Actions of General Anesthetics on Acetylcholine Receptor-rich
Membranes from Torpedo californica

Leonard L. Firestone, M.D.,* J.-F. Francois Sauter, Ph.D.,† Leon M. Braswell, B.S.,‡ Keith W. Miller, D.P.H.§

The molecular mechanisms by which general anesthetics act on postsynaptic membranes can only be worked out in a highly purified, homogeneous system. The nicotinic acetylcholine receptor-rich membranes from the electric tissue of Torpedo californica are currently the only postsynaptic membranes that fulfill this condition. Is this peripheral synapse acted on with a pharmacologic specificity similar to that for general anesthesia, and how much less sensitive is it to anesthetic action than the unknown central site? To answer these questions, the authors studied the effects of 13 anesthetic compounds, including volatile general anesthetics, alkanols, and urethane, on the equilibrium binding of 3H-acetylcholine to these nicotinic receptors. As the anesthetic concentration was raised, all the agents first increased acetylcholine binding steeply and then, with few exceptions, decreased it again at higher concentrations. Anesthetics increased acetylcholine binding by decreasing acetylcholine's dissociation constant without changing the Hill coefficient or the number of sites. To a first approximation, the relative ability of these agents to increase 3H-acetylcholine binding parallels that of anesthetics in vivo as predicted by the Meyer-Overton lipid solubility rule. On average, they produced half maximal increases in acetylcholine binding (EC50) at about four times the concentration that causes loss of righting reflex in one-half of a group of animals (ED50). However, a few agents deviated from this relationship. They were the agents with greatest general anesthetic potency in both the volatile anesthetic series (thiometoxylfluorane) and the normal alcohol series (octanol), and required up to 17 times their ED50s to achieve a half effect on acetylcholine binding. Although the concentrations required were high, these effects were reversible. These systematic deviations in the Torpedo model suggest either that: 1) lipid solubility is not a sufficient criterion for activity in Torpedo; 2) lipid solubility in Torpedo membranes deviates from that at the anesthetic site; or 3) more than one effect underlies the binding assay. (Key words: Alcohols. Anesthetics, volatile. Receptors: acetylcholine. Theories of anesthesia.)

CONSIDERABLE PROGRESS has been made in devising theoretical models of general anesthetic action that successfully account both for the relative potencies of structurally diverse agents and for the pressure reversal of their
effects.1-3 These theoretical models suggest that anesthetics act at a hydrophobic site, or sites, which exhibit such weak structural constraints that it (or they) may be successfully modeled by simple bulk solvents. Much effort has been directed to the hypothesis that this hydrophobic site is the lipid bilayer of some excitatory membrane. Such efforts, using readily available lipid bilayer models, have met with some, not entirely unequivocal, success. One unattainable requirement for the more detailed development of any hypothesis remains a physiologic definition of the site of general anesthetic action. Such a requirement is also faced by alternative approaches that suppose that general anesthesia is produced by direct interaction between anesthetics and hydrophobic sites on excitatory proteins. Thus, at present, a direct test of these theories remains impossible.

An alternative approach to the molecular mechanisms by which anesthetics act is to take advantage of their lack of pharmacologic specificity and to study some accessible structure from the peripheral nervous system. This is, for example, the approach adopted by most detailed electrophysiologic studies.4 In return for a defined locus of action at which molecular mechanisms may be approached, one encounters the problem of assessing the relationship of such mechanisms to those that produce general anesthesia centrally. Currently, the only criterion for so doing is the pharmacologic definition of general anesthesia. This problem is particularly pertinent with such nonspecific agents as the general anesthetics. They exhibit a wide spectrum of pharmacologic effects and can interact with both lipid and protein components of membranes. In addition, given agents can often exhibit quite selective effects on particular structures. Thus, it is important in studies of the effects of general anesthetics to assess critically the relationship of the mechanisms involved to those occurring centrally. In this article, a wide range of agents is examined for this reason.

One peripheral structure at which electrophysiologic studies have shown that a wide variety of general anesthetics do exert an effect is the postsynaptic membrane of the neuromuscular junction. (See reference 4 for a review.) For molecular level studies, the nicotinic receptor that is found in the electroplaque of many electric fish has considerable advantages. Membranes from this tissue can be prepared in large quantity with high specific activity of the cholinergic receptor; in relative terms, homogenates from rat brain contain 104-fold less acetylcholine recep-
The Torpedo receptor, purified to homogeneity and functionally reconstituted, has been found to be a 280,000-dalton transmembrane protein sharing considerable structural homology with the mammalian acetylcholine receptor.

The early demonstration of the noncompetitive effects of local anesthetics on this membrane was encouraging, and subsequent reports have shown that several clinical general anesthetics also exert effects (by a different mechanism from the local anesthetics) on the cholinergic receptor obtained from Torpedo electroplaques. The volatile anesthetics and alcohols have been found to shift the population of acetylcholine receptors to a state with high affinity for cholinergic ligands, but which is functionally inactivated or "desensitized." It has been suggested that facilitation of receptor desensitization could underlie the inhibition of synaptic transmission by anesthetics. In this article, we extend these studies to 13 anesthetic compounds. In each case we have obtained detailed concentration–response curves that enable us to compare these actions to anesthetic potency and to lipid solubility.

**Methods**

**Preparation of Receptor-rich Membranes**

The procedure used was a previously described adaptation of the method of Cohen et al. Briefly, electroplaques from freshly killed *Torpedo californica* were homogenized, and receptor-rich membranes were obtained by differential and sucrose density gradient centrifugation. These membranes usually contained 1–2 μmol of acetylcholine binding sites per g of membrane protein. This suspension was kept at 4°C and used within 3 weeks. If more prolonged storage was required, membrane suspensions in 0.3 M sucrose were frozen in liquid nitrogen and stored at −55°C until needed. Storage did not significantly affect results.

**Equilibrium Binding of 3H-Acetylcholine**

Binding was measured at room temperature by centrifugation (volatile agents) or filtration assay. The assay was performed under conditions where binding caused significant depletion (in some cases up to 80%) of the unbound 3H-acetylcholine. This was necessary because both the high dissociation rate of acetylcholine from its receptor and the lability of anesthetics in the membrane raise questions about the conventional procedure of washing and then counting the filter. The bound acetylcholine concentration was calculated as the difference between total and free acetylcholine concentrations. Displaceable binding was estimated after prior incubation with excess α-Bungarotoxin. Full experimental details of these assays have been given previously. Gas chromatography was used to establish the aqueous concentrations of all volatile agents.

**Determination of Anesthetic Potencies**

The potency of each of the anesthetics was determined at 20 ± 1°C using the loss of righting reflex (LRR) of tadpoles bathed in aqueous anesthetic solution as previously described. LRRs were determined at five different concentrations, and all concentrations of volatile agents were checked by gas chromatography. Dose–response curves were analyzed by the method of Waud for quantal responses.

**Materials**

3H-acetylcholine (50–3200 mCi/mmol) was obtained from Amersham-Searle (Arlington Heights, IL). Specific activity was confirmed by isotope dilution experiments as described by Neubig and Cohen. Disopropylfluorophosphate (DFP) was from Sigma Chemicals (St. Louis, MO). Sources of volatile anesthetics were: halothane (clinical grade, Ayerest, NY), chloroform (reagent grade, American Scientific Products, McGraw Park, IL), methoxyflurane (clinical grade, Abbott, North Chicago, IL), isoflurane (Ohio Medical Products, Madison, WI), and diethylether (Mallinckrodt, Paris, KY). Triethoxyflurane and fluroxetine were gifts of Dr. R. C. Terrell, Research and Development, Anaquest, Inc. (Murray, Hill, NJ). Urethane (ethycarbamate) was from Fisher Scientific (Fairlawn, NJ), and alcohols were spectroscopic grade from Aldrich (Milwaukee, WI). Gas chromatography was performed on a Beckman GC-72, using Poropak® P (Waters, Inc., Milford, MA) column packing.

**Results**

**Effects of General Anesthetics on Acetylcholine Binding**

Pilot experiments confirmed previous findings that 3H-acetylcholine binds to Torpedo membranes in a saturable manner in the concentration range of 1–100 nM. Controls in which the membranes were preincubated with α-Bungarotoxin showed that only a small percentage of the total 3H-acetylcholine binding was not displaceable. Corrections for this binding were made in all experiments and did not change significantly in the presence of any of the anesthetics studied.

In order to survey conveniently a range of anesthetics, assays were set up in such a way that approximately 50% of the receptors were occupied by acetylcholine in the absence of anesthetic. To provide a sensitive index, the conditions were also arranged so that the ratio of the
bound and 20 nM free (bound/free = 1.0), a 50% increase in specifically bound $^3$H-acetylcholine (from 20 to 30 nM) would reduce the free $^3$H-acetylcholine (from 20 to 10 nM) and change the ratio of bound/free (from 1.0 to 3.0). (A small correction for nondisplaceable binding has been omitted to simplify this example.) To facilitate presentation of the data, small day-to-day deviations of the bound/free ratio from one were removed by the normalization of the control ratio to one.

The effect of halothane on $^3$H-acetylcholine binding is shown in figure 1. At the lowest effective concentrations, it increased the binding of $^3$H-acetylcholine. The effect first became significant at about 0.4 mM and reached a peak at 2.0–2.5 mM. At higher concentrations, binding decreased precipitously, returning to the control value at 7–8 mM and to zero at 14 mM (the saturated solubility ($C^\text{sw}$) of halothane in Torpedo Ringer was 17 mM). Preincubation-dilution experiments showed that these effects of halothane were reversible up to 6–8 mM, but that higher concentrations caused some irreversible loss of sites.

Six other volatile anesthetics were examined in the same way (fig. 2), and all were found to increase $^3$H-acetylcholine binding. Only thiomethoxyflurane was more potent than halothane, while fluoro*xene and, particularly, diethylether were much less potent. Chloroform, isoflurane (not shown), diethylether, methoxyflurane, and thiomethoxyflurane all acted biphasically, decreasing binding at higher concentrations in much the same way as halothane. Fluoro*xene’s solubility was too limited to establish a downturn unequivocally.

Five alkanols were also studied (fig. 3). These agents all increased $^3$H-acetylcholine binding in a manner analogous to the volatile anesthetics. The potencies of the normal alkanols increased with chain length. The range of potencies was greater than that encountered with the volatile anesthetics, as ethanol was even less potent than

![Figure 1](http://anesthesiology.pubs.asahq.org/pdfaccess.ashx?url=/data/journals/jasa/931762/)

![Figure 2](http://anesthesiology.pubs.asahq.org/pdfaccess.ashx?url=/data/journals/jasa/931762/)
Diethyl ether. Solubility limitations prevented unequivocal
demonstration of biphasic effects in the case of octanol (C\text{sat} = 4 mM) and of hexanol (C\text{sat} = 60 mM), but all
other 1-alkanols exhibited biphasic effects.

In order to provide a more quantitative description of these
effects, the bound to free concentration ratio was
normalized between the control value and the maximum
value. The data on this scale were then fitted by a non-
linear least squares routine to the logistic function of
Berkson, which approximates the Hill equation. This
analysis was adopted in order to provide a convenient
measure of the slope and half effect concentration and is
not meant to imply any particular model for the anesthetic
action. The results of this analysis are given in table 1.

All of the volatile anesthetics, alkanols, and urethane
produced slopes of greater than one. The average slope
and its standard deviation for seven volatile anesthetics
was 2.50 ± 0.61 and for the five alkanols was 2.87 ± 0.55.
The decrease in binding at higher anesthetic concentra-
tions following the initial increase in binding was not
analyzed in detail because these concentrations can cause
irreversible effects (see previous discussion).

**ORIGIN OF GENERAL ANESTHETIC EFFECTS ON
CHOLINERGIC LIGAND BINDING**

In order to define the origin of these effects, represen-
tative anesthetics were studied at fixed concentration in
the presence of varying amounts of \(^3\)H-acetylcholine. The
\(\alpha\)-Bungarotoxin displacable \(^3\)H-acetylcholine binding is
plotted as a function of the free \(^3\)H-acetylcholine binding in
fig. 4. The effect of 1.5 mM halothane was to displace
the binding curve to the left. The two curves were run
separately, but controls showed there was no change in
the number of sites. Analysis of these data by the method
of Hill indicates that binding did not have a Hill coefficient
of one. The control experiments in table 2 show a mean
Hill coefficient of 1.5 ± 0.20 (SD) and half occupancy
\((K_{1/2})\) of 15 ± 4.1 nM. \(K_{1/2}\) varied somewhat between
membrane preparations and batches of \(^3\)H-acetylcholine,
so that the relative changes caused by anesthetics should
be inferred from the control on the same preparation,
which is always the nearest control value above it in table
2. Our control values are similar to those previously re-
ported.7

**Table 1. Analysis of the Effects of General Anesthetics on \(^3\)H-acetylcholine Binding**

<table>
<thead>
<tr>
<th>Agent</th>
<th>Slope</th>
<th>Half Effect Concentration (E_{50} mM (±SD))</th>
<th>General Anesthesia E_{D_{50}} (mM (±SE))</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diethyl ether</td>
<td>2.1 ± 0.64</td>
<td>57 ± 13</td>
<td>25 ± 2.5</td>
</tr>
<tr>
<td>Fluroxene*</td>
<td>2.9 ± 0.28</td>
<td>5.6 ± 0.24</td>
<td>2.3 ± 0.31</td>
</tr>
<tr>
<td>Chloroform</td>
<td>1.7 ± 0.29</td>
<td>1.3 ± 0.15</td>
<td>0.9 ± 0.1†§</td>
</tr>
<tr>
<td>Isoflurane†</td>
<td>2.0 ± 0.55</td>
<td>1.0 ± 0.13</td>
<td>0.29 ± 0.004</td>
</tr>
<tr>
<td>Methoxyflurane‡</td>
<td>2.7 ± 0.19</td>
<td>1.14 ± 0.035</td>
<td>0.21 ± 0.016**</td>
</tr>
<tr>
<td>Halothane§</td>
<td>2.7 ± 0.71</td>
<td>0.90 ± 0.093</td>
<td>0.23 ± 0.016**</td>
</tr>
<tr>
<td>Thiopentothylurane¶</td>
<td>3.5 ± 0.65</td>
<td>0.64 ± 0.05</td>
<td>0.063 ± 0.0084</td>
</tr>
<tr>
<td>Ethanol</td>
<td>3.6 ± 0.61</td>
<td>320 ± 21</td>
<td>190 ± 10</td>
</tr>
<tr>
<td>Butanol</td>
<td>3.5 ± 0.41</td>
<td>44 ± 1.7</td>
<td>12 ± 1††</td>
</tr>
<tr>
<td>Hexanol</td>
<td>2.2 ± 0.31</td>
<td>5.5 ± 0.42</td>
<td>0.7 ± 0.1††</td>
</tr>
<tr>
<td>Octanol</td>
<td>3.2 ± 0.54</td>
<td>1.03 ± 0.067</td>
<td>0.060 ± 0.009††</td>
</tr>
<tr>
<td>Benzyl alcohol</td>
<td>2.4 ± 0.64</td>
<td>13 ± 1.3</td>
<td>2.0 ± 0.21**</td>
</tr>
<tr>
<td>Urethane</td>
<td>2.5 ± 0.44</td>
<td>96 ± 13</td>
<td>13.6 ± 0.69‡‡</td>
</tr>
</tbody>
</table>

Data from figures 1–4 were analyzed as described in the text.

* CF₃·CH₂·O·CH₂CH₂
† CF₃·CHCl·O·CH₂F₂
‡ CF₃·CH₂·CF₂·O·CH₃
§ CF₃·CHClBr

\(\uparrow\) Data are for tadpoles from Kita et al.50
\(\uparrow\) Data are for tadpoles from Pringle et al.51
\(\uparrow\) Data are for tadpoles from Dodson et al.52
\(\uparrow\) Data are for newts from Miller et al.53
\(\uparrow\) Data are for newts from Miller et al.53

Downloaded From: http://anesthesiology.pubs.asahq.org/pdfaccess.ashx?url=/data/journals/jasa/931762/ on 11/27/2018
Halothane was studied at two concentrations. It decreased the $K_{1/2}$ for $^3$H-acetylcholine binding two-fold at 1.5 mM. At 8 mM halothane, where binding is decreased once more to near control levels (fig. 1) but without any irreversible loss of sites, the $K_{1/2}$ is barely decreased, but the Hill coefficient is reduced significantly. These results are independent of the ligand used to monitor receptor affinity because quantitatively similar halothane effects have been obtained using $^3$H-d-tubocurarine.9 Thiomethoxyflurane was studied at two concentrations: one near its EC$_{50}$ and the other near the maximum effect. Thiomethoxyflurane caused the $K_{1/2}$ to decrease while the Hill coefficient remained unchanged. Both alcohols studied decreased the $K_{1/2}$ without changing the Hill coefficient. Ethanol (700 mM) and octanol (1.1 mM) produced 1.8- and 2.9-fold decreases in $K_{1/2}$, respectively.

All the previously mentioned binding curves were determined by centrifugation in capped tubes because of the volatile nature of the anesthetics. However, filtration assays are more convenient because the number of samples is not limited. Filter assays with halothane proved impossible because of its high vapor pressure, but filtration of octanol solutions resulted in volatilization of only about 10%.16 The data for octanol in table 2 show that the results are independent of the method employed in this case. Similarly, the control curves obtained on the same membrane preparation on the same day for ethanol by centrifugation and for urethane by filtration were identical within error (table 2).

**Discussion**

All 13 general anesthetics studied here altered ligand binding. At their lowest effective concentrations, they caused a decrease in the dissociation constant of the cholinergic ligand without any change in the number of binding sites or the slopes of the binding curves. At higher concentrations, most of the alcohols and the volatile agents

---

**TABLE 2. Effect of General Anesthetics on Acetylcholine Binding**

<table>
<thead>
<tr>
<th>Anesthetic</th>
<th>Concentration</th>
<th>$K_{1/2}$ ± SD (nM)</th>
<th>$n_H$ ± SD</th>
<th>Assay</th>
</tr>
</thead>
<tbody>
<tr>
<td>Halothane</td>
<td>0</td>
<td>19 ± 4.8</td>
<td>1.28 ± 0.084</td>
<td>C</td>
</tr>
<tr>
<td></td>
<td>1.5</td>
<td>9 ± 2.5</td>
<td>1.25 ± 0.12</td>
<td>C</td>
</tr>
<tr>
<td></td>
<td>0</td>
<td>5.4 ± 0.65</td>
<td>1.48 ± 0.076</td>
<td>C</td>
</tr>
<tr>
<td></td>
<td>8</td>
<td>4.1 ± 0.61</td>
<td>1.19 ± 0.071</td>
<td>C</td>
</tr>
<tr>
<td>Thiomethoxyflurane</td>
<td>0</td>
<td>16 ± 1.8</td>
<td>1.39 ± 0.099</td>
<td>C</td>
</tr>
<tr>
<td></td>
<td>0.6</td>
<td>12 ± 2.4</td>
<td>1.45 ± 0.087</td>
<td>C</td>
</tr>
<tr>
<td></td>
<td>1.0</td>
<td>10 ± 1.1</td>
<td>1.49 ± 0.049</td>
<td>C</td>
</tr>
<tr>
<td>Ethanol</td>
<td>0</td>
<td>16 ± 2.8</td>
<td>1.38 ± 0.065</td>
<td>C</td>
</tr>
<tr>
<td></td>
<td>700</td>
<td>9 ± 1.1</td>
<td>1.36 ± 0.058</td>
<td>C</td>
</tr>
<tr>
<td>Octanol</td>
<td>0</td>
<td>8 ± 2.4</td>
<td>1.8 ± 0.17</td>
<td>C*</td>
</tr>
<tr>
<td></td>
<td>1.1</td>
<td>2.8 ± 0.44</td>
<td>1.5 ± 0.14</td>
<td>C</td>
</tr>
<tr>
<td></td>
<td>0</td>
<td>6.4 ± 0.82</td>
<td>1.49 ± 0.069</td>
<td>F</td>
</tr>
<tr>
<td></td>
<td>1.0</td>
<td>3.0 ± 0.48</td>
<td>1.8 ± 0.14</td>
<td>F</td>
</tr>
<tr>
<td>Urethane</td>
<td>0</td>
<td>14 ± 2.5</td>
<td>1.49 ± 0.071</td>
<td>F</td>
</tr>
<tr>
<td></td>
<td>120</td>
<td>6.5 ± 0.69</td>
<td>1.46 ± 0.056</td>
<td>F</td>
</tr>
</tbody>
</table>

Binding curves were performed with $^3$H-acetylcholine as described in the text either by centrifugation (C) or filtration (F) assay. Each curve should be compared with the nearest control curve above it (see text).

* Data previously published in reference 16.
decreased $^3$H-acetylcholine binding. We did not study this effect in detail because slightly higher concentrations often caused irreversible effects; however, at 8 mM halothane, where binding had fallen to near control values, the binding curve was similar to that in the absence of halothane (Table 2).

Our findings are similar to those from laboratories employing kinetic methods$^{11,14,15}$ or other probes.$^{24}$ All of these studies demonstrated that alcohols induce an increase in receptor affinity, and some$^{14,24}$ reported the decrease seen at higher concentrations. In spite of the differences in probes and temperatures employed, agreement on half-effect concentrations is fair. For example, half-effect values for butanol ranged from 30 to 105 mM,$^{11,14,24}$ straddling our value of 44 mM. In the only study which examined volatile anesthetics,$^{11}$ EC$_{50}$ values are three-fold higher than presented here. Such high values could result from failure of the buffer to reach equilibrium with the anesthetic, which was applied in the vapor state, or from subsequent volatilization. Our own data were obtained in closed tubes, and the buffer was analyzed by gas chromatography; however, we cannot rule out that the difference lies in the use of a kinetic$^{11}$ rather than an equilibrium assay.

The actions of these general anesthetics could result a priori either from their partitioning into lipid or from direct interactions with some hydrophobic regions on the acetylcholine receptor protein, or from interactions at the lipid–protein interface. The simplest hypothesis to test is that their cholinergic actions are directly proportional to their lipid solubility, although one must always bear in mind that the range of compounds under test may be inadequate to rule out the generation of false correlations.$^{25}$ Unfortunately, no systematic study of partition coefficients in the Torpedo membrane has been undertaken at this time. The best one can do is to use the data that are available. Partition coefficient data in lipid bilayers are available for nine of the 15 agents. The hypothesis predicts that a logarithmic plot of EC$_{50}$ (Table 1) versus partition coefficient should produce a straight line of slope minus one. Figure 5A tests this hypothesis. A fair correlation is obtained for EC$_{50}$s varying over two and one-half orders of magnitude, which predicts that the EC$_{50}$ of any anesthetic occurs when its concentration in the lipid is 150 mM. Because partition coefficients in lecithin are usually several times greater than those in biomembranes,$^{26}$ this concentration should be regarded as an upper limit.

Close inspection of the figure shows small, systematic deviations from the solid line, which has the theoretically expected slope of −1.0. The unconstrained fit (dashed line) turns out to have a slope of $-0.90 \pm 0.054$ (SD); $r = -0.985$. This is not statistically significantly different from the expected value of $-1.0$ ($P > 0.1$). However, the partition coefficients for ethanol, urethane, and butanol, which strongly influence the slope, must be considered in
light of the experimental uncertainties in determining small partition coefficients. For example, we have used partition coefficients for butanol and ethanol from a single careful study.27 This partition coefficient for ethanol (in dimerystoillecithin) is 0.44, but a recently reported value in synaptic membranes is 1.0.28 It is probable that one of these values is in error because in all other alcohols examined to date, partitioning into lipid bilayers has proven stronger than partitioning into biomembranes.26

One way of attempting to resolve this issue is to compare the ED50 of these agents to their general anesthetic potency. This strategy has two rationales. First, anesthetic potency can be measured readily and the errors involved are independent of the anesthetic. Second, the potency of some two dozen general anesthetics correlates very well with their membrane/buffer partition coefficients.26 Thus, to a first approximation a correlation with anesthetic potency would imply a correlation with membrane solubility.

We have not solved the technical problems of determining equilibrium anesthetic concentrations in Torpedo. Further, their availability is inadequate for statistically satisfactory results. For this comparison we have, therefore, used a set of data for tadpoles.29 These small animals can be readily assayed under equilibrium conditions at the same temperature as our in vitro work was performed, and because species variation in anesthetic potency is generally small, the tadpoles provide a useful approximation. The data are plotted in figure 5B and provide a test using all 13 general anesthetics. Once again, the fit to the solid line, which has the expected slope of 1, is good, but there are small systematic deviations from the hypothesis. The slope of the unconstrained fit, shown as a dashed line in figure 5B, is 0.83 ± 0.066 (SD); r = 0.981. This slope differs significantly from one (P = 0.03), and closer inspection of figure 5B reveals that a group of the most potent anesthetics at bottom left whose EC50 vary only two-fold (from 1.2 mM for chloroform to 0.64 mM for thiopenthal) have anesthetic potencies that vary 14-fold (from 0.9 mM for chloroform to 0.063 mM for thiopenthal). This anomaly can be seen more clearly when the abscissa is replotted with the EC50 expressed as multiples of the ED50 for LRR in tadpoles (fig. 6). In general, the least potent anesthetics have EC50 that are two to four times higher than their ED50s. However, the volatile anesthetics with ED50 less than 0.2 mM and the alcohols with ED50 less than 0.5 mM require five or more times the ED50 to achieve an EC50, and this deviation appears to increase with increasing anesthetic potency. The carbamate family was not examined, but urethane is clearly also deviant. This general conclusion also holds for the alcohols when membrane concentration is considered, as can be seen from the systematic deviations from the solid line (slope = 1) in figure 5A.

That the electromotor synapse should be less sensitive to anesthetic action than a synapse on an unknown neuron in the central nervous system is not surprising.29 The problem could, however, be of experimental rather than theoretical origin and might arise from the biphasic actions of some of the anesthetics (figs. 1–3). It seems probable that the observed primary increase in acetylcholine binding does not reach the maximum achievable before the secondary decrease begins to occur. This makes it difficult to resolve these two actions and to assign relative potencies with a high degree of accuracy. The problem is well illustrated by the normal alcohols (fig. 3). Neither octanol nor hexanol produces a significant decrease in acetylcholine binding at high concentrations, and they cause much greater increases in binding than do butanol and ethanol, which both produce biphasic effects. If we assume that butanol and ethanol would have achieved a maximum effect equal to that of octanol in the absence of the secondary decrease in binding, reanalysis yields EC50s of 61.8 and 617 mM, respectively. These represent increases of 1.9- and 1.4-fold, respectively, which does not alter the fact that it takes supra-anesthetic concentrations for these agents to enhance acetylcholine binding. Without making arbitrary assumptions, other methods of deconvoluting biphasic effects on binding could not be applied because of the absence of a well-defined plateau between the component effects.

Our unexpected finding, that the most potent anes-
thetic compounds (thiometoxyflurane and octanol) required much larger anesthetic multiples to produce their half effect on binding than do the least potent (diethyl ether and ethanol) (fig. 6), may be at least partially explained by more detailed kinetic studies that would resolve the biphasic effects. Consistent with this expectation is a previous kinetic study\(^\text{11}\) using α-Bungarotoxin binding to reflect receptor conformation which showed that octanol required less than twice as high a multiple of anesthetic concentration to cause enhancement of high affinity binding as did ethanol. In contrast, in the present study, that same ratio (i.e., (octanol [EC\(_{50}/\text{ED}_{50}]/\text{ethanol} [\text{EC}_{50}/\text{ED}_{50}]) was greater than ten. It remained greater than five even when the EC\(_{50}\) for ethanol was recalculated as before, assuming the same maximum effect as octanol. Such differences may be reflecting the complex, dynamic processes underlying receptor conformational changes and their modulation by drugs. In the face of these uncertainties, it seems premature to consider in detail other explanations for the anomalous effects on acetylcholine binding by the potent compounds.

**Conclusion**

In summary, our data show that to a first approximation, there is a fair correlation between anesthetic potency and the ability to increase high affinity binding of acetylcholine to its receptor. The latter action, however, required higher concentrations than those required for general anesthesia at comparable temperatures in another species. Thus, although this action on cholinergic receptors, which is also pressure reversible,\(^\text{16}\) may be caused by a similar type of mechanism to that causing anesthesia, it would not itself be sufficiently developed at anesthetic concentrations to cause anesthesia if central nicotinic receptors have a similar sensitivity to those from Torpedo electroplaques.

However, a closer look at the data (fig. 6) reveals trends not entirely consistent with this mechanistic conclusion. Further experiments will be needed to see if this remains true when: 1) the primary action of these anesthetics on acetylcholine receptors is studied independently of their secondary inhibitory action; and 2) the effects of anesthetics on the lipids of Torpedo membranes themselves are determined.

The authors thank Dr. R. C. Terrell of Anaquest for kindly providing thiomethoxyflurane and fluroxene, and Elizabeth Ambalavanar for technical help.

**References**


32. Dodson BA, Furmaniak ZW Jr, Miller KW: The physiologic effects of hydrostatic pressure are not equivalent to those of helium pressure on Rana pipiens. J Physiol (Lond) 362:233–244, 1985