asymmetric change in the surface potential [29].

Lipophilic anaesthetics affect time constants also. For the study of mechanisms, it is necessary to separate these shift-induced changes as discussed above (fig. 5) from those caused by other effects. This can be done by considering the effect of a particular anaesthetic on the peak values of the activation and inactivation time constants. When this is done, it becomes clear that a decrease in the peak values of activation and inactivation time constants is found for most of the anaesthetics examined. A tempting explanation is that membrane fluidity increases in the presence of the anaesthetics and that the movements of proteins becomes more rapid. There is, however, relatively little direct evidence for this in the squid giant axon.

In summary, the effects of purely lipophilic substances, including cyclopropane, on the variables of the Hodgkin-Huxley equations suggest that suppression of the sodium current by these substances originates from several different effects. None of these appears to be highly specific. They may be tentatively explained in terms of adsorption of the hydrocarbons into the lipid regions of the membrane and the consequent perturbations of the sodium channel. Both the shifts in the steady state variables

and the loss of maximal conductance appeared to be accounted for by thickening of the bilayer parts of the membrane, which has two effects: change of the internal electric field after the thickening and affecting the steady state variables, and a thickness-tension mechanism reducing maximal conductance. The reduction in time constants may result from increased "fluidity" which small hydrocarbons are expected to have produced in the chain region of the membrane lipid.

*Alcohols and other polar volatile substances.* Alcohols do not make good clinical anaesthetics. They and other surface active substances differ from hydrocarbons in that, as a consequence of their polar groups, (hydroxyl groups for alcohols), they have only one end of the molecule localized in the bilayer interface. In contrast to hydrocarbons, for most polar substances there is little evidence of membrane thickening. Alcohols do not thicken "solvent-free" lipid bilayers [12], electrical capacity changes in squid giant axons are small and so are changes in the voltage-independent conductance,  $\bar{g}_{Na}$ , at anaesthetic concentrations where sodium currents are half-suppressed [30]. However, there are exceptions, such as methyl mono-octanoate, which produces a comparatively large reduction in  $\bar{g}_{Na}$ , suggesting perhaps a local thickening, as discussed below.

The polar substances examined in voltage-clamp experiments included the series of n-alkanols, from pentanol to decanol, and several neutral octanol derivatives with varying headgroup sizes, such as n-octyl (oxyethylene)<sub>3</sub> alcohol, methyl n-octanoate, glycerol 1-mono-octanoate and dioctanoyl phosphatidylcholine [30]. The alcohols produced very small effects on the steady-state inactivation at the concentrations at which sodium currents were half-suppressed (fig. 4). This is consistent with the evidence from lipid bilayer experiments and electrical capacity measurements on axons, that little or no thickness changes occur. Again, methyl mono-octanoate was an exception, leading to substantial shifts of the inactivation curve in the hyperpolarizing (negative) direction and reduction of its slope. A possible explanation for this [30] is that one or both oxygen atoms in the ester group interact with the sodium channel protein via a group embedded in the interior of the membrane. A local membrane thickening would result that could not be detected readily by other means, such as electrical capacity measurements. Therefore, it now appears necessary to invoke a site of action in or on the channel itself, in addition to interactions with the adjacent lipids.

All the polar substances examined produced a shift of the steady state activation curve in the depolarizing (positive) direction, opposite to the one observed for lipophilic substances. Following the reasoning for the hydrocarbons, the absence of membrane thickening by polar substances would cause the second mechanism that produced a depolarizing (positive) shift in the activation curve to dominate. If changes in the steady state properties of the membrane are to be invoked, dipole potentials at the surface rather than surface tension are possible candidates [30]. Benzyl alcohol has been demonstrated to modify dipole potentials in lipid bilayer

membranes [53]. However, at present there are no compelling reasons to rule out dipole potentials originating from the sodium channel protein itself. Allowing for shifts along the voltage axis, the substances within this group decreased the time constants of activation and inactivation.

In conclusion, alcohols and surface active substances also suppress sodium currents by a number of effects. They appear to act in a lipophilic environment, such as the membrane lipid, but a non-polar region of a protein cannot be ruled out as a possible site of action. While several effects may be explained by a non-specific mechanism of action, there appears to exist at least one much more specific interaction, involving a site of action in or on the channel itself.

*Inhalation anaesthetics.* The inhalation anaesthetics examined in this series included isoflurane, enflurane, methoxyflurane, diethyl ether, halothane and chloroform [31]. These are intermediate to hydrocarbons and alcohols in physico-chemical properties. The dielectric constants are greater than for hydrocarbons, resulting in a lesser tendency to adsorb into the lipophilic interior regions of the membrane. Yet they are also less surface active than the alcohols because they have only weak hydrogen bonding groups by which they may interact with polar molecules. Consequently, they should be less strongly localized at the membrane surface.

Suppression of voltage-independent conductance,  $\bar{g}_{Na}$ , is similar for the different anaesthetics and it is small in relation to suppression of the peak inward current. It resembles the suppression produced by the alcohols and other surface active substances, much more than that for hydrocarbons (fig. 4).

At the concentrations which half-suppressed the peak sodium current, the shifts in the steady state inactivation curves along the voltage axis ranged from very small values, which were scarcely different from the results for alcohols, to values which were much larger and more than half the corresponding shifts for the hydrocarbons. When another group of 11 halogenated ethers was considered also, a structure-activity correlation emerged [66]. When hydrogens in a CH<sub>3</sub> or CH<sub>2</sub> group are replaced by halogen atoms, to yield groups such as CF<sub>2</sub>H or CClH, the remaining hydrogen(s) become acidic and capable of forming hydrogen bonds [9]. Halogenated ethers with only one strongly acidic hydrogen, such as methoxyflurane, produced large shifts in the inactivation parameters. Substances with two acidic hydrogens, such as isoflurane and enflurane, produced only small shifts. If the large shifts reflected membrane thickening then it would appear that strongly hydrogen bonding molecules could partition into the membrane interior by forming a bond with a protein group in the non-polar part of the membrane, such that the otherwise unfavourable partitioning between aqueous and hydrocarbon environments was offset. The adverse effect of two acidic hydrogens would merely suggest that there are not two suitably placed proton acceptors in the relevant region of the membrane. Halothane appears to spoil this correlation, since it has one acidic hydrogen atom, yet it causes only a small shift.

Features which distinguish it from the halogenated ethers are a smaller molecular volume and a bromine atom on the same carbon as the acidic hydrogen. Molecular volume of the hydrophobic portion of an anaesthetic molecule appears to play a role, as will be seen in a later discussion on the effects of long chain alcohols on potassium currents.

All the steady state activation curves are shifted in the depolarizing (positive) direction. In this respect, these inhalation anaesthetics also resemble the alcohols and other surface active substances much more than the hydrocarbons. Lipid bilayer studies suggest that chloroform appears to modify the surface dipole potential [53]. This would be consistent with the suggestion made when discussing the effects of the hydrocarbons and alcohols, that an unequal effect on the surface dipole potentials at the two sides of the axon membrane may be responsible. The peak values for both activation and inactivation time constants were lowered. This decrease in time constants, allowing for shifts along the voltage axis, is an almost uniform feature for all the substances in this section and the preceding sections on hydrocarbons and alcohols.

In summary, as expected from their physico-chemical properties, the inhalation anaesthetics show effects on the Hodgkin-Huxley variables that are largely intermediate between those of the hydrocarbons and alcohols. Again, several effects appear to result from non-specific actions of these drugs. However, halogenated ethers, such as isoflurane, enflurane and methoxyflurane, appear to be capable of an additional specific interaction, possibly resulting from their abilities to form hydrogen bonds. Again, the conclusion is reached that several factors contribute to the changes produced in the sodium current.

#### *Anaesthetic effects on voltage-gated potassium channels*

*Comparing different ion channels within the same membrane.* If the finding of non-specific interactions as an important component in the molecular mechanisms of anaesthetic action is to be generally applicable, it should find its parallel when anaesthetic actions on other ion channels are examined. With the exception of esters, ketones and halogenated ethers, no strong reasons have emerged to invoke specific interaction between the gaseous and volatile anaesthetics with either lipids or sodium channel, but several factors normally contribute to changes in sodium current. Since the above mentioned anaesthetics interact non-specifically with sodium channels, the expectation appears natural that other membrane proteins might be affected similarly. Apart from voltage-activated sodium channels, the squid giant axon membrane also contains voltage-activated potassium channels of the delayed rectifier type. There are many other types of potassium channel [35]. The potassium channels of the delayed rectifier type are important during the decreasing phase of the action potential when the membrane potential is repolarized towards the resting potential. To compare the actions of anaesthetics on ion channels within the same membrane has the ad-

vantage that differences in anaesthetic concentration may be ruled out, as explanations for the observed differences in anaesthetic sensitivity.

There are relatively few studies on interactions of general anaesthetics with voltage-gated potassium currents of the delayed rectifier type, probably because sodium currents are usually more sensitive. However, there are reasons why such a difference in sensitivity may be expected. While voltage-gated potassium channels in squid giant axons function similar to sodium channels, they do not normally inactivate. As has been discussed in the previous section, the action of anaesthetics on voltage-independent conductance, the activation and inactivation system summate. Since the action of anaesthetics on the inactivation system has resulted generally in sodium current suppression, the absence of an inactivation system would take away a mechanism by which anaesthetics may depress the ion channel function. In order to compare non-specific anaesthetic actions on sodium and potassium currents, the effects on sodium inactivation mechanism have therefore to be isolated. Using the Hodgkin-Huxley formalism, such comparisons may be accomplished easily.

*Purely lipophilic, polar and inhalation anaesthetics.* As in the case of sodium currents, the observation is made again that anaesthetics affect several functions of the potassium current system, reducing the voltage-independent conductance, as well as shifting steady state activation curves and changing activation time constants (fig. 6). There are striking parallels between the sodium and potassium systems. In both current systems, at concentrations that half-suppressed sodium currents, the voltage-independent conductances were reduced by lipophilic substances by a similar amount, that is about one-third, while the group of n-alcohols produced decreases of, at most, 10%. A similar observation was made with another ion channel, the antibiotic gramicidin. The time-averaged conductance of this channel, corresponding to the membrane voltage-independent conductance of sodium and potassium channels, was also much more depressed by lipophilic compounds than by alcohols [34, 63]. As discussed before, the thickness-tension hypothesis may provide an explanation for this similar behaviour of three very different membrane ion channels. There are differences also for inhalation anaesthetics. Methoxyflurane and diethyl ether reduced voltage-independent potassium conductance more effectively. Also, at larger concentrations, the normal alcohols decreased significantly both voltage-independent potassium and sodium conductance and the suggestion was made that this effect could arise from a weaker interaction with another site, perhaps inside the channel [33].

Most of the anaesthetics examined affected steady state activation by shifting it in the depolarizing (positive) direction. Comparing these shifts with those for sodium currents showed a broad qualitative correlation, but the quantitative agreement was poor. For sodium currents it had been argued that dipole potential changes could explain these shifts [30]. If these dipole potentials originate in membrane lipids

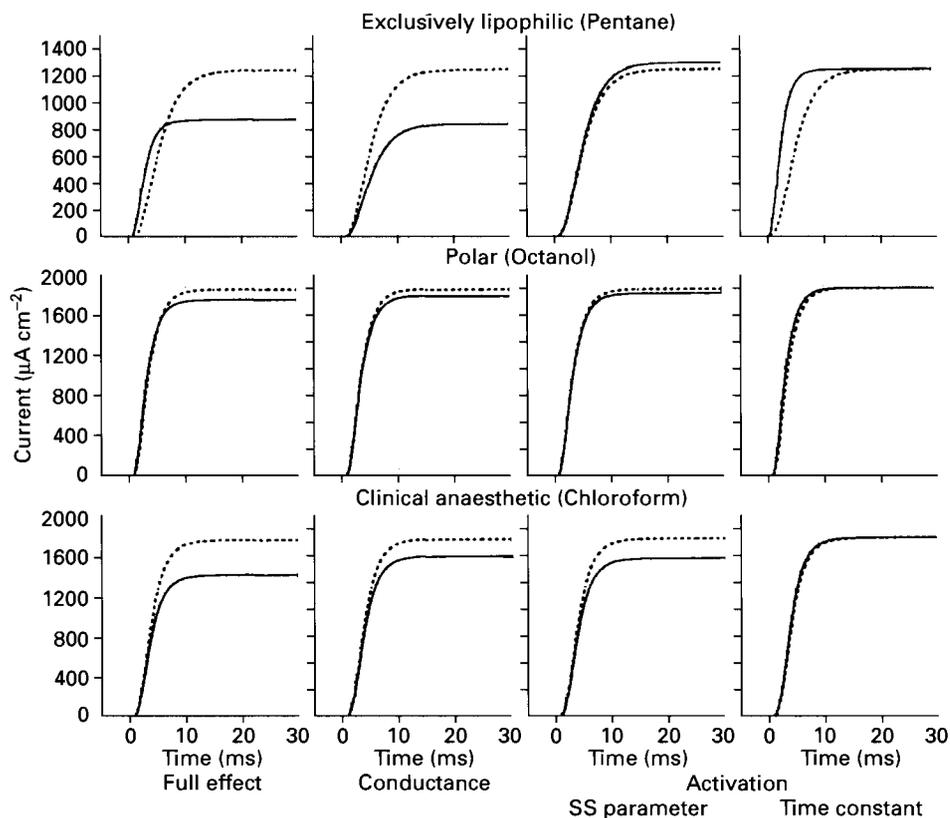


FIG. 6. Suppression of  $K^+$  currents by typical representatives of three groups of anaesthetics (left column of panels) at concentrations comparable to those in figure 4: pentane  $275 \mu\text{mol litre}^{-1}$ , octanol  $330 \mu\text{mol litre}^{-1}$  and chloroform  $5 \text{ mmol litre}^{-1}$  at  $0 \text{ mV}$  membrane potential [33]. Based on the Hodgkin-Huxley formalism, the effects of three groups of anaesthetics on potassium currents are dissected (remaining three columns of panels). A set of three Hodgkin-Huxley variables each were obtained for the control and drug-suppressed currents by fitting the Hodgkin-Huxley equations to the experimental data [33]. The Hodgkin-Huxley variables of the control currents are then replaced, only one in each of the following panels, with the corresponding variable of the drug-suppressed current, the remaining two variables in each panel are those describing the control current. The resulting current (continuous line) is shown.

then, in order for the anaesthetics to have an effect, they would have to affect the dipoles on the extra- and intracellular membranes differently, since otherwise there would be no additional potential decrease across the membrane. If this were the case, the lipid environment of sodium and potassium channels would have to be different. The alternative would involve the dipoles attached to the membrane channel itself. The latter explanation would mean a direct interaction between anaesthetic and ion channel.

Further evidence for direct interaction with the channel protein stems from experiments with carboxylic esters [14] and halogenated ethers [66]. Most of these substances form hydrogen bonds while, in addition, the halogen atoms in substituted substances, such as isoflurane or halothane, may undergo specific interactions with chemical groups that exhibit electron donor properties [43]. The observed differences in the shifts of sodium and potassium current steady state activation could be correlated [33] in terms of electron-accepting anaesthetics shifting more potassium current activation, while anaesthetics that can donate electrons shift more sodium current activation.

As for sodium currents, changes in the activation time constants may be understood as a combination

of increased rate constants (possibly as a result of changes in membrane fluidity) together with a shift along the voltage axis.

In conclusion, the effects of a range of gaseous and volatile anaesthetics on the Hodgkin-Huxley variables suggest that depression of potassium currents, similar to depression of sodium currents, originates from more than one type of interaction. The comparison of the two current systems is consistent with the idea that, as well as interacting non-specifically with the adjacent lipid, certain anaesthetics may have an additional site of action in or on a membrane channel itself.

*More than one site of action.* There is further evidence for specific anaesthetic interactions. Normally, potassium currents in the squid giant axon do not inactivate, except in special circumstances [12]. However, potassium currents at larger membrane depolarizations exhibit a pronounced maximum (fig. 7) in the presence of methoxyflurane, and certain other, but not all, halogenated ethers and *n*-decanol. Although other *n*-alcohols and benzyl alcohol also cause a decrease in potassium currents, the effect is most noticeable with the longer chain alcohols, such as *n*-nonanol and *n*-decanol [33]. Mathematically, inactivation of the potassium current may be described by an inactivation term [33], analogous to the

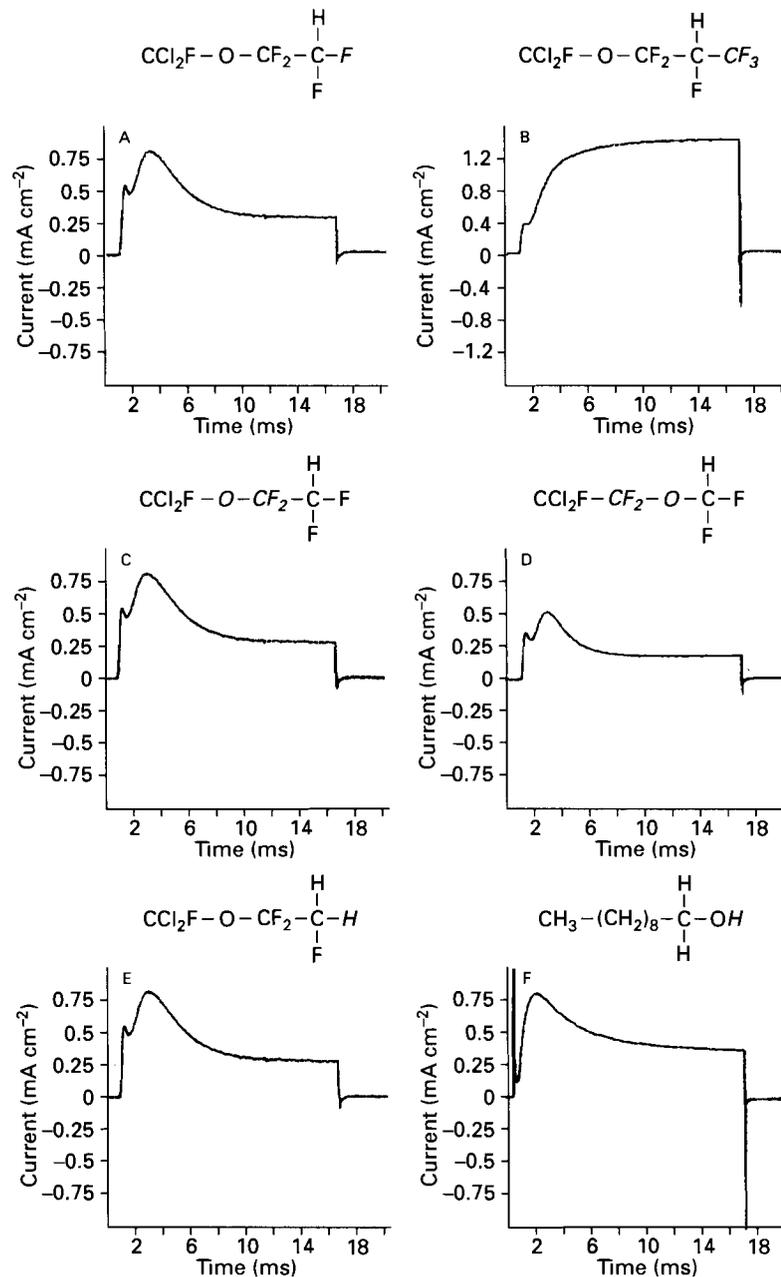


FIG. 7. Structure-function analysis of inactivation-like behaviour of potassium currents induced by different halogenated ethers and decanol [66]. Membrane potentials were depolarized to +75 mV. In the upper two panels, from left to right, only one (*italics*) chemical group has been changed in the halogenated ether molecule. In the bottom panels, the acidic hydrogen in the halogenated ether (left) and the alcohol decanol (right) have been changed (see text for full explanation).

one used for the description of sodium current inactivation (fig. 3). The physical basis for this apparent inactivation is not yet clear. It could be the consequence of a channel block [2, 11, 47], but it is also possible that the compounds that lead to inactivation-like behaviour in potassium currents simply accelerate a process (just as inactivation in sodium currents is generally speeded up, see fig. 4) that is latently present [12] but too slow to be obvious under normal conditions.

The presence of an acidic hydrogen atom in the halogenated ether molecule appears to be necessary but not sufficient to produce potassium current inactivation. The compound in figure 7B differs from that in figure 7A only by an additional  $\text{CF}_3$

group that replaces a terminal fluorine atom. In contrast to lipophilic interactions, polar hydrogen bonding interactions are directed. Therefore, they may be compromised by inappropriate molecular dimensions (e.g. the above bulky  $\text{CF}_3$  group) or competing interactions, such as the formation of hydrogen bonds by other acidic hydrogen atoms within the same substituted ether molecule. This could be the explanation as to why inactivation-like behaviour is not seen with isoflurane (two strongly acidic hydrogens), hardly present with enflurane (two strongly acidic hydrogens), but is pronounced with methoxyflurane (only one acidic hydrogen of comparable strength to the ones in isoflurane or enflurane). These findings may also be related to the

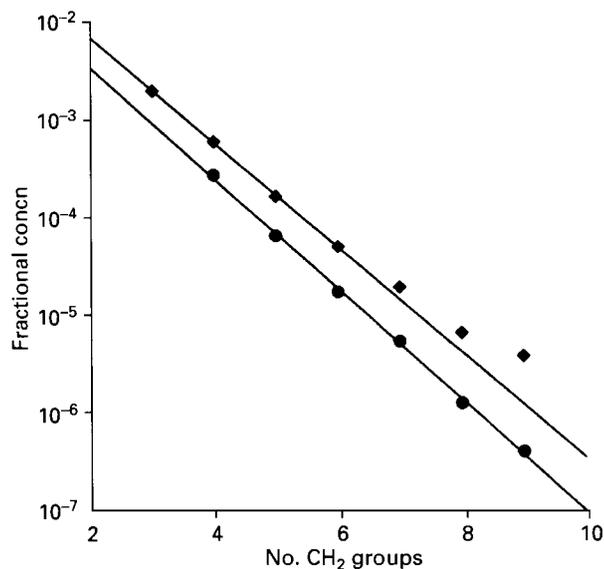


FIG. 8. Chain length dependence of n-alkanol concentrations (expressed as mole fractions) required to half-suppress peak sodium (●) or potassium (◆) current (at large depolarized membrane potentials) [33]. The slopes of the curves yield a standard free energy of adsorption per methylene ( $\text{CH}_2$ ) group to the site of anaesthetic action. The values of  $-3.04 \text{ kJ mol}^{-1}$  (sodium current) and  $-2.91 \text{ kJ mol}^{-1}$  (potassium current, low chain length) are very similar and suggest a lipophilic environment for the adsorption site. Note the deviation from linearity in the potassium current response to longer chain alcohols.

observation that, depending upon the position of the halogen substitution, halogenated ethers may have anaesthetic or convulsive potency [58]. The halogenated ethers that produce pronounced inactivation of potassium currents share certain structural features with the longer chain alcohols. In particular, they contain a lipophilic chain, since their oxygen atoms (fig. 7C and 7D) have lost their basicity and no longer have polar but rather alkane character. Because of this, the position of this oxygen within the chain no longer matters, both derivatives in figures 7C and 7D lead to inactivation. This lipophilic chain is terminated at one end by a polar group (an acidic hydrogen, fig. 7E) with a hydrogen bonding capability of comparable strength of alcohols (the H in the  $-\text{OH}$  can hydrogen bond, fig. 7F). In these, the sizes of the molecules are similar to n-nonanol and n-decanol. The sizes of the molecules relate to the strength of their lipophilic interactions, which appear to be important in explaining their mode of action. It appears that the molecular volume of the hydrophobic portion of the anaesthetic molecule is critical.

While the observation of inactivation-like behaviour strongly suggests at least two different sites of action, more evidence comes from analysis of sodium and potassium current suppression by n-alkanols (fig. 8). When anaesthetic potencies (half-suppression concentrations) are plotted against the number of methylene groups, the slopes of the resulting curve yield, under certain assumptions, the free energy of adsorption per methylene group to the site of anaesthetic action [33]. The values for the free energies (fig. 8) reveal a lipophilic site and are almost the same as for adsorption into phospholipids or an

n-dodecane-aqueous solution interface. For the potassium current, however, the magnitude of this free energy decreases for longer chain alcohols, as if they were progressively excluded from a lipophilic environment, suggesting a second site of action. The fact that no such exclusion occurs for the action of the same alcohols on sodium currents suggests that although the sodium and potassium current systems reside in the same membrane, they are surrounded by different lipids. Alternatively, these sites are on the channel proteins themselves. Whichever possibility proves correct, it appears clear that there must be more than one site of anaesthetic action.

#### CONCLUSION

##### *Diversity of anaesthetic mechanisms and sites of action*

Not all anaesthetics act alike. Their actions depend on their physico-chemical properties. Functionally and structurally different groups may be distinguished for small organic compounds with anaesthetic actions. Any one anaesthetic suppresses membrane ion currents by more than one action, while anaesthetics from different groups have distinct spectra of anaesthetic actions. When the different effects are summed, the suppression of sodium and potassium currents correlates with lipid solubility (fig. 9).

While the case for more than one site of anaesthetic action is strong, their locations have not been unambiguously identified. Based on consistency arguments, the lipid bilayer interior, in addition to the bilayer interface, as well as polar groups at or around the sodium channel protein, appear to be probable candidates. Even hydrophobic domains on the sodium channel protein itself cannot be excluded. Evidence from macroscopic current measurements concerning the underlying molecular mechanisms is indirect, but surface potentials, membrane thickness, surface tension, microviscosity and hydrogen bonding have been invoked; however, molecular rather than macroscopic techniques appear to be required in order to unambiguously identify anaesthetic actions on a molecular scale.

The pattern that emerged when comparing anaesthetic actions on sodium, potassium and gramicidin channels, may suggest that different anaesthetic compounds have similar lipophilic interactions with different ion channels, but that they differ in terms of additional polar interactions, for example, hydrogen bonding. However, lipophilic interactions appear to be important. As has been discussed, sufficient lipophilic volume of the anaesthetic appears to be required before certain anaesthetic actions are observed. However, it may be the polar interaction that gives an anaesthetic molecule its distinct characteristics and may lead to differentiation between different groups of anaesthetics. In this sense, isoflurane and enflurane may have become more specialized general anaesthetics than their parent compound ether, while propofol may be in some ways more closely related to relatively undifferentiated inhalation anaesthetics than to other i.v. anaesthetics.

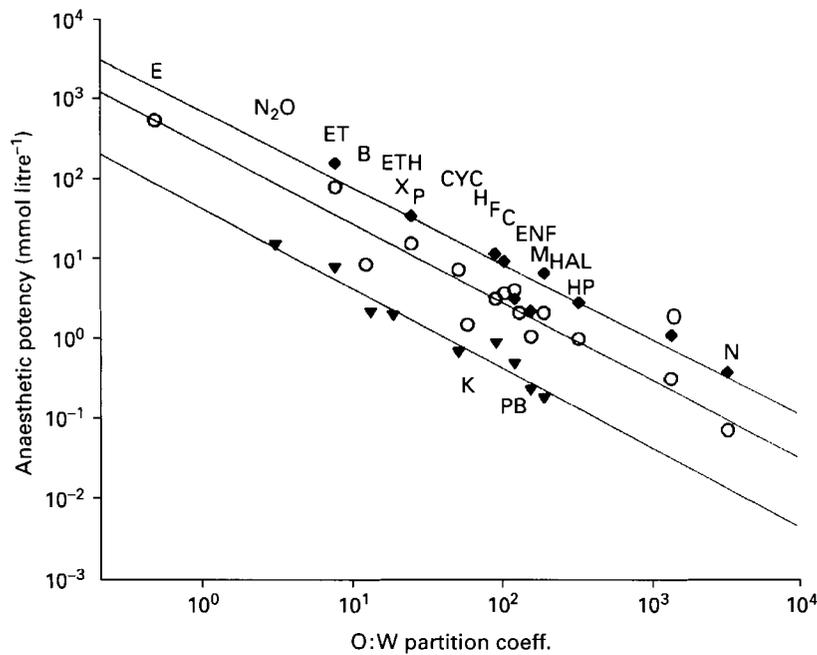


FIG. 9. Meyer-Overton correlation for human MAC equivalent ( $\blacktriangledown$ ) (data as in fig. 2, slope  $-0.991$ ) as well as  $\text{Na}^+$  current ( $\circ$ ) (slope  $-0.975$ ) and  $\text{K}^+$  current ( $\blacklozenge$ ) (slope  $-0.945$ ) half-suppression [22, 33, 60]. Octanol:water (O:W) partition coefficients from Roth and Miller [56]. Note the deviation of enflurane and methoxyflurane data from the correlation for potassium current suppression. E = Ethanol;  $\text{N}_2\text{O}$  = nitrous oxide; ET = ether; B = benzyl alcohol; ETH = ethylene; X = xenon; P = pentanol; CYC = cyclopropane; H = hexanol; F = fluorene; C = chloroform; ENF = enflurane; M = methoxyflurane; HAL = halothane; HP = heptanol; O = octanol; N = nonanol; K = ketamine; PB = pentobarbitone.

Clearly, ion channels may differ in their sensitivities to different groups of anaesthetics. The comparison of anaesthetic actions on voltage-gated sodium and potassium channels discussed above has shown that in peripheral nerve, many of the gaseous and volatile anaesthetics suppress sodium currents more than potassium currents, with the group of halogenated ethers forming at least one exception (fig. 9). Thus it has become clear that in order to fully understand the molecular mechanisms of anaesthesia, the whole range of anaesthetics has to be studied and that caution should be exercised before general conclusions are drawn from examining only a few anaesthetics on a single type of preparation.

#### *Potential correlations between molecular and clinical or physiological effects*

It appears that each individual species of membrane ion channel has its own characteristic set of responses when exposed to a range of anaesthetics. Like fingerprints, these responses should be useful in identifying ion channels and possibly neuronal circuits that play an important role in a clinical or physiological reaction during anaesthesia. There are several examples of potential correspondences. Artusio observed that the clinical excitement phase was seen only during diethyl ether induction but not when patients returned to consciousness [4]; in the squid giant axon, excitability often increased during induction and then returned to normal because different anaesthetic effects appeared to reach their steady state values more rapidly than others [27, 28]. Electroencephalographic studies of seizure activity in dogs during anaesthesia reported seizure-like EEG tracings with ether derivatives but not with

non-ethers [37], correlating with the absence or presence of spontaneous action potentials in the squid giant axon when exposed to the same anaesthetic [66]. Halogenated ethers may be qualitatively different in their actions on sodium as well as on potassium channels; the number and position of the acidic hydrogens these halogenated ethers possess appear to be important in determining their actions and may also be the explanation for why some halogenated ethers act as anaesthetics, but others act as convulsants [58]. The importance of hydrogen bonding in the anaesthetic response is also consistent with a recent finding of stereospecificity in the action of optical isoflurane isomers on molluscan potassium conductance and nicotinic acetylcholine receptor [21]. The spontaneous firing of action potentials observed when exposing peripheral nerve to certain hydrocarbons [28] may be connected with cardiac arrhythmias observed with cyclopropane. Finally, the temporal dispersion increase in heart rate in the presence of local anaesthetics observed by Kasten [40] may be related to the variability increase in steady state activation of sodium channels caused by a barbiturate [23].

In order to elucidate the molecular mechanisms of anaesthetic interactions with membrane proteins, at least three different types of structure-function studies are required in which the following components are systematically varied: (i) the anaesthetic molecule, (ii) the membrane protein, and (iii) the lipid bilayer composition. Studies of the first two types have already been carried out successfully using conventional macroscopic electrophysiological techniques. However, rather than varying membrane protein species, which has been done previously and

represents a large change, it will be of great interest to systematically alter the structure of a given protein using molecular cloning techniques [41] and monitor the resulting functional changes. Electrophysiological studies of this and the third type require a different approach, one of which will be discussed in another article in this volume.

## ACKNOWLEDGEMENTS

Supported by NIH grant NS22602. The author gratefully acknowledges the excellent technical assistance of Ms Sabine Schmitz in the production of the figures and thanks Drs Frenkel and Rehberg for critically reading the manuscript.

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