Br. J. Pharmacol. (1989), **96**, 9–16

Anaesthetic potencies of primary alkanols: implications for the molecular dimensions of the anaesthetic site

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1 We have redetermined the anaesthetic potencies (EC_{50}s) for a series of primary alkanols, to resolve uncertainties about the molecular dimensions of the anaesthetic site resulting from the use of data from different laboratories.

2 For each alkanol, concentration-response relationships for loss of righting reflex (LRR) were plotted for over one hundred tadpoles, and the median effective concentrations determined. Aqueous concentrations present during potency assays were determined independently, and for alkanols with chain length greater than nonanol, correction was made for depletion from the aqueous phase.

3 The EC_{50}s were found to decrease logarithmically with increasing number of carbon atoms in the hydrocarbon chain of the alkanol (C_N), such that, on average, each additional methylene group was associated with an approximately four fold increase in potency.

4 The relationship between log EC_{50} and C_N was best described by the quadratic equation, $\log EC_{50} = 0.022\ (\pm 0.0038)\ C_N^2 + 0.76\ (\pm 0.051)\ C_N + 3.7\ (\pm 0.14)$ ($r^2 = 0.9951$).

5 A previously described correlation between the apparent changes in the free energy of binding of an additional methylene group both to luciferase and to the sites for LRR in tadpoles was not confirmed.

6 A cut-off in potency beyond dodecanol was established in experiments where tadpoles were maintained in supersaturated solutions of tridecanol for 20 h without demonstrable LRR.

7 These findings indicate that the soluble enzyme firefly luciferase does not adequately model the anaesthetic site. Specifically, there are discrepancies in the position of cut-off, and the apparent changes in the free energy of binding, per methylene group, of an alkanol to luciferase do not parallel that for tadpoles.

Introduction

It is still not known whether general anaesthetics produce their effects by acting on central nervous system neurotransmitter receptor proteins directly, or indirectly through the lipids which surround these receptors. One approach to resolve these alternatives is to apply pharmacological criteria derived from observations of anaesthesia in animals to well-characterized model systems. For example, a particularly useful criterion is the 'cut-off' in anaesthetic potency, that is, the aburpt loss of potency among the higher molecular weight members of homologous families of anaesthetics. The primary alkanols are one such series that can be used to probe structure-activity relationships in these model systems. In animals, cut-off was thought to occur beyond tridecanol at tetradecanol (Meyer & Hemmi, 1935; Pringle *et al.*, 1981), but it remains to be established whether cut-off occurs due to depletion of the aqueous alkanol concentration or loss of intrinsic anaesthetic activity (Lee, 1976).

Proponents of the protein-based theories have used data obtained with the lipid-free enzyme, firefly luciferase, to argue that anaesthetics act directly at central nervous system receptors. Specifically, luciferase not only manifests a cut-off in inhibitory potency for the primary alkanols and alkanes, but also demonstrates changes in the free energy for binding of alkanols which parallel those found in

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animals (Franks & Lieb, 1985). However, the validity of this latter point has recently been questioned (Elliott & Haydon, 1986) because the potencies of the primary alkanols *in vivo* are not known with a high degree of precision. Problems with these values in the literature are that: (i) data have never been derived in a single laboratory for the complete homologous series of n-alkanols (Vernon, 1913; Meyer & Hemmi, 1935; Pringle *et al.*, 1981); (ii) the potencies were roughly estimated by a 'bracketing' method rather than quantal concentration-response analysis, so that no standard errors could be derived; and (iii) measurements of the actual free concentration of alkanol in solution are lacking. This is especially important for the higher molecular weight compounds where adsorption might lead to significant depletion of the free concentration. Although such values in the literature may be useful for a first approximation, they are inadequate to define the fine structure of their molecular targets. Therefore, we have systematically determined the loss of righting reflex (LRR) potencies in tadpoles for the complete homologous series of primary aliphatic alkanols. With these values, we show that *in vivo* potency data from tadpoles do not parallel the inhibition of firefly luciferase by the primary alkanols, and we firmly establish the position of the cut-off in anaesthetic potency for the series of primary alkanols.

Methods

Early pre-limb bud tadpoles, *Rana pipiens*, approximately 1.0–1.5 cm in length (Carolina Biological Supply Co., Burlington, NC), were used for determination of anaesthetic concentration-response curves as previously described (Alifimoff *et al.*, 1987). Briefly, groups of five tadpoles were placed in covered 100 ml beakers in neutral, oxygenated, aqueous solutions of alkanols prepared in twice distilled water. No tadpole was used more than once. Anaesthesia was defined as the loss of righting reflex (LRR). After allowing 15 to 240 min for equilibration with the alkanol, the tadpoles were tipped manually with a flame polished glass pipette. Unresponsiveness for greater than 5 s was scored as LRR, and this test was repeated at 15 min intervals. After each experiment, all tadpoles were placed in containers of neutral distilled water until recovery of righting reflex was confirmed.

[^{14}C]-tetradecanol was assayed for its ability to induce LRR in three ways. First, two groups of five tadpoles each were incubated for 20 h in separate beakers containing 100 ml of oxygenated, saturated solutions, and then assayed for LRR. Samples of these solutions were obtained for liquid scintillation counting at 30 min intervals for the first 4 h, and then again at 20 h. Second, ten tadpoles were incubated in a beaker containing 500 ml of an oxygenated, supersaturated solution for 72 h; both LRR and aqueous concentration were assayed at 24 h intervals. Thirdly, to determine whether tetradecanol shifted the concentration-response curve of another anaesthetic, tadpoles were preincubated in an oxygenated, supersaturated solution of tetradecanol for 48 h (50 ml of solution per animal), then assayed for their responses to [^{14}C]-octanol. Tridecanol was also assayed for its ability to induce LRR. Two groups of ten animals were incubated in 1000 ml of an oxygenated, saturated solution of tridecanol and in 1000 ml of a supersaturated solution prepared from an ethanolic stock solution. Tadpoles were assayed for LRR at hourly intervals for 6 h and then again at 20 h. Samples were obtained for determination of free aqueous concentrations by gas chromatography at the start of the experiment and at 20 h.

Concentrations of alkanols present during assays were verified either by gas chromatography or liquid scintillation spectroscopy. Concentrations of the short chain alkanols (C_1–C_9; C_N = number of carbon atoms in the hydrocarbon chain of the alkanol) were measured with a Beckman GC 72B Gas Chromatograph (Porapak P column packing (Waters Co., Milford, MA) with column temperatures ranging from 130 to 215°C), while concentrations of undecanol and tridecanol were measured on a Varian 3700 Gas Chromatograph (Dexsil-300 column packing (Supleco Inc., Bellfonte, PA) with column temperatures from 140 to 200°C). Actual concentrations of the short chain alkanols were within 5% of their nominal values during the time course of assays. For the long-chain alkanols (C_{10}–C_{14}), the relationship between nominal and measured concentrations during concentration-response assays was more complex, and is described in the Results section.

For all agents, concentration-response assays were performed using a minimum of 10 animals at each of 5 different concentrations. Entire studies were repeated at least once, and the data pooled. The respective effective concentrations (EC_{50}) and slopes were obtained using a logistic method for quantal responses (Waud, 1972).

With the more potent agents, it was convenient to make aqueous solutions of alkanols from ethanolic stock solutions. To determine whether this made any difference in potency, the EC_{50} of [^{14}C]-octanol was determined when alkanol solutions were prepared (i) by dilution from ethanolic stock solutions, (ii) directly in water or (iii) from a [^{14}C]-alkanol directly in water. EC_{50}s obtained for octanol by each method were compared. The estimated variance in the respective EC_{50} values was calculated from the

Table 1 Time-course of [^{14}C]-tetradecanol depletion during a concentration-response assay

Time (h)	[^{14}C]-*tetradecanol* (μmol l^{-1} water)	% *LRR*
0	48.5*	0
24	43.1	0
48	22.2	0
72	12.7	0

See Methods for details. LRR = loss of righting reflex.
* Saturated solubility = 1.5 μM (Bell, 1973).

standard errors; the sum of these then yielded the estimated variance of the difference in the EC_{50} between the octanol prepared from an ethanolic stock solution, and radiolabelled or unlabelled octanol prepared directly in water. The ratio of these differences to their respective standard errors was then referred to a standard normal distribution. Statistical significance was assumed at the level of $P < 0.05$.

Drugs and solutions

All alkanols were the highest quality available from their respective manufacturers. Aqueous solutions of methanol (Mallinckrodt, Paris, KY; purity 99.8%), ethanol (Pharmco, Dayton, NJ; >99%), propanol (Mallinckrodt, St. Louis, MO; 99.8%), butanol (Baker, Phillipsburg, NJ; 99.7%), pentanol (Aldrich, Milwaukee, WI; 99%), and hexanol (Sigma, St. Louis, MO; 99%) were prepared in gas-tight vials by stirring weighed aliquots of each compound into twice distilled water for 1 h at room temperature. Solutions of heptanol (Eastman Kodak, Rochester, NY; 99%) and octanol (NuChek Prep, Elysian, MN; >99%) were prepared by stirring for longer (>12 h) in gas-tight vials at 40°C. Solutions of undecanol and tridecanol (NuChek Prep; >99%) were prepared from ethanolic stock solutions. Gas chromatography revealed no impurities in saturated stock solutions of these compounds.

[^{14}C]-octanol (4.5 Ci mol^{-1}), [^{14}C]-decanol (7.5 Ci mol^{-1}), [^{14}C]-dodecanol (56 Ci mol^{-1}) (ICN Biomedicals, Irvine, CA) and [^{14}C]-tetradecanol (58 Ci mol^{-1}) (NEN, Boston, MA) were used to determine free aqueous concentrations of the longer chain alkanols during concentration-response assays. Radiochemical purities were >98.5% as determined by thin layer chromatography, high performance liquid chromatography and/or autoradiography by the manufacturer. Aqueous solutions of each radiolabelled alkanol were prepared by diluting concentrated stock solutions made in ethanol; final ethanol concentrations were always <25 mM. Samples were counted in Liquiscint (National Diagnostics, Manville, NJ), and efficiency was determined using

Table 2 Loss of righting reflex potency of primary alkanols in *Rana pipiens* tadpoles at 20 ± 1°C

Alcohol	EC_{50} ± s.e.	*Slope* ± s.e.	n/*T*
Methanol	590 ± 41 mM	2.7 ± 0.44	3/170
Ethanol	190 ± 16 mM	2.3 ± 0.31	4/270
Propanol	73 ± 2.4 mM	7 ± 1.1	3/170
Butanol	10.8 ± 0.77 mM	2.2 ± 0.34	3/230
Pentanol	2.9 ± 0.11 mM	5.0 ± 1.2	3/150
Hexanol	570 ± 37 μM	3.9 ± 0.72	2/120
Heptanol	230 ± 11 μM	6 ± 1.1	2/140
Octanol	57 ± 2.5 μM	5 ± 1.0	3/150
Octanol[a]	55 ± 3.1 μM	3.6 ± 0.50	4/220
Octanol[b]	59 ± 3.1 μM	5 ± 1.1	3/170
Nonanol	37 ± 2.4 μM	2.3 ± 0.40	4/250
Decanol[a,b]	12.6 ± 0.48 μM	5.4 ± 0.81	3/170
Undecanol[a]	8.1 ± 0.81 μM	2.5 ± 0.49	2/110
Dodecanol[a,b]	4.7 ± 0.33 μM	3.1 ± 0.47	3/160
Tridecanol[a]	Not anaesthetic		
Tetradecanol[a]	Not anaesthetic		
Range:	126,383 ×		

EC_{50} was determined after 15–30 min for methanol through to nonanol; after 60 min for decanol and undecanol, and after 120 min for dodecanol. See Methods for curve-fitting procedure and estimation of standard errors. All concentrations were verified by gas chromatography unless otherwise noted.
n = number of experiments, T = total number of animals in *n* experiments.
[a] Aqueous alkanol solutions were prepared from concentrated ethanolic stock solutions; the final concentration of ethanol was <25 mM. See Methods for details.
[b] A [^{14}C]-alkanol was used to measure the aqueous concentrations.

quenched samples of [^{14}C]-toluene as an external standard.

Results

The short-chain alkanols (C_1–C_9) produced a reversible LRR in tadpoles which plateaued in 10 min and remained unchanged when assayed at 15 and 30 min. For decanol and undecanol, a steady LRR response was not observed until 60 min; for dodecanol, 120 min was required. Accordingly, the EC_{50} values presented for decanol and undecanol were obtained at 60 min, while the EC_{50} value for dodecanol was obtained at 120 min. In contrast, tridecanol did not produce LRR during 20 h of exposure to both saturated and supersaturated solutions. Tetradecanol, on the other hand, did not produce LRR in tadpoles within 72 h even when concentrations in excess of saturation were present (Table 1). At intervals greater than 72 h, tetradecanol was irreversibly toxic. However, preincubation of tadpoles in tetradecanol for 48 h, did not shift their response to octanol (EC_{50}: 52 ± 5.2 with preincubation vs 59 ± 3.1 μM without preincubation; $P > 0.19$).

For octanol, results monitored by gas chromatography were in close agreement with those obtained using [^{14}C]-octanol (Table 2). However, the free aqueous concentrations of [^{14}C]-dodecanol decreased by about 15% during 2 h of incubation with tadpoles (Figure 1). Depletion of [^{14}C]-dodecanol from aqueous solution continued between 2 and 4 h, but resulted in little further change in either the aqueous concentration or in EC_{50} values (4.7 ± 0.33 (s.e.) vs 4.6 ± 0.73 μM, determined at 2 and 4 h, respectively, $P > 0.80$). In comparison, free aqueous concentrations of [^{14}C]-tetradecanol decreased by approximately 18% and 30% at 2 and 4 h, respectively. Depletion of free aqueous concentrations continued so that there was approximately 13, 50, and 68% depletion of decanol, dodecanol and tetradecanol, respectively, at 20 h.

Final concentrations of ethanol present during concentration-response assays for the long-chain alkanols (C_{10}–C_{14}) were always <25 mM; however, even as much as 40 mM ethanol does not produce LRR in tadpoles. Moreover, when results from assays performed in the presence and absence of 25 mM ethanol were compared (Table 2), it was clear that small concentrations of ethanol did not shift the concentration-response curves of other alkanols. There was no significant difference in either the EC_{50} or slope of concentration-response curves for octanol in the presence or absence of 25 mM ethanol (EC_{50}: 55 ± 3.1 vs 57 ± 2.5 μM, respectively, $P > 0.61$; slope: 3.6 ± 0.50 vs 5 ± 1.0 respectively, $P > 0.66$). Likewise, the EC_{50} (59 ± 3.1 μM, $P > 0.62$) and slope (5 ± 1.1, $P > 0.16$) for [^{14}C]-octanol were not different (Table 2).

The EC_{50}s for the series of primary alkanols, standard errors, and slopes are presented in Table 2. The EC_{50}s decreased in the series from methanol through to dodecanol, such that dodecanol was some 126,000 times more potent than methanol. The log concentration-response curves were sigmoidal with slopes ranging from 2.3 ± 0.40 to 7 ± 1.1 (Figure 2), but no systematic trends (for example, as a function of C_N) were observed. Similar degrees of

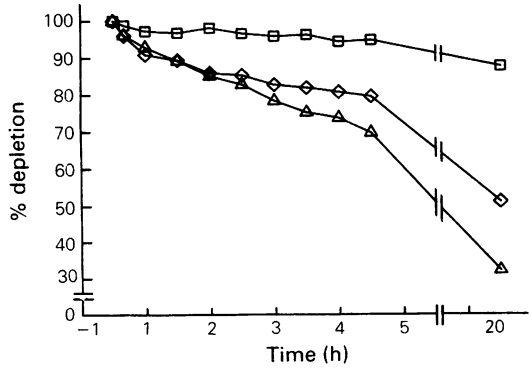

Figure 1 Time-course for depletion of long-chain alkanols from aqueous solution; decanol (□), dodecanol (◇), and tetradecanol (△). Each point represents the mean of five separate determinations during a single experiment under the conditions of the concentration-response assay (see Methods for details). In all cases, the standard deviations were <10% of the mean values presented.

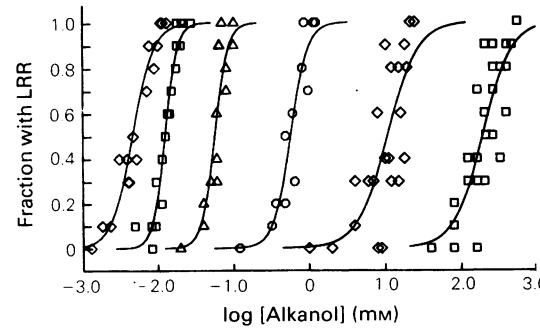

Figure 2 Log concentration-response relationships for the primary alkanols with an even number of carbon atoms. Dodecanol is the left-most, while ethanol is the farthest to the right. Curves were fitted according to the method of Waud (1972). Each symbol represents the mean response of ten animals, scored quantally as described in Methods. In some instances, symbols overlap. The total number of animals tested appears in Table 2. LRR = loss of righting reflex.

variation in the slopes of concentration-response curves for tadpoles have been previously found in our laboratory (Dodson *et al.*, 1985; Alifimoff *et al.*, 1987).

The coefficients of variation in EC_{50} values (s.e./EC_{50}) × 100%) ranged from 3.3 to 9.9%, which are comparable to those previously obtained by us (Pringle *et al.*, 1981; Dodson *et al.*, 1985; Alifimoff *et al.*, 1987) and by others measuring either righting response or minimum alveolar concentration (MAC) in mice (Deady *et al.*, 1980), or MAC in man (Saidman *et al.*, 1967). A similar degree of variation was found between experiments. For example, the mean of five individual ethanol concentration-response experiments which were fitted independently was 190 ± 22 mM (s.e.).

The relationship between EC_{50} and the alkanol carbon chain length (C_N) was logarithmic (Figure 3a), and a least-squares line fitted to log EC_{50} versus C_N had a slope of -0.49 ± 0.024 (s.e.) ($r^2 = 0.9764$). The deviations of the observed values for EC_{50} from the fitted line were assessed by calculating the residual of each point (Figure 3b). This suggested that the data might be better fitted by a quadratic rather than a linear equation. Fitting the data to a quadratic equation (log $EC_{50} = 0.022$ (± 0.0038) C_N^2 ± 0.76 (± 0.051) $C_N + 3.7$ (± 0.14) ($r^2 = 0.9951$)) resulted in a significantly better fit ($P < 0.002$; F test).

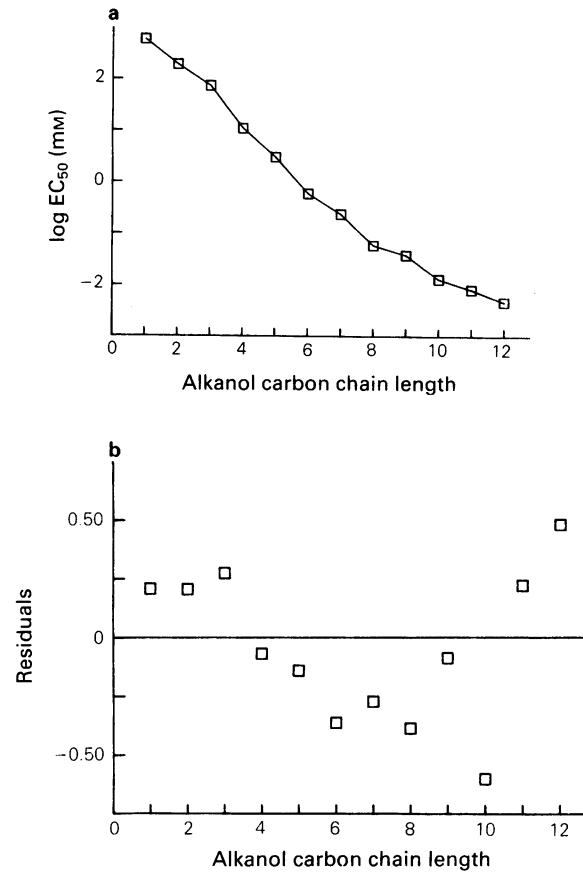

Figure 3 (a) Relationship between log EC_{50} and the number of carbons in the hydrocarbon chain of the primary alkanols (C_N). Error bars are smaller than the symbols. (b) Residual analysis of (a). The residuals (y axis) are the difference between the observed value of log EC_{50} and the calculated value from a linear fit of the data in (a).

Discussion

This study was undertaken to resolve deficiencies in the anaesthetic potency values for the primary alkanol series available from the literature. The problems mentioned in the Introduction have been addressed in this study as follows: (i) the EC_{50}s presented in Table 2 represent the only complete series of anaesthetic potencies for primary alkanols from a single laboratory; (ii) the potencies were derived by analysis of quantal concentration-response curves so that estimates of standard errors around both EC_{50}s and slopes could be made; and (iii) free aqueous concentrations of all alkanols were monitored either by gas chromatography or liquid scintillation spectroscopy. Finally, monitoring the free concentrations allowed the exact position of the cut-off in anaesthetic potency of primary alkanols to be established.

Any model of the anaesthetic site should share the pharmacological properties of general anaesthesia itself, including pressure reversal (Lever *et al.*, 1971); lack of stereoselectivity between enantiomeric pairs of secondary alkanols (Alifimoff *et al.*, 1987); and cut-off (Janoff & Miller, 1982). Anaesthetic cut-off has been observed in several series of homologous compounds, including the primary alkanols, alkanes, and fluorocarbons (for a review see Miller, 1987). While luciferase has also been shown to manifest a cut-off in inhibitory potency for the primary alkanols, this occurs at a higher molecular weight member of the series (beyond hexadecanol) than in animals (beyond dodecanol). One conceivable explanation for this discrepancy involves the depletion of anaesthetic from the aqueous phase, wherein the cut-off *in vivo* might actually occur at a longer chain length than previously observed. Our data, however, clearly establish that cut-off *in vivo* is not an artifact of depletion and that it occurs at tridecanol. This was demonstrated by the inability of both tridecanol and tetradecanol to produce LRR. Tridecanol did

Table 3 Comparison of anaesthetic potencies of the primary alkanols obtained by different investigators

C_N	Vernon (1913) (mM)	Meyer & Hemmi (1935) (mM)	Pringle *et al.* (1981) (mM)	Present study (mM)
1	990	—	—	590
2	410	330	120	190
3	104	110	54	73
4	22.3	30	12	11
5	—	7.0	—	3
6	0.9	—	0.7	0.57
7	0.35	0.38	—	0.23
8	0.127	0.13	0.06	0.057
9	—	0.025	—	0.037
10	—	0.010	0.013	0.013
11	—	0.005	—	0.0081
12	—	0.0075	0.0054	0.0047
13	—	—	0.037	Not anaesthetic

Dashed lines indicate that values were not determined.

not induce LRR after exposure of tadpoles for 20 h to either saturated or supersaturated aqueous solutions of tridecanol. Even after 20 h of exposure to supersaturated solutions, aqueous concentrations still remained above saturated solubility (Bell, 1973). Likewise, tetradecanol did not induce LRR despite (i) long-term exposure of tadpoles to solutions containing in excess of 0.9 μM, and (ii) exposure for 72 h to supersaturated solutions of [^{14}C]-tetradecanol (Table 1). Although 74% of the total amount of [^{14}C]-tetradecanol initially added was depleted during the 72 h, the aqueous phase still contained more tetradecanol than the published saturated solubility of approximately 1.5 μM (Bell, 1973).

The observed percentage depletion increased with the alkanol chain length. *A priori*, this might arise from increasing lipophilicity and surface activity coupled with decreasing aqueous solubility. Simple calculations show that uptake into the fatty tissues of the tadpoles would account for much of the depletion within the range of error of the published lipid-buffer partition coefficients (Sallee, 1978; Franks & Lieb, 1986).

When our results are compared to those previously obtained by Pringle *et al.* (1981), the majority of their values are within the precision of our measurements. However, the values for both ethanol and propanol observed by Pringle *et al.* (1981) were significantly lower than in the present study. In addition, they demonstrated that tridecanol was a partial anaestheic, but in the present study, it did not induce LRR even after prolonged exposure to supersaturated solutions. Because the purities of the anaesthetics used in that study as well as their free aqueous concentrations were not monitored by gas chromatography, we can only explain the discrepancies by possible contamination with potent higher molecular weight anaesthetic alkanols.

That the relationship between log EC_{50} and C_N is best described by a quadratic equation rather than a straight line can be interpreted to mean that the approach to cut-off is gradual rather than abrupt, whereas the phenomenon of cut-off itself is even more abrupt than previously appreciated, in that potency disappears completely after dodecanol, i.e., there are no partial anaesthetics. This gradual approach to cut-off implies that the intrinsic anaesthetic efficacy of dodecanol per methylene group is less than that of the other alkanols. Whether this somewhat gradual loss of potency per methylene group occurs in other homologous series of anaesthetics which display cut-off remains to be investigated.

While the approach to cut-off is gradual for the primary alkanols in that the higher molecular weight members of the series significantly deviate from a linear relationship, we found no such deviations from linearity in the region of hexanol and heptanol. Indeed a fit of the EC_{50}s of propanol through octanol showed a strong linear correlation ($r^2 = 0.9912$). This is in contrast to the results of Franks & Lieb (1985) who compared a collection of data for tadpole anaesthesia with the inhibitory potencies of primary alkanols on luciferase (IC_{50}). They observed that the chain-length dependence of the IC_{50}s levelled off in the region of hexanol and heptanol (i.e., these two alkanols were approximately equal in potency) and that the change in the free energy of binding ($\Delta\Delta G°$) of alkanols to luciferase appeared to parallel that found in animals. This was interpreted to mean that the alkanol binding site on luciferase, and the *in vivo* anaesthetic site, share

similar molecular dimensions. However, recalculation of their $\Delta\Delta G^\circ$ with our self-consistent set of EC_{50}s shows that the reported correlation between anaesthetic activity in tadpoles and luciferase does not exist (Figure 4). The disparity between our EC_{50}s and those used by Franks & Lieb (1985) (Vernon, 1913; Meyer & Hemmi, 1935) are made apparent in Table 3. It appears that Vernon's and Meyer's laboratories employed different anaesthetic endpoints (suppression of spontaneous movement vs suppression of reflex response, respectively) as well as different indices of relative potencies (EC_{100} vs EC_{50}, respectively). In addition, as pointed out by Elliott & Haydon (1986), the value shown for pentanol (Vernon, 1913) is actually an estimate based on that of isopentanol. Thus, it would seem that combining these two sets of data for the purposes of molecular level arguments is inappropriate.

Linearity of log EC_{50} versus C_N for the lower molecular weight members of the series has been observed in mice (LRR for ethanol through hexanol, $r = 0.993$ (Lyons *et al.*, 1981)) and the freshwater shrimp *Gammarus* (butanol through octanol, $r^2 = 0.998$, Elliott *et al.*, 1987). In the latter study, hexanol and heptanol were not equipotent, as they are shown to be for luciferase. Thus whatever such anomalies observed in luciferase may imply about the molecular features of the alkanol site *in vitro*, inferences regarding the site *in vivo* must be made with caution.

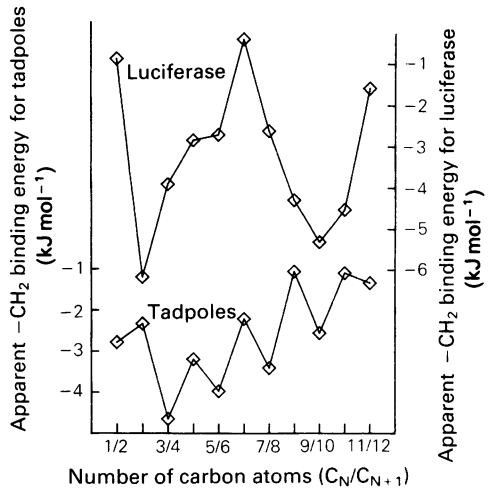

Figure 4 The apparent changes in the free energy of binding ($\Delta\Delta G^\circ$) for a methylene group to luciferase (top) and for loss of righting reflex (LRR) in tadpoles (bottom) as a function of C_N/C_{N+1}. Note that the vertical axis scale for luciferase is shown on the right while that for tadpoles is shown on the left. Points were calculated using the differences in thermodynamic free energy,

$$\Delta\Delta G^\circ = -RT \log[EC_{50}(N)/EC_{50}(N+1)]$$

for luciferase inhibition and LRR. The EC_{50} values for luciferase inhibition are from Franks & Lieb (1985 and personal communication). The EC_{50} values for LRR are from Table 2.

One previous explanation for cut-off, derived from lipid based theories of anaesthesia, was based on the reported decrease in the membrane/buffer partition coefficient for tetradecanol, such that the maximum membrane concentration obtained was less than that necessary for anaesthesia (Pringle *et al.*, 1981). However, recent data obtained in cholesterol-containing bilayers (Franks & Lieb, 1986) revealed that partition coefficients continue to increase beyond dodecanol, making it unlikely that cut-off is a consequence of inability to achieve a sufficient membrane concentration. Therefore, either biomembranes behave differently in this respect from lipid bilayers, which seems unlikely, or the alternative explanation, that cut-off may arise from a loss in the intrinsic pharmacological efficacy must be considered (Pringle *et al.*, 1981; Janoff & Miller, 1982). Indeed, observation of a gradual loss of lipid disordering potency, or even an increase in order, by long chain alkanols in spin labelled lipid bilayers (Richards *et al.*, 1978) and postsynaptic membranes (L. Firestone, Miller, K.W., unpublished observations) support the latter alternative.

In summary, we present LRR potency values for the complete series of anaesthetic primary alkanols. In contrast to the previously published values, the aqueous concentrations of all the alkanols were monitored, and the EC_{50} values were obtained from quantal concentration-response relationships. The EC_{50}s show a relationship between potency and alkanol chain length from methanol through to dodecanol which is best described by a quadratic equation, suggesting that the *approach* to cut-off is gradual. However, after dodecanol potency abruptly cuts off in that there are no partial anaesthetics. Depletion of longer chain alkanols, while significant, is unable to account for the potency cut-off.

This work was supported in part by grants from the National Institute of General Medical Sciences to the Harvard Anaesthesia Research Center (GM-15904, GM-07592) and the Department of Anaesthesia, Massachusetts General Hospital. J.K.A. was supported in part by an Anesthesiology Young Investigator Award and is a Foundation for Anesthesia Education and Research Investigator. L.L.F. was supported by a Starter Grant from the American Society of Anesthesiologists and a Fellowship from the Charles A. King Trust, Boston, MA. The authors thank Dr David C. Hoaglin, Department of Statistics, Harvard University, for assistance in statistical analysis.

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(Received July 14, 1987
Revised June 22, 1988
Accepted August 1, 1988)