Anesthetic Cutoff in Cycloalkanemethanols

A Test of Current Theories

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Background: N-alkanols containing up to 12 carbons are anesthetic; however, those with more than 12 carbons are not. This phenomenon has been termed cutoff. Lipid disordering theories of anesthetics suggest that cutoff occurs because the alkyl chains of long-chain alcohols approach the length and shape of the lipids of neuronal membranes and, therefore, intercalate into membranes without perturbing them. Protein theories suggest that cutoff occurs because the size of long-chain alcohols exceeds that of a protein binding site having finite dimensions. These theories were tested with a new series of alcohols, the cycloalkanemethanols: \(6\) c(C\(_\text{n}H\text{2n+1}\))·CH\(_2\)·OH.

Methods: Anesthetic potency was measured in *Rana pipiens* tadpoles using the reversible loss of righting reflexes as the endpoint. The change in order parameter induced by cycloalkanemethanols and n-alkanols in lipid bilayers made of egg phosphatidylcholine and cholesterol was measured with electron paramagnetic resonance spectroscopy.

Results: On ascending the series from cyclopropanemethanol (EC\(_50 = 54 \pm 3.2\) nM) to cycloundecanemethanol (EC\(_50 = 7.0 \pm 0.2\) μM) anesthetic potencies first increased exponentially but then decreased sharply at cyclododecanemethanol (EC\(_50 = 13 \pm 0.2\) μM). Cyclotetradecanemethanol was found not to cause anesthesia in tadpoles, even after 48 h of exposure, although saturated solutions shifted the dose-response curve of octanol from 66 ± 2.6 to 47 ± 2.8 μM. A linear loss in the ability to disorder lipid bilayers was observed on ascending both alcohol series such that cyclohexadecanemethanol and n-tridecanol actually increased bilayer order.

Conclusions: Molecular length does not correlate with anesthetic cutoff in these two alcohol series. Cutoff is predicted by the ability of both series of alcohols to disorder lipid bilayers and correlates with their molecular volume. (Key words: Anesthetics, alcohols: cycloalkanemethanol. Potency: cutoff; lipid order parameter.)

GENERAL anesthesia is induced by a wide variety of compounds possessing great structural diversity. Volatile liquids, gases, alcohols, steroids, and barbiturates all induce anesthesia. In spite of this great diversity, the potencies of these anesthetics can be correlated with their hydrophobicity over a great range (Meyer-Overton rule). This has led to the concept that anesthetics partition into neuronal membranes and cause anesthesia by perturbing the lipid bilayer. Anesthesia could be induced by changes in membrane physical properties such as lipid fluidity, lateral phase separation, ion permeability, or thickness. Alternatively, anesthesia could result from the direct interaction between an anesthetic and a hydrophobic region on a membrane protein. Such an interaction would necessarily be nonspecific to allow for the binding of structurally dissimilar anesthetic compounds. Because anesthetics are known to interact with both lipid and protein components of membranes, identifying the specific site responsible for anesthesia has proven difficult. This has led to the introduction of new pharmacologic tools to distinguish between competing theories.

Cycloalkanemethanols are cyclic analogues of primary n-alkanols, the anesthetic properties of which have not previously been examined. They are potentially highly valuable tools for studying molecular mechanisms of anesthetic action, because their effects on both living organisms and model systems can be compared with the effects of n-alkanols and differences between the two series attributed to cyclization of the alkyl chain.

The phenomenon of cutoff in anesthetic potency refers to the absence of anesthetic activity in the higher members of a homologous series of molecules, despite their being extremely lipid soluble, and is an exception

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to the Meyer-Overton rule. The study of such exceptions allows us to test theories of anesthesia and to gain insight into the molecular nature of the site of general anesthesia. One of the best studied examples of cutoff occurs in n-alkanols, in which anesthetic potency increases with the addition of each methylene group between ethanol and dodecanol, but even saturated solutions of tridecanol and higher homologues do not produce anesthesia.\textsuperscript{11}

Based on older partition coefficient measurements, it was suggested that cutoff occurs because long-chain alkanols could not achieve a sufficient concentration in membranes (~25–50 mM) to cause anesthesia.\textsuperscript{12} However, more recent studies have shown that nonanesthetic n-alkanols do, in fact, dissolve into membranes and model lipid bilayers and reach concentrations comparable to their anesthetic counterparts.\textsuperscript{13,14} Therefore, lipid theories can be consistent with cutoff only if long-chain n-alkanols do not perturb the lipids that comprise neuronal membranes. Because the alkyl chains of the nonanesthetic n-alkanols are similar in length and composition to the alkyl chains of the phospholipids in neuronal membranes, these compounds may, indeed, intercalate into membranes without causing the perturbation necessary for anesthesia.

Protein theories of anesthesia suggest that cutoff occurs in a series of homologous compounds because the addition of successive methylene groups increases anesthetic potency of higher homologues to a lesser degree than it decreases aqueous solubility. In the case of a hydrophobic protein pocket of fixed dimensions, this can occur if added methylene groups sit outside of that pocket and, consequently, do not contribute to the binding energy or if they result in steric hindrance.

Using cycloalkanemethanols, we have chosen to test whether cutoff in primary alcohols is related to molecular length or volume and whether changes in lipid order parameter could predict cutoff. The polar headgroup of members of both the n-alkanol and cycloalkanemethanol series are identical, but each cycloalkanemethanol is approximately one-half the length of its n-alkanol analogue (fig. 1). Therefore, if cutoff were determined by length, it should occur in the cycloalkanemethanol series at approximately twice the number of carbons as the n-alkanol series. Alternatively, if cutoff were related to volume, it would occur at a similar number of carbons in both series.

Anesthetic potencies of cycloalkanemethanols were determined in tadpoles, which are the standard model for comparing the potencies of a wide range of general anesthetics, including volatile, liquid, and solid agents. The reversible loss of righting reflexes was used as the criterion for anesthesia. The ability of higher members of this anesthetic series to disorder lipid bilayers was examined and compared with that of the n-alkanols. This property was chosen because, in previous studies, lipid disordering has been shown to correlate remarkably well with anesthetic potency in structurally diverse compounds,\textsuperscript{2,15,16} and to predict pressure reversal of anesthesia,\textsuperscript{1} anesthetic tolerance,\textsuperscript{17} and cutoff in long-chain n-alkanols.\textsuperscript{14}

\textbf{Materials and Methods}

Prelimb \textit{Rana pipiens} tadpoles (1–1.5 cm in length) were obtained from Carolina Biological Supply Company, Burlington, NC. Cyclopropemethanol through cycloheptanemethanol, cyclooctanemethanol, and cycloundecanemethanol were purchased from Aldrich Chemical Co. (Milwaukee, WI). Cyclooctanemethanol was purchased from Columbia Organic Chemicals (Cassatt, SC). With the exception of cycloundecanemethanol, these alcohols were 95–99% pure and, therefore, were used without further purification. Cycloundecanemethanol was purified on a silica column to 99% purity as determined by gas chromatography before use (the available commercial purity is only

\begin{figure}[h]
\centering
\includegraphics[width=0.5\textwidth]{figure1.png}
\caption{Typical structures of a cycloalkanemethanol and n-alkanol, each with nine carbons.}
\end{figure}
80%). Cyclononanemethanol, cyclodecanemethanol, and cyclooctadecanemethanol were synthesized according to the method of Vineck et al.\textsuperscript{18} The structures of compounds synthesized in our laboratory were confirmed by nuclear magnetic resonance spectroscopy and their purities determined to be greater than 99% by gas chromatography. Cycloctadecanemethanol could not be synthesized because the required precursor, cyclooctadecanol, was unavailable. Egg phosphatidylcholine (EPC) was from Avanti Polar Lipids (Alabaster, AL). Cholesterol, from Calbiochem (La Jolla, CA), was recrystallized from methanol before use. The spin label 12-doxyl stearate was purchased from Molecular Probes (Eugene, OR).

**Determination of Anesthetic Potencies**

With approval from the Subcommittee on Animal Studies of the Massachusetts General Hospital, the anesthetic potencies of cycloalkanemethanols were determined in tadpoles. Groups of five tadpoles were placed in covered 100-ml beakers containing various concentrations of the desired cycloalkanemethanol in water at 20.0 ± 1.0° C. Anesthesia was defined as the reversible loss of righting reflexes after tipping with a flame polished pipette as previously described.\textsuperscript{11} For the higher members of the series (cyclononanemethanol through cyclooctadecanemethanol), alcohols were added as ethanolic solutions. The final concentration of ethanol was always less than 10 mm. This concentration does not cause anesthesia (the EC\textsubscript{50} for ethanol in tadpoles is 190 mm) nor shift the concentration–response curve of coadministered anesthetics.\textsuperscript{11} At least ten different concentrations using a total of 75–125 tadpoles per cycloalkanemethanol were used for the determination of anesthetic potency. Partitioning of the most hydrophobic cycloalkanemethanols into tadpoles can potentially reduce their concentration in the aqueous phase. Therefore, 1 l of water for each group of five tadpoles was used for the determination of anesthetic potency for cyclooctadecanemethanol and cyclooctadecanemethanol. An EC\textsubscript{50} for each anesthetic was calculated as described by Waud for quantal responses.\textsuperscript{19}

Saturated solutions of cyclooctadecanemethanol were prepared by adding an excess of the compound as an ethanolic solution to distilled water, stirring for 48 h at 20.0 ± 1.0° C, and filtering through glass wool to remove undissolved cyclooctadecanemethanol. The saturated solubility of cyclooctadecanemethanol in water was determined by gas chromatography. The small quantity of cyclooctadecanemethanol in aqueous solution necessitated extracting 1 l of solution into 4 ml of hexane, evaporating the hexane under a stream of nitrogen, and resuspending the cyclooctadecanemethanol in 100 μl of hexane. Ten microliters of solution were injected onto a Hewlett Packard 5890 gas chromatograph equipped with a J and W (Folsom, CA) DB-WAX 122-7033 column. Standard curves, prepared by injecting 10-μl aliquots from standard solutions of cyclooctadecanemethanol in hexane onto the column, were linear over the concentration range studied. The reported saturated solubility of cyclooctadecanemethanol is the average of 11 injections onto the column from 4 separate experiments.

The anesthetic efficacy of cyclooctadecanemethanol was tested in 55 tadpoles. To minimize depletion of this cycloalkanemethanol from the water, only one tadpole was used in each 250 ml of a saturated solution of cyclooctadecanemethanol. Air was bubbled through the water during the course of the experiment. The loss of righting reflexes was tested every 2 h for the first 12 h and then at 8–12-h intervals for the next 36 h. The concentration of cyclooctadecanemethanol in solution was determined by gas chromatography at the end of the experiment to monitor for depletion.

For additivity experiments, 50 tadpoles that had been exposed to saturated solutions of cyclooctadecanemethanol for 48 h were transferred from the saturated cyclooctadecanemethanol solution directly to 100-ml beakers containing various concentrations of octanol in water. The EC\textsubscript{50} of n-octanol was determined in the manner described above. An EC\textsubscript{50} was simultaneously determined in a control group of 50 tadpoles that had not been exposed to cyclooctadecanemethanol. To increase the reliability of our results, octanol solutions for both groups were prepared from the same stock solutions.

**Preparation of Lipid/Anesthetic Samples**

The abilities of higher members of the cycloalkanemethanol and n-alkanol series to disorder lipid bilayers were determined by measuring changes in lipid order parameter. Each sample was prepared by combining a methanolic solution of the spin-labelled probe 12-doxyl stearate, 6 mg EPC/cholesterol (2:1 molar ratios), and the appropriate alcohol in chloroform. We chose the fatty acid 12-doxyl stearate probe because, in a previous study using postsynaptic membranes, it accurately predicted cutoff in alkanols; whereas, in a separate study, a 5-doxyl fatty acid probe did not accurately predict cutoff in model membranes of EPC.
and cholesterol. The mixture was then gently shaken, dried to a thin film under a stream of nitrogen, and placed under vacuum for 2 h to remove residual solvent. This was deemed sufficient to remove residual solvent, because additional drying was not found to alter the fluidity of EPR spectra. Vesicles were made by adding 100 μl of buffer (0.01 M MOPS, pH 7.4 in 0.1 M NaCl), vortexing for 2 min, then bath sonicating in ice water for 15 min. The final concentration of alcohol was 33 mole percent in the bilayer where:

\[
\text{mole percent} = 100 \times \frac{\text{alcohol}}{\text{alcohol} + \text{EPC} + \text{cholesterol}}.
\]

The final lipid-to-spin-label ratio was greater than 100:1 to minimize spin–spin interactions. The vesicles were transferred into capillary tubes, flame sealed on ice, and sedimented (6,000 × g for 10 min) before obtaining electron paramagnetic resonance (EPR) spectra.

**EPR Spectroscopy and Order Parameter Calculations**

Spectra were obtained on a Bruker ER200 spectrometer interfaced with an IBM system 9000 computer. A thermostated insert within the EPR cavity maintained the sample temperature at 20.0 ± 0.1°C. Order parameters were calculated by the method of Gaffney et al. with correction for solvent polarity:

\[
\text{Order parameter} = \left( \frac{A_\parallel - A_\perp}{A_\perp} \right) \cdot \left( \frac{a_0}{a_0} \right),
\]

where

\[
A_\parallel = A_\perp + (1.4) \cdot \left( 1 - \frac{A_\parallel - A_\perp}{A_\perp} \right),
\]

and 2A_\parallel and 2A_\perp are the outer and inner hyperfine splittings in gauss, respectively, as defined in figure 4,

\[
a_0' = \frac{A_\perp + A_\parallel}{3}
\]

and \(a_0 = A_\parallel + 2A_\perp\). Splittings determined by eye and by using a gaussian fit of the appropriate spectral peaks were not systematically different. Values for \(A_\perp\), \(A_\parallel\), and \(A_\perp\) for doxyl stearate are 6.3, 5.8, and 33.6 gauss, respectively. For each anesthetic, the reported change in order parameter is the average of two separate samples.

**Results**

**Anesthetic Potencies of Cycloalkanemethanols**

Members of the homologous series of cycloalkanemethanols from cyclopropanemethanol to cyclodecane methanol are anesthetic (table 1, fig. 2). The lower members of the series (cyclopropanemethanol through cyclooctanemethanol) produced loss of righting reflexes after approximately 15 min. Tadpoles required successively longer exposures to higher members of the series (1–2 h for cyclodecane methanol) before a stable anesthetic response was observed. Return of righting reflexes was likewise longer after exposure to the higher members (30–60 min) than the shorter ones (15–20 min). Log (concentration) versus response curves for anesthetic cycloalkanemethanols were sigmoid in shape. The fitted slopes were steep, as has previously been reported for other anesthetic agents (table 1), and varied from 4 to 18.1, 22 This variation in slopes among anesthetics probably reflects the sensitivity of the fit to the spacing of points on the concentration axis as well as to the number of animals used for each point. Cycloundecanemethanol, the most potent anesthetic, was nearly 8,000 times more potent than cyclopropanemethanol. Anesthetic potency increased in an exponential manner between cyclopropanemethanol and cycloundecanemethanol, with each methylene group increasing anesthetic potency, on average, by a factor of 3 (fig. 3). Significantly, cycloundecanemethanol was less potent than cycloundecanemethanol.

![Fig. 2. Log_{10} (concentration)–response curves for cycloalkanemethanols with an even number of carbons from cyclopropanemethanol to cycloundecanemethanol. Each symbol represents the response of five tadpoles to a given cycloalkanemethanol concentration. In some cases, symbols overlap. Curves are drawn using the values listed in table 1.](image-url)
Table 1. EC₉₀ and Slopes for Members of the Cycloalkane Methanol Series Calculated from Concentration–Response Curves for Loss of Righting Reflex in *Rana pipiens* Tadpoles

<table>
<thead>
<tr>
<th>Anesthetic Agent</th>
<th>No. of Carbons</th>
<th>EC₉₀</th>
<th>Slope</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cyclopropanemethanol</td>
<td>4</td>
<td>54 ± 3.2 mM</td>
<td>5 ± 1.5</td>
</tr>
<tr>
<td>Cyclobutanemethanol</td>
<td>5</td>
<td>8.4 ± 0.64 mM</td>
<td>4 ± 1.3</td>
</tr>
<tr>
<td>Cyclopentanemethanol</td>
<td>6</td>
<td>2.7 ± 0.19 mM</td>
<td>5 ± 1.6</td>
</tr>
<tr>
<td>Cyclohexanemethanol</td>
<td>7</td>
<td>910 ± 17 μM</td>
<td>16 ± 4.5</td>
</tr>
<tr>
<td>Cycloheptanemethanol</td>
<td>8</td>
<td>320 ± 14 μM</td>
<td>6.3 ± 1.8</td>
</tr>
<tr>
<td>Cyclooctanemethanol</td>
<td>9</td>
<td>120 ± 2 μM</td>
<td>16 ± 4.0</td>
</tr>
<tr>
<td>Cyclononanemethanol*</td>
<td>10</td>
<td>41 ± 1.3 μM</td>
<td>10 ± 3.2</td>
</tr>
<tr>
<td>Cyclodecanemethanol*</td>
<td>11</td>
<td>15 ± 0.4 μM</td>
<td>10 ± 3.4</td>
</tr>
<tr>
<td>Cycloundecanemethanol*</td>
<td>12</td>
<td>7.0 ± 0.12 μM</td>
<td>16 ± 4.8</td>
</tr>
<tr>
<td>Cyclododecanemethanol*</td>
<td>13</td>
<td>13 ± 0.2 μM</td>
<td>18 ± 5.8</td>
</tr>
<tr>
<td>Cyclotetradecanemethanol*</td>
<td>15</td>
<td>Not anesthetic†</td>
<td>Not anesthetic†</td>
</tr>
</tbody>
</table>

Data are mean ± SE.

* These cycloalkanemethanols were added from ethanolic stock solutions. The final concentration of ethanol was always <10 mM.
† Partial anesthetic.

The saturated solubility of cyclotetradecanemethanol was determined to be 4.5 ± 0.61 μM (±SD). Depletion of cyclotetradecanemethanol from saturated solutions after 48 h of contact with tadpoles (250 ml/tadpole) was approximately 10%, as determined by gas chromatography. The compound was stable over the course of the experiment, as judged by gas chromatography.

Saturated solutions of cyclotetradecanemethanol did not cause anesthesia in any of the tadpoles after 48 h. The EC₉₀ of n-octanol in tadpoles that were exposed to saturated solutions of cyclotetradecanemethanol for 48 h was 47 ± 2.7 μM (slope = 7 ± 2.8), which is significantly lower than the value of 66 ± 2.6 μM (slope = 12 ± 5.3) obtained in the control group (P < 0.005, Student’s t test). Righting reflexes returned in both groups after approximately 30 min in distilled water. Irreversible toxicity occurred in tadpoles exposed to saturated solutions of cyclotetradecanemethanol containing a large excess of undissolved cyclotetradecanemethanol that had not been removed by filtration through glass wool. It also occurred in some tadpoles (5 out of 55) exposed to saturated solutions at 48 h.

**Lipid Disordering Efficacies of Cycloalkanemethanols and n-alkanols**

The lipid disordering efficacy of each cycloalkanemethanol and n-alkanol, defined as the change in lipid order parameter induced by a compound at a defined membrane concentration, was determined in lipid bilayers of EPC and cholesterol (2:1 molar ratio). Higher members of each series were examined, because they could be added directly to the lipid without appreciable partitioning into the aqueous phase during the addition of buffer. Therefore, lipid perturbation could be examined at a defined alcohol concentration in the bilayer (33 mole percent). This membrane con-

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**Fig. 3.** The log₁₀ of the EC₉₀ of the cycloalkanemethanols and n-alkanols as a function of the number of carbons. The values for the EC₉₀ of the n-alkanols were taken from Allfinoff et al. In all cases, the symbols are larger than the reported errors. Anesthetic potency increases logarithmically between cyclopropanemethanol and cycloundecanemethanol with a slope of −0.47 ± 0.017 (r = 0.996), which is nearly identical to that of the n-alkanols (−0.49 ± 0.024).
CUTOFF AND LIPID DISORDERING IN CYCLOALKANEMETHANOLS

Fig. 4. The EPR spectra obtained with a 12-doxyl stearate spin label in EPC/cholesterol (2:1) bilayers without cycloalkanemethanol (top), with cyclooctadecanemethanol (middle), and with cyclooctanemethanol (bottom). The cycloalkanemethanol concentration in the bilayer is 55 mole percent. Cyclooctadecanemethanol ordered, while cyclooctanemethanol disordered, the lipid bilayer. Dashed lines demonstrate the spectral splittings for the control (without cycloalkanemethanol) spectrum used to calculate the order parameter. Solid lines demonstrate the splittings used to calculate A and A in the samples with the indicated cycloalkanemethanol. Spectroscopy was performed at 20.0 ± 0.1°C on a Bruker ER200 spectrometer operating at 9.4 GHz, microwave power = 10 mW, magnetic field strength = 3550 gauss, modulation amplitude = 2 gauss, and sweep width = 120 gauss.

centrations, approximately 10 times that needed to produce anesthesia, was used because the effects of anesthetics on membrane order at clinical concentrations are quite small. The anesthetic cycloalkanemethanols from cyclooctanemethanol through cycloundecanemethanol were found to disorder lipid bilayers of EPC and cholesterol, as reflected in a decrease in $2A_1$ and an increase in $2A_2$ (fig. 4). The disordering efficacy decreased with the addition of each methylene group to the ring. Cyclooctadecanemethanol produced no significant change in order parameter, while cyclooctadecanemethanol actually increased it. A linear least-squares fit of the disordering efficacy versus the number of carbons yielded a x-intercept of 13.0 carbons and a slope of 0.015/methylene group ($r = 0.996$) (fig. 5).

At a given number of carbons, the n-alkanols disorder the bilayer consistently less than the cycloalkanemethanols. Nonetheless, their disordering efficacy also decreased linearly with the addition of each methylene group. The anesthetics nonanol through undecanol decreased, while the nonanethetics tridecanol, tetradecanol, and pentadecanol increased the order parameter of EPC/cholesterol bilayers. No significant change in order parameter was produced by dodecanol. A linear regression of this data produced a x-intercept of 11.3 carbons and a slope of 0.015/methylene group ($r = 0.990$).

**Discussion**

The Meyer-Overton rule relates the potency of a general anesthetic to its hydrophobicity and, hence, can be expressed as a double logarithmic plot of oil/water partition coefficient versus $EC_{50}$. It is remarkable for its good correlation over a 100,000-fold range of potencies, which implies that the concentration of an anesthetic in the lipid needed to produce general anes-

![Change in Order Parameter x100 vs Number of Carbons](http://example.com/fig5.png)

Fig. 5. Change in lipid order parameter as measured with 12-doxyln stearate. The lipid bilayer concentration of all alcohols was 55 mole percent. Each value represents the average of two separate samples and the error bars indicate the range. Nonanesthetic alcohols are shown as open symbols. Lines are least-squares fits. The x-intercept is 11.3 ± 0.14 and 13.0 ± 0.11 carbons for the n-alkanols and cycloalkanemethanols, respectively. On ascending either alcohol series, order parameter increases by 0.015/methylene group. The control order parameter was 0.435 ± 0.0023 (±SD, n = 4).

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thesis is approximately constant for a wide range of compounds. For any anesthetic, this concentration is the product of the lipid/water partition coefficient and the EC₅₀. For the cycloalkanemethanols cyclopropanemethanol through cyclooctanemethanol, only oleyl alcohol/water partition coefficients at 4°C are available. Using these data, together with representative data from the literature for a diverse range of agents, we demonstrate that these cycloalkanemethanols obey the Meyer-Overton rule because they fit to a straight line with a slope that is not different from the theoretical slope of −1 (fig. 6). The calculated values for the concentration of cycloalkanemethanol in oleyl alcohol at the EC₅₀ ranges from 7 to 21 mM (mean = 13 ± 5.4 mM) over a 450-fold range of potencies and is fairly close to the mean value of 29 ± 19.4 mM for the other anesthetics given in figure 6. Anesthetic potency for the shorter members of the cycloalkanemethanol series increases in parallel with those in the n-alkane series (fig. 3), indicating that there is little discrimination between methylene groups added to a cyclic ring and those added to a normal chain.

Cutoff in anesthetic potency is an exception to the Meyer-Overton rule, because the higher members of both series are expected to be very potent anesthetics but, in fact, do not produce anesthesia. In the cycloalkanemethanol series, it occurs between cyclododecanemethanol and cyclotetradecanemethanol. The approach to cutoff is gradual in that the increase in anesthetic potency per methylene group is essentially constant up to cyclodecanemethanol, but decreases as the series is ascended further from cyclodecanemethanol to cyclododecanemethanol. Cyclododecanemethanol is actually less potent than cycloundecanemethanol and is an order of magnitude less potent than would be predicted by extrapolation of the potencies of lower members of this series.

Preincubation of tadpoles for 48 h in saturated solutions of cyclotetradecanemethanol decreased the EC₅₀ of n-octanol from 66 to 47 μM. This suggests that cyclotetradecanemethanol may be a “partial anesthetic” with an extrapolated EC₅₀ of approximately 16 μM (three and a half times its saturated solubility). This is two orders of magnitude higher than that predicted by extrapolation of the potencies of lower members of the series. Although it appears that cyclotetradecanemethanol is a partial anesthetic, we cannot rule out the possibility that the shift in the octanol concentration–response curve results from toxic effects of cyclotetradecanemethanol or its metabolites. We judge that cyclotetradecanemethanol is a partial anesthetic because: 1) tadpoles surviving the additivity experiments appeared to recover normally (in 30 min) after transferring them from anesthetizing concentrations of octanol to water, and 2) there was no significant difference in the slopes of the octanol dose–response curves determined for control and cyclotetradecanemethanol-exposed tadpoles.

The gradual approach to cutoff in cycloalkanemethanols is broadly similar to that observed for n-alkanols. Nonanesthetic n-alkanols are taken up in tadpoles and reach concentrations similar to their anesthetic counterparts. When large tadpoles (2–3 cm in length) were used, we observed significant depletion of cyclotetradecanemethanol over 48 h, indicating that this compound is taken up in tadpoles. However, we cannot say whether metabolism might significantly reduce the concentration of cyclotetradecanemethanol in tadpoles.

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such that equilibrium with the aqueous solution is never reached.

Order parameter has a value between 0 (isotropic or no order) and 1 (completely ordered). Anesthetics disorder membranes as reflected in a decrease in order parameter. At clinical concentrations, this decrease is small. Therefore, studies that examine the effects of anesthetics on membrane order generally employ high anesthetic concentrations. Nevertheless, the ability of a compound to decrease lipid order correlates remarkably well with its ability to induce anesthesia. For example, anesthetic n-alkanols disorder the postsynaptic membranes from Torpedo electroplaques while nonanesthetic n-alkanols do not, anesthetic steroids decrease the order parameter of liposomes composed of EPC and cholesterol significantly more than their nonanesthetic counterparts. In fact, lipid bilayers can discriminate between anesthetic and nonanesthetic steroids even when the anesthetic and nonanesthetic steroids are enantiomeric pairs; the anesthetic 3α-hydroxy-5α-pregnane-11,20-dione is anesthetic and strongly disorders model lipid bilayers, but 3β-hydroxy-5α-pregnane-11,20-dione is not anesthetic nor does it disorder bilayers. Similarly, NMR studies reveal that the rotational mobility of anesthetic steroids is much greater than nonanesthetic analogues.

Lipid disordering correctly predicts both that there should be a cutoff in anesthetic potency in each alcohol series we examined and that it will occur at a greater number of carbons for cycloalkanemethanols than for n-alkanols. The actual values for anesthetic cutoff in both series are remarkably close to those predicted by order parameter changes, considering the simplicity of the model. The inability of the last full anesthetic in each series (dodecanol and cyclododecanemethanol) to disorder membranes does not rule out a membrane site, because the lipid bilayer used in this study may not exactly model the behavior of the neuronal membrane in which anesthetics exert their effects. For example, in a previous study that examined the membrane-disordering effects of n-alkanols in postsynaptic membranes from Torpedo, anesthetic cutoff was accurately predicted as occurring between dodecanol and tetradecanol.

Does the inability of higher members of these series to disorder bilayers correlate with molecular length or shape? Dodecanol and cyclododecanemethanol have molecular lengths of 15 and 8 Å, respectively. However, they have similar molecular volumes of 224 and 204 cm³/mole, respectively, suggesting that molecular volume may be more important than length in determining when a homologous series of alcohols switches from disordering to ordering bilayers.

It is puzzling that lipid bilayers predict the pharmacology of general anesthetics so well, because the measured changes in bulk membrane properties at clinically relevant concentrations of anesthetics seem too small to directly cause anesthesia. Trudell has suggested that the observed change in membrane fluidity is just an indication of membrane perturbation. The important action of anesthetics may be to disrupt the lateral phase separation at the lipid–protein interface that is thought to be crucial for neuronal protein function. Alternatively, anesthetics may alter neuronal protein function by simply displacing lipid from the lipid–protein interface. In either case, significant alterations at the lipid–protein interface could occur with small changes in such bulk membrane properties as order parameter.

Theories of anesthesia that invoke direct anesthetic–protein interactions may also account for cutoff in cycloalkanemethanols. The luciferases are currently the best protein models for such interactions. In the firefly luciferase, anesthetics compete with a cofactor, luciferin, for a binding site on the luciferase protein. This results in a suppression of luminescence. The anesthetic binding site is a hydrophobic pocket that exhibits little structural specificity. For firefly luciferase, the potency of n-alkanols increases with chain length up to tridecanol. Beyond this point, the potency achieves a plateau; additional methylene groups decrease aqueous solubility without enhancing potency. Cutoff occurs when an anesthetic's aqueous solubility falls below that needed to produce suppression of luminescence. This occurs between hexadecanol and heptadecanol in the n-alkanol series.

In the cycloalkanemethanols, the approach to anesthetic cutoff in tadpoles follows a somewhat different pattern from that observed for suppression of firefly luciferase activity in that the last full anesthetic in the series is actually less potent than the previous member. Within the framework of a protein model, this would imply that steric hindrance plays a role in decreasing the anesthetic potency of cycloalkanemethanol below that predicted by simple extrapolation of lower homologues; once the ring fits tightly in the protein pocket, an additional methylene group encounters steric constraints that results in a decrease in binding affinity between the anesthetic and the protein. Cycloalkanemethanol's large molecular volume would
almost completely prevent its binding to a hydrophobic pocket of fixed dimensions. The molecular volumes of the first nonanesthetic in each homologous series, tridecanol and cyclotetradecanemethanol, are approximately 240 and 234 cm³/mole, respectively. Therefore, a hydrophobic pocket with a volume of approximately 235 cm³/mole would completely exclude the nonanesthetic tridecanol (which is not even a partial anesthetic) and nearly exclude cyclotetradecanemethanol (which may be a partial anesthetic). This correlates remarkably well with predictions by Franks and Leib that the size of a protein binding site for general anesthesia would be somewhat less than the 250 cm³/mole volume of the luciferin binding site on firefly luciferase.  

In summary, we have introduced a new series of general anesthetics that, when combined with the n-alkanols, enables one to more rigorously test theories of general anesthesia and, in particular, the importance of such steric parameters as length and volume. Anesthetic potency in this series of anesthetics increases exponentially, on average, with the addition of successive methylene groups. This increase occurs in parallel with previously reported values for n-alkanols. Anesthetic cutoff occurs between cyclododecanemethanol and cyclotetradecanemethanol. Cutoff is not related to molecular length, but does correlate with molecular volume. We cannot rule out the possibility that other molecular parameters that correlate with volume (i.e., molecular surface area or hydrophobicity) cause anesthetic cutoff. Cutoff is generally predicted by EPR measurements of order parameter of lipid bilayers composed of EPC/cholesterol. Although this is consistent with membrane lipid being the molecular site of anesthesia, it does not exclude the possibility that the site is a hydrophobic pocket on a protein. Future studies examining the effect of cycloalkanemethanols on better-defined targets may help to identify that site.

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